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CASHEWNUT MOISTURE RELATIONS

By T. N. OKWELOGU and P. J. MACKAY

Investigations on the moisture relations of raw cashewnuts at tropical temperatures (about 27°) are described; a description is included of a laboratory method suitable for use as a reference method, which involves the use of toluene in a Dean & Stark distillation apparatus. This method was found to be reliable to within 0·3%.

There was some evidence that differences in the moisture contents of these nuts stored under identical conditions may be partly due to differences in the kernel/shell weight ratio, and that this ratio is probably higher in small than in larger nuts. This might explain the slight tendency for the moisture content of heavy nuts to be greater than that of lighter nuts.

Nuts stored in moving air took up moisture more rapidly and attained equilibrium moisture content much faster than in apparently still air.

It was found that at an equilibrium moisture content the shell moisture content could be used to predict the moisture contents of the whole nut and kernel.

Above 75% R.H., cashewnuts at 27° became visibly invaded by microftora including members of the genera *Aspergillus, Fusarium, Paecilomyces, Penicillium* and *Rhizopus.* Sorption and desorption curves were produced, relating the ambient relative humidities to the corresponding equilibrium moisture contents of fresh nuts stored in apparently still air at 27°. From this relationship the maximum safe moisture content for raw cashewnuts was estimated to lie between 8·9 and 9'2%, being that in equilibrium with surrounding air at 70% R.H. and 27°.

Introduction

The cashew, *Anacardiul/1 occidelllale,* has become an important 'cash crop' in many countries, notably India (where it accounts for about 9% of the agricultural export earnings!), Mozambique, Tanzania, Kenya and Brazil. In a number of these countries, production has been increased in recent years or is projected for the future: for example, in Tanzania, the target set by the 5-year development plan[2] is 85,000 tons by 1970.

After collection, the nuts are usually sun-dried and are then stored in bags for use throughout the year. If this initial drying is inadequate and/or the nuts are subsequently stored in very humid surroundings, as is frequently the case, deterioration sets in within a few weeks, owing to attack by moulds, bacteria and possibly insects. This usually results in decayed or discoloured kernels, which are highly objectionable to the trade.

Although it is recognised in the industry that cashewnuts should be adequately dried to avoid spoilage during storage, there is little published research on the necessary moisture relations. The present studies have been carried out to investigate these relations and, in particular, to establish the maximum moisture content for the safe storage of cashewnuts under tropical conditions.

It has been necessary to establish a reference method of determining the moisture content of cashewnuts.

Experimental

Nuts

The terms 'nut' and 'kernel' have been used with their botanical connotations, and, unless otherwise stated, the term 'kernel' includes the testa. The nuts used were obtained from Tanzania between 1966 and 1968.

To obtain nuts with different levels of moisture content *(MC),* the nuts were first dried at 40–45° in a forced-draught air-oven for 24 hours, and then cooled over silica gel in a desiccator. The initial *MC* was determined and samples were exposed to air at selected relative humidities (R.H.) maintained in standard laboratory desiccators by suitable saturated salt solutions.[3] Nuts were periodically withdrawn for determination of *MC,* until the desired level was reached, or until the equilibrium *MC* was attained, depending upon the objective. This was the sorption technique. Alternatively, the desorption technique, in which the initial *MC* of the nuts was higher than the desired final levels, was used. In this case, also, nuts were conditioned to the different R.H. For some of the experiments, the nuts were conditioned to the desired *MC* by exposure to the air in a room maintained at the appropriate constant temperature and humidity. In the present work a constant temperature and humidity (c.t.h.) room with air maintained at about 27° and 75% R.H., was used to provide approximately tropical conditions. Continuous air movement was obtained by means of a ceiling fan.

Determination of cashewnut moisture content

Cashewnuts contain volatile constituents, and in an oven-drying method, these, as well as moisture, would be driven off. A distillation method was used to avoid this problem. Toluene (b.p. 110°) was used as the entraining solvent since it is immiscible with water and would presumably take up any organic constituents of the nut which might distil over with water.

Procedure

A Dean & Stark distillation apparatus was used, essentially as described by the World Health Organisation.[4]

The entire apparatus was cleaned with potassium dichromate/sulphuric acid solution to minimise the adherence of water droplets to the sides of the condenser and receiver; it was rinsed thoroughly with distilled water and was dried completely (e.g. in a forced-draught air oven) before use.

The nuts were prepared for analysis as quickly as possible. The nut was held with gloved fingers (for protection against the vesicant action of the shell liquid) with the suture line uppermost in a pit carved in a wooden block. The nut was cut with a scalpel, first along the suture and then across it, into quarters. The splits were transferred into a weighing container and the same operation was carried out quickly on the remainder of the nuts to be analysed together. The material was weighed immediately (by the differential method) at least to the nearest 0·I g and was transferred to the distillation flask. Sufficient dry toluene was added to cover the sample, and the mixture was swirled.

After application of Vaseline to the ground joints of the condenser and receiver, the apparatus was assembled and the receiver was filled with toluene poured through the condenser until it began to overflow into the distillation flask. A loose absorbant cotton wool plug was inserted in the top of the condenser to prevent condensation of atmospheric moisture within the tube.

Cold water was kept in continuous circulation around the condenser. The flask was heated at such a rate that about 170-190 drops of distillate were collected per minute. The sample was completely covered by the toluene while the distillation was in progress and heating was continued until no more water collected (end-point).

During the distillation, the condenser was purged occasionally with small (~ 5 ml) portions of toluene in order to wash down any water adhering to its walls. A copper wire was moved periodically up and down in the condenser and receiver, thus causing all the water to settle to the bottom of the trap. When the water level in the receiver remained unchanged for 30 min, using the copper wire again, the condenser was flushed down with some toluene and heating was continued for a further few minutes. Any water still not in the column was brought down within the trap using the wire. After the receiver cooled to room temperature, the volume of water collected was read.

The moisture content was calculated from the following equation:

$$M\,C\ (\text{wI.}\ ^{0}/_{0}) = \frac{100 \times V}{W}$$

where V = volume of water collected (ml) and W = wI. of sample (g).

Distil/ation time

When distillation was carried out at the prescribed rate, and the collected proportion (as %) of the total amount of water in the material was plotted against the distillation time, a curvilinear relationship was obtained (Fig. I); about 12 hours of distillation were required to collect all the available water. Since a shorter distillation time would be preferred in practice, a faster means of obtaining the end-point result was examined.

When moisture losses after a 2 h distillation were plotted against the corresponding moisture losses after distillation to end-point, a rectilinear relationship was obtained (Fig. 2) which could be used as a reference for subsequent analyses. However, for the present studies, distillation to end-point was adopted.

Results

Table I shows the results of five different sets of analyses carried out on three different batches of nuts (I, 2 and 3). For each analysis, not less than four nuts were used.

In batch 3, ten nuts were used for each analysis. Only nuts with sound kernels were used in batch 3 (a), whereas samples of batch 3 (b) consisted of nuts which had been randomly

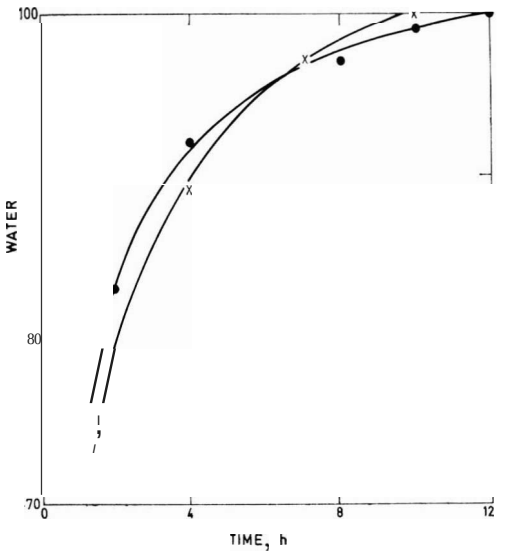


FIG. I. *Relationship between distillation time alld proportioll* (%) *oj total water collected Jrom cashewllllls*
Each point represents the average of 4 readings
• Whole nut; x Shell

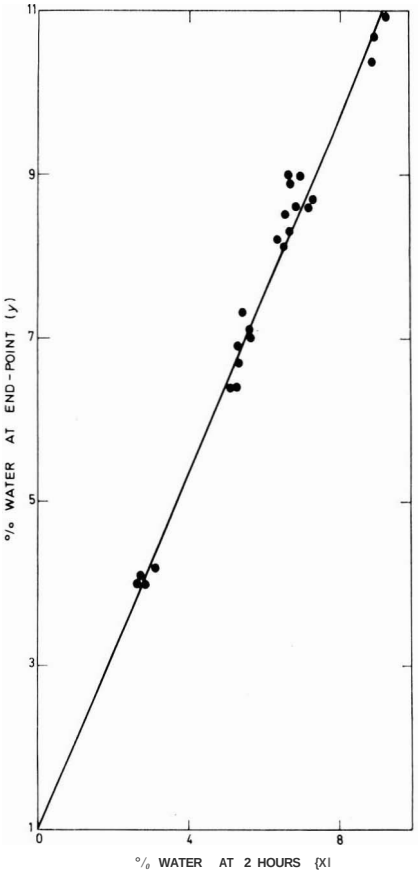


FIG. 2. *Relatiollship beMeell the 1lll0isture cOlltelll oj whole cashew-nuts bUJed Oil 2 h alld elld-poilll distillatioll*
J' 1·1x + 1·0

TABLE I

Repeatability of the method of determining the moisture content of cashewnuts by distilling with toluene

Batch	Description	*Me* (%) in replicate No. 2		4		Mean	S.E. of mean
\	Wholenut	8·3	8·8	8·4	8·6	8·5	± O'II
2	Wholenut	4·2	4·0	4·2	4·0	4·1	± 0'06
3(a)	Wholenut (S)	6·8	6·9	7·0	6·9	6·9	± 0'13
3(b)	Wholenut (Us)	7·0	6·9	7·\	7·0	7·0	± 0'13
3(c)	Shell	7·5	7·5	7·5	7·7	7·6	± 0'16

Average S.E. of mean == ± 0·\2; 99% confidence limits = ± 0·3
S = selected; Us = unselected

TABLE Il

Average percenlage moisture contents of raw cashewnuts exposed to moving and still air at 27° and 75%R.H.

Time	Still air Group A	Moving air Group B	Group C
Initial	4·1	5·3	6·6
I day		7·6	7·6
1 week	7·2	7·9	8·1
2 weeks	8·2	7·7	7·8
3 weeks		8·0	8·1
4 weeks	9·0	7·9	8·3
6 weeks	9·1		

selected after successive quartering, and the percentage contents, by count, of decayed or otherwise bad kernels were 20,0,40 and 10 for replicate numbers 1,2, 3 and 4, respectively.

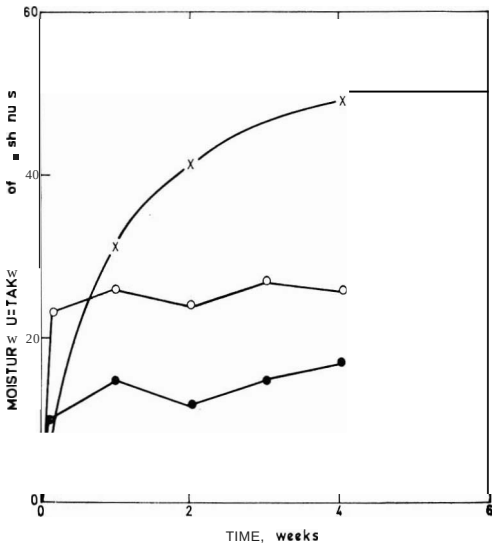
An examination of the above data shows that the distillation method described gives results repeatable to within 0·3% in at least 99% of all cases. The results also suggest that, provided the samples are as representative as possible, the reliability of the analytical method is altered neither by the kernel (i.e., sound or decayed) nor by whether the wholenut or the shell is analysed for water.

Uptake of moisture by cashewnuts

The rate of uptake of moisture by cashewnuts in apparently still air and in moving air at 27° and 75° R.H. was investigated. In the experiment in still air, fresh nuts (group A) were dried to 4·1% moisture content. They were then divided into four portions, each of which was conditioned to 75% R.H. (over saturated solutions of common salt) in separate air-tight desiccators kept in the c.t.h. room. The moisture contcnt *(MC)* of the nuts was determined after I, 2, 4 and 6 weeks using only the nuts from one desiccator on each occasion so that no desiccator was opened more than once. On each occasion, two samples of four nuts each were analysed for water and the average *MC* of the eight nuts was calculated.

In the experiment in moving air, two groups of nuts (8 & C) at 5·3 and 6·6% *MC,* respectively (but otherwise from the same consignment and given the same initial drying and wetting pre-treatment) were exposed one-nut deep in two shallow, open metal trays in the c.t.h. room. Moisture contents were determined after 24 hours and then after I, 2, 3 and 4 weeks. On each occasion and for each group, two samples each of four nuts were analysed for water and the average *MC* of the eight nuts was calculated. The results of the investigation are shown in Table II. Finally, the data were converted to amounts of moisture taken up by I g weight of nuts in the course of the study (Fig. 3).

Fig. 3 shows that the uptake of moisture was slower in still air than in moving air, and that, particularly in the latter situation, the initial *MC* of the nuts tended to influence the rate of moisture uptake, even though the time taken to reach equilibrium *MC* did not appear to be affected. Thus, in still air, the group A nuts attained equilibrium *MC* in about 5 weeks, whereas the two groups of nuts with the initial *MC* of 5·3 and 6·6% attained equilibrium *MC* within a week of exposure to moving air.



FtG.3. *Rate of uptake of moisture at 27° alld 75% R.H. perg wt. offresh cashewlluts, whell three groups were exposed to still air* (x) *alld movillg air* (e *alld 0)*

x (group A) 4·'%; • (group B) 5·3%; 0 (group C) 6·6% Me

Effect of differences in nut weight on moisture content

Fresh wholenuts were exposed one-nut deep in a shallow, open tray and kept continuously in the c.t.h. room for 12-13 days. Just before being split for analysis (still in the c.t.h. room), the nuts were weighed individually and then grouped into eight batches of four nuts each. In no case was the weight of the batch after splitting for analysis less than 99·7% of the weight before splitting. The percentage *MC* of each batch was determined and was taken to represent the *MC* of an average nut in the batch. The weight of an average nut in a batch was then calculated from the batch weight. The results are shown in Table Ill.

The results suggest that in at least six out of every ten cases differences in nut weight probably account for about a third of the observed differences in the *Me*. In a further experiment, using nuts from the same batch used in the previous experiment, two groups of ten small (A) and ten large (B) nuts were selected. Each nut was then split, and the weights of the shell and kernel were determined. The shells of all the large nuts were collected together, as were the kernels. The average *MC* of the shell and of the kernel were then determined. These procedures were repeated for the shell and kernel of the small nuts. On the assumption that all the shells and kernels were identical in all relevant respects for the large and small nuts, respectively, the appropriate *MC* was used to calculate the dry weights of the shell and kernel. The dry weight for each nut was obtained by adding the dry weights of the corresponding shell and kernel. Finally the kernel/shell weight ratio (*Kr*) was calculated for each of the 20 nuts (Table IV).

The results suggest that in at least nine cases out of ten the kernel/shell weight ratio tends to decrease with increasing nut weight. They also indicate that probably a third of the difference in the *Kr* values is due to differences in nut weight. A heavy nut usually has a lower *Kr* value than that of a lighter nut.

TABLE lit

Relationship between the weight of a cashewnut and its *Me* after exposure to moving air at 27° and 75% R.H. for 12-15 days

Parameter	Nut No.							
				4		6		
Wt., g	4·0	4·4	5·4	6·3	7·5	7·7	7·8	7·9
MC, %	8·5	8·3	8·5	8·2	8·9	8·5	8·7	8·7

r = 0'58; 't' distribution = 0'9; probability = 0·38

TABLE IV

Relationship between the nut weight and kernel/shell weight ratio (*Kr*) in two groups of cashewnuts

Nut No.	Group A		Group B	
	Dry wt, g	*Kr*	Dry wI., g	*Kr*
1	2·4	0'63	4·7	0·46
2	2·6	0·53	5·3	0·46
3	2·7	0·61	5·5	0·50
4	2·7	0·48	5·5	0·50
5	2·8	0·50	5·6	0·53
6	3·2	0·53	5·6	0·42
7	3·3	0·42	5·6	0·41
8	3·3	0·33	5·7	0·48
9	3·4	0·51	6·0	0·41
10	3·8	0·46	6·3	0·43
Mean	3·0	0·50	5·6	0·45
r	−0·61		- 0,585	
't' distribution	2·17		1·99	
Probability	0·07		0·09	

Relationship between moisture contents of shell, wholenut and kernel

Two sets of cashewnuts from two different consignments were conditioned to equilibrium moisture contents. In the first set, each of the four batches used contained eight nuts for determination of the *MC* of the shell and kernel which were analysed separately. In the second set, each of the eight batches used (four for determination of the *MC* of the shell and four for the wholenut) contained four nuts. The results are shown in Table V.

These results show that a high wholenut or kernel *MC* is closely associated with a high shell *MC*, and that for nuts the *MC* of which are in equilibrium with the ambient R.H., the *MC* of the wholenut and kernel can be predicted from that of the shells with more than 99% confidence.

Equilibrium MC/R.H. relationship for nuts at 27°

The nuts used in this investigation were fresh 1967 Tanzanian crop. The average *MC* at the time of receipt in Britain was found to be 13·1%. The nuts were divided into two batches. The first batch was dried to 6·3% *MC*, and then divided into three portions, which were conditioned to equilibrium *MC* at R.H. of 58,75 and 90% to obtain sorption data. The second batch of nuts was used as received, because 13'1% was considered to be a higher *MC* than would be expected at the highest R.H. used in the desorption experiment. These nuts were therefore dried to equilibrium *MC* at 58, 71 and 81% R.H., the *MC* being determined after 6, 7 and 8 weeks of conditioning, using four nuts for each determination. A graph (Fig. 4) was drawn on which the average percentage *MC* for each set of three results (Table VI) was plotted against the corresponding R.H. No individual result differed from the average by more than 0·3% *Me*.

It was observed that, under the conditions of the investigation, nuts conditioned to relative humidities above 75% became mouldy within a few weeks, nuts at 90% R.H. being the worst affected. The invading micro-organisms were subsequently isolated and identified (Table VII).

It can be seen from Fig. 4 that the *MC* of the nuts corresponding to 70% R.H. (below which level they are reasonably safe from most moulds) is 9·2%.

Discussion

The development, advantages and shortcomings of the methods for determining the moisture content of materials by entrainment distillation with an organic liquid immiscible

TABLE V

Relationship between the moisture content of the shell and those of the wholenut and the kernel, after conditioning the nuts to equilibrium moisture contents in still air at 27° and different R.H.

Part	*MC* (%) for batch number				
				4	
Shell (*x*)	6·8	8·2	10·1	10·5	Regression equation: $y = x - 0'5$
Wholenut (*y*)	6·4	7·6	9·4	10·3	't' = 10·9 (P = 0,01)
Shell (*x*)	5·4	6·8	8·1	10·4	Regression equation: $y = 0·8x + 0·9$
Kernel (*y*)	5·2	6·1	7·7	9·3	't' = 12·2 (P = 0'01)

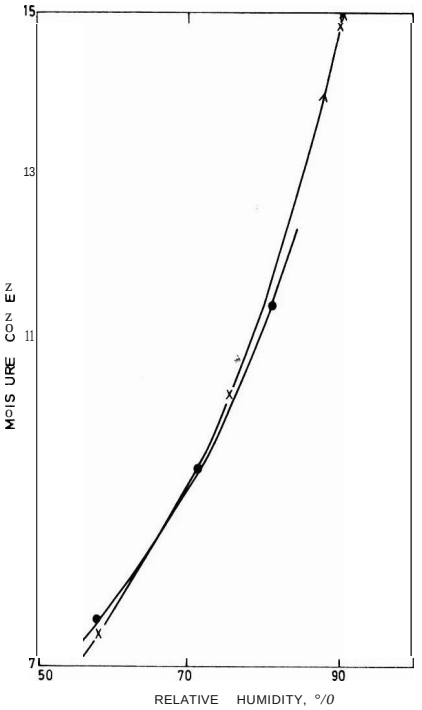


FIG. 4. *Mois/llre colllellls oj whole cashewlluls* ill *equilibrium wilh lhe ambielll re/alive humidilies at 27°*

Each point represents 3 measurements with 4 whole nuts in each
• Desorption; × sorption

TABLE VI

Average percentage equilibrium moisture contents of whole cashew nuts at **27°**

R.H., %	Desorption (from 13·1% *Me)*	Sorption (from 6'3% *Me)*
58	7·6	7·4
71	9·4	
75		10·3
81	11·4	
90		14'8

TABLE VII

Micro-organisms isolated from Tanzanian cashewnuts kept at above 75% R.H. and **27°**

\+ Present; - absent

Species of micro-organism	Outer surface of shell	Between shell and kernel	Within kernel
Aspergillus amsle/adami		+	
A. /lavus	+	+	
A. japollicus	+	+	+
A. niger	+	+	+
A. sejunctus		+	+
Fusarium moniliforme		+	
Paecilomyces variot;		+	+
Pellicillium jrequelllalls	+	+	+
Penicillium crustosum		+	
Penicillium funiculosum			+
Rhizapus arrhizus	+		

with water have been reviewed in detail by Tate & Warren.[5] One chief source of inaccuracy is the loss of moisture as droplets on the sides of the condenser and receiver above the column of water in the trap. The magnitude of this loss was reduced by the precautions discussed previously. Nevertheless, from the experience gained in the present work, the permanent error in the method could be estimated at 2% of the reading.

Reproducibility with split cashewnuts and toluene is estimated to be within 0·3%, which agrees closely with the 0'4% estimated by Esteves." One other difficulty with the method as recommended here for cashewnuts is the long distillation time involved. This can be reduced considerably if a reference graph (see Fig. 2) is first prepared, by means of which results obtained from a shorter distillation time can be converted to the desired end-point values.

During storage in the tropics and sub-tropics, cashewnuts are usually subject to the effect of successions of air currents and apparently still air, at varying combinations of temperature and humidity. Results from the present work suggest that in moving air the nuts take up moisture very rapidly in the first 24 hours, and then very slowly until equilibrium moisture content is reached. In this case, equilibrium appeared to have been reached within a week of exposure. On the other hand, under identical conditions, but with the air apparently still, the nuts took up moisture at a steadily decreasing rate until equilibrium *MC* was reached after nearly five weeks. The practical implication is that the equilibrium *MC* of nuts may be reached in a much shoner time by installing a fan in the conditioning chamber and that for practical storage under humid tropical conditions, there should be minimum ventilation in the godowns, since excessive movement of damp air around the product will tend to raise its *MC* to undesirable levels in a relatively short time. On the other hand, with the air reasonably still, the attainment of high moisture contents in a large stack of adequately dried nuts will be much delayed. The study under moving air revealed further that the lower the initial *MC* of the nuts, the greater is the rate of moisture uptake, probably owing to the lower vapour pressure of the water within the nut; the higher the *MC,* the greater is the vapour pressure of the water within the nut, and the less rapid will be the uptake. However, the time taken to reach equilibrium did not appear to have been greatly affected by the differences in the initial *Me.*

There seems to be some evidence that higher moisture contents tend to be associated with heavier nuts. This slight effect of nut size or weight appears to arise because a given weight of shell is associated with more of the kernel tissue in the small nut than it is in the large nut. About a third of the shell is an oily cashewnut shell liquid, which is presumably hydrophobic, while 38-47% of the kernel is fat' suggesting that the kernel is more hydrophobic than the shell. Consequently a higher kernel/shell weight ratio (or *Kr)* will tend to be accompanied by a lower *MC* and *vice versa.* It therefore appears that the slight variations in the *MC* of cashewnuts may be in some part due to variations in the amount of kernel tissue associated with a given weight of shell in a nut.

For cashewnuts which have been conditioned to an equilibrium moisture content, a knowledge of the *MC* of the shell has been shown to be adequate for predicting the *MC* of the wholenUl and of the kernel. This is of interest in developing a moisture meter which measures only the *MC* of the cashew shell and does not involve the splitting of the nut, thereby speeding up the process of moisture determination.

It has been estimated from one of the present studies that the equilibrium moisture content of freshly collected raw cashewnuts is 9·2% at **70%** R.H. and **27°**. This figure did not take into account the insignificant inaccuracy found to be inherent in the method. However, provided that the prescribed procedure is employed, 9·2% may be accepted as the *MC* of cashewnuts in equilibrium with **70%** R.H. This level of R.H. is about the minimum required to promote deterioration of produce by mites, most fungi and bacteria at normal tropical temperatures. **In** view of the problems of representative sampling, and because the method of determining the *MC* as described here gives values reliable to within O·3%, the maximum *MC* for safeguarding fresh nuts against attack by micro-organisms during storage should be taken as 8·9%. As it is preferable, in practice, to reject a dry batch of nuts rather than risk accepting a wet one, this level of acceptable *MC* may be lowered further.

It has been suggested[8] that the maximum permissible figure for the safe storage of oilseeds is 14% of moisture calculated on the non-oleaginous portion of the seed. As stated above, about **38–47%** of the cashew kernel is fat, one-third of the wholenut is kernel, and about one-third of the shell is a non-fatty oily liquid. Therefore, for a cashewnut with a kernel of the lower value of 38% oil content, the total non-oil portion of the wholenut would be approximately 65%, and the calculated maximum permissible *MC* would be 14/100 of 65%, i.e., 9·1%. Similarly, for a nut the kernel of which contains the higher value of **47%** oil, the total non-oil portion of the wholenut is approximately 62%, and the calculated maximum permissible *MC* would be 14/100 of 62%, i.e., 8·7%. Hence the calculated limits for the maximum 'safe' moisture content for raw cashewnuts is 8·7-9' I%, which agrees very closely with the limits established in the present studies.

Acknowledgments

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Slough,
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EFFECTS OF HEAT PROCESSING ON THE NUTRITIONAL VALUE OF GROUNDNUT PRODUCTS

I.-Protein quality of groundnut cotyledons for rats

By K. ANANTHARAMAN and K. J. CARPENTER

The trypsin inhibitor activity of raw groundnut cotyledons was approximately 20% of that found in raw soya beans but was apparently sufficient to cause significant pancreatic hypertrophy in rats receiving 15% protein from this source in their diet. Autoclaving at 121° for 0·5 h reduced the inhibitor activity to one-fifth; dry heating for the same time at this temperature reduced it to one-half. There was a tendency for 0·5 h heating. wet or dry, at 100-121° slightly to increase the net protein utilisation *(NPU)* value of the protein for rats. Dry heating at 121° for 4 h severely reduced *NPU;* fluorodinitrobenzene-available lysine in the severely heated material was similarly reduced but there was little change in the level of available methionine assessed using *Streptococcus zymogenes.*

Introduction

Since the observation by Osborne & Mendel' of beneficial effects of heat processing on the protein nutritional value of raw soyabean for rats, numerous reports have appeared confirming the effect, for a number of species.[2,3] Such improvements are now recognised as being due to the heat inactivation of the naturally occurring trypsin inhibitors and other deleterious factors in the soyabean.[4,5] One natural consequence of these findings is that similar studies have been carried out on a variety of other protein-rich vegetable materials.

With groundnuts *(Arachis hypogaea)* it has been elaimed that an improvement in protein quality results from wet-heat processing. Cama & Morton" and Balasundaram ,'t *al.*[7] observed increases in the protein efficiency ratios *(PER),* biological value *(BV)* and digestibility with rats on expeller-pressing of groundnuts, after 'slight' initial cooking or on moderate steaming of the expeller meal. 15 and 25% increases in the growth-promoting value for rats of a hydraulic press meal on open steaming for O'5 h and on autoelaving at 15lb/in^2 for a similar period, were reported by Kuppuswamy & Subrahmanyan;[8] more severe autoelaving impaired its growth-promoting value. However, Borchers & Ackerson[9] found that autoelaving at 15 lb/in^2 for 0·5 h had no beneficial effect on groundnu! protein.

Dry-heating or roasting conditions have only been reported to lower the protein nutritional value of groundnuts; this effect has been associated with a lower availability of certain essential amino acids, particularly lysine.[10,11]

In the present paper further work is reported on the effects of heat processing of groundnuts on their protein value for rats, and the possible correlation of that value with *il/ vitro* trypsin inhibitor activity. The test materials have also been analysed for fluorodinitrobenzene (FDNB)-available lysine[12] and assayed for available methionine\3 by microbiological means to investigate whether these tests can be used to predict the nutritional value of groundnut products.

Experimental

Materials

Decorticated groundnuts of the Spanish light-skinned variety were bought locally and sorted by hand to eliminate fungus-damaged and shrivelled nuts. They served as the starting material (X.427) for the preparation of the heat-processed samples llsed in most of the work. A further batch of similar nuts was used for experiment 3. The compositions of the heated and unheated preparations are given in Table I.

COl/trol groul/dl/ut /lour (X.428)

10 kg X.427 were kept in a forced-draught oven for 8 h at 45 ± I° to effect a differential drying between the cotyledons and the skins. They were then rubbed in a coarse jute bag to loosen the skins, which were separated from the cotyledons by a cyelone separator device assembled with the aid of a laboratory hot-air blower. The cotyledons were finally sorted manually.

Attempts to hammer-mill the material through a standard screen reduced it to a buttery consistency which made subsequent fat extraction difficult. The skin-free nuts were therefore passed through a laboratory mill (Christy & Norris, Ltd., Chelmsford) equipped with 8 in hammers but with the screen removed. Most of the material then passed readily through a 12 mesh BSS sieve. Batches of 500 g were fat-extracted with 3 I portions of petroleum spirit (b.p. 40-60°) for 8 h in a large Soxhlet extractor, dried for 16 h at 45 ± 1° in a forced-draught oven, and milled to a flour (X.428).

For experiment 3 a similar flour (X.742) was prepared from the second batch of nuts referred to above.

Wet-heat processed preparatiol/s

Raw groundnuts (X.427) were boiled in four volumes of water for 0·5 h, drained and dried in a vacuum oven at 45° and then freed from skins, milled and fat-extracted as for X.428.

For processing at higher temperatures, successive samples of milled nuts (X.427) were spread in aluminium trays (each 4 x 8 x I in deep) and autoelaved at each of the combinations of time and temperature plotted in Fig. 1. At the end of each heating period the steam pressure in the autoelave was quickly released to avoid condensation on the samples. A metal baffle placed above the sample trays provided additional protection from the condensate without affecting contact between milled nuts and steam during heating.

TABLE I
Composition of heated and unheated groundnut preparations

Heat treatment		Nil (X,428)	Wet processed			Dry processed		
			Boiled 0·5 h	107°C 0·5 h	121 °C 0·5 h	107 °C 0·5 h	121 °C 0·5 h	121 °C 4 h
Moisture, %	before heating	8·8	7·0	7·0	7·0	4·2	4·2	4·2
	after heating		32·9	14·0	14·4	4·5	4·1	6·6
	after drying		5·4	6·5	7·5	•	•	•
Residual fat, %		2·7	2·2	6·1	J·3	7·6	2·1	1·9
Crude protein (N x 6·25), %		55·0	60·2	57·9	57·8	53·2	59·2	57·1
Crude protein (moisture and fat-free basis), %		62·2	65·2	66·2	62·9	63·4	63·1	62·4

• These samples were not dried further

These materials contained about 14% moisture; they were then dried under vacuum to about 5–7% moisture and finally fat-extracted with petroleum spirit as for X,428.

Dry-heat processed preparations

The milled nuts (X.427) were again used. The material was packed in aluminium foil envelopes 4 x 8 x O'3 in thick, sealed and heated in the autoclave at the various conditions plotted in Fig. I and, in addition, at 121 ° for 4 h. Each heated sample was then fat-extracted without prior drying. The use of foil envelopes for heating minimised change in moisture content during heating.

'Expeller' flours

X.210 was a commercial, pre-pressed and solvent-extracted meal manufactured from decorticated nuts with the skins still present, for feeding to farm animals.

X.367 was an expeller flour made for human consumption from de-skinned nuts in the pilot plant of the Central Food Technological Research Institute, Mysore (India), by the method of Subrahmanyan *et al*.,[14] and stored at - 20° for many months. At no time during processing had the material been subjected to a temperature above 80°.

Methods

Moisture, fat and nitrogen

Moisture in samples was determined as loss of weight after 5 h in a vacuum oven at 70°; the samples were checked for constant weight after a further 2 h in the oven. Fat was determined as 'ether extract' by extraction for 8 h with petroleum spirit (b.p. 40-60°) in a Soxhlet apparatus. Nitrogen in feeds and in carcasses was determined by the Kjeldahl method using a macro-digestion procedure,ls followed by semi-micro distillation of the ammonia produced into 1% (wt.jvol.) boric acid containing a mixed indicator for titration with 1/70 N-HCl16

'Aflatoxin' toxicity

The common starting material (X.428) for heat processing was tested after fat extraction. The material was extracted with chloroform by the method of Lee,[17] and the extracts were chromatographed on thin-layer silica gel (Kieselgel G, Macherey Nagel & Co., Duren, Germany).!8 Aflatoxin was not detected, nor was it detected in the expeller flour X.210. X.367, however, exhibited a slightly positive fluorescence test for presence of aflatoxin, but was estimated to be too low to influence the growth of rats in a 2-week trial.

In vitro *trypsin inhibitor activity*

This was measured with the synthetic substrate benzoyl oL-arginine p-nitroanilide (BAPA) method!9 as described elsewhere.[20] 200 mg of each test material was extracted with 100 ml 0·0025 N-HCl (pH 3'0), with continuous shaking at 37° for I h, followed by filtration. The inhibitor extract was complexed with trypsin solution before reacting with substrate. In Fig. I the trypsin inhibitor activity in each heated preparation has been expressed as a percentage of the original activity in the control flour (X.428).

Available lysine

This was measured in the preparations of groundnut flour by the procedure of Carpenter" using FDNB. The values were corrected by recovery factors obtained by internal addition of ε-DNP lysine before the acid-hydrolysis stage.

Available methionine

This was assayed by the procedure of Ford!3 using *StreptococClls zymogenes*, NCOO 592 the only modification consisting of the use of 0·4% (wt.jvol.) crude papain for the digestion.[21]

Rat experiments

Experimental diets

The basal protein-free diet, used in each experiment, consisted of groundnut oil 15, potato starch 10, glucose 15, vitamin-corn starch mixture 5, mineral premix[22] 5 and maize starch *ad* 100. Each kg of the vitamin–corn starch premix contained 0·06 g thiamine HCl, 0·06 g riboflavine, 0·04 g pyridoxine, I.2 g calcium pantothenate, 4·0 g nicotinic acid, 4·0 g inositol, 12·0 g p-amino benzoic acid, 12·0 g choline chloride, 0·04 go-biotin, 0·04 g folic acid, 0·001 g cyanocobalamine, 20·0 g Rovimix A50 + 03 (containing 50,000 I.U. retinol and 12,500 I.U. calciferol jg; Roche Products Ltd., Welwyn Garden City) and 20·0 g Rovimex E (containing 10% o-tocopheryl acetate; Roche Products Ltd.).

The protein sources under test were incorporated into the above diet at the expense of maize starch at levels designed to contribute either 10% crude protein to the diet (experiments I & 2) or 15% crude protein (experiment 3).

Experiment I

Twelve litters of Wistar rats (SPF strain, 21-24 days old and weighing on average 40–45 g) with 3 males and 3 females in each litter were obtained from Animal Suppliers (London)

Ltd., Welwyn. They were kept on a preliminary diet containing 10% protein from casein until their average body weight was 50 g when they were allocated to six experimental treatments. The distribution of rats to the different treatments, the feeding pattern and the management of rats were as described in a previous paper.[23] Rats allocated to the first treatment were killed immediately and stored frozen.

The remaining rats were allocated to the five dietary treatments, one protein-free and the others each containing a test protein source as set out in Table II. Every diet was fed to 6 cages, each containing a pair of rats, I male and I female. The diets were fed *ad libitum* in powder form. After 10 days the rats were killed, and all (including those killed at randomisation) were dried and then ground for analysis in pairs.

Protein efficiency ratio *(PER)* for each diet was calculated as mean live weight gain/weight of dietary protein eaten over the 10-day experimental period.[24] Net protein ratio *(NPR)* was calculated as 'liveweight gain of test group + weight loss of protein-free group'/weight of protein eaten on the test group.[25]

Net protein utilisation *(NPU)* of the test proteins was determined from carcass analysis of the rats. It was calculated as 'final carcass N of rats receiving test protein - final carcass N of rats on protein-free diet'/l 00 g dietary proteins eaten by the test rats,[22.2H.27] with a correction for differences in initial weight of the rats, using the analysis of those killed at randomisation.[23]

The 'appetite quotients' of the rats receiving the different 'groundnut' treatments were calculated as weight of diet eaten/day/(mean body weight, g)0.88. The term, as originally introduced[28] referred to metabolisable energy *(ME)* consumed instead of weight of diet. In this series of experiments the *ME* values of the diets were not known, but they were expected to be similar.

Experimel/t 2

The general procedure was similar to that of experiment 1. Twelve litters of Wistar rats, with 4 males and 4 females in each litter, were used. They were allocated between eight treatments on randomisation. The rats in the first treatment were again killed immediately and the remainder received experimental diets for 10 days. Six of these are listed in Table III; there was some doubt about the seventh treatment and results are not included.

Experiment 3

Twelve litters of Wistar rats with 3 males and 3 females in each litter were obtained and maintained on the 10% protein casein diet until they reached 50 g body weight. Allocation of rats to the treatments followed the pattern for the previous experiments. Each treatment finally had six pairs of rats. Only five treatments were tcsted and after randomisation the sixth set of rats was discarded. The groundnut preparations were then fed at a level contributing 15% crude protein to the diet (instead of the 10% used in experiments I & 2) and feeding continued for 14 days (Table IV). The rats were then killed with ether and their pancreases were removed and weighed, but the carcasses were not analysed.

Results

In vitro trypsin inhibitor activity

The control flour X.428 gave a value of 13 BAPA units/mg as compared with values of approximately 60 units/mg obtained with samples of extracted raw soya flour by the same procedure.[29] The results for 15 groundnut samples heated in various ways have been calculated as a percentage of the value for X.428 and are plotted in Fig. I. As expected, wet heating was more efficient than dry heating in reducing the trypsin inhibitor activity in the groundnut cotyledon. It is seen from Table IV that the expeller flour X.367 prepared at low temperature still has essentially the full trypsin inhibitor activity of the two unheated flours (X.428 and X.742) that have been tested, whilst thc other meal X.210 processed under ordinary commercial conditions has shown no residual activity.

Rat experiments

It is seen from Table II that the three wet-heated samples tested in experiment I all gave *NPU* values that were **3–4** units higher than the value obtained with the unheated flour X.428. However, a difference of at least **5–7** units would have been required for it to be statistically significant ($P < 0.05$). There was no consistent tendency in any of the other measures.

The results from experiment 2 are summarised in Table III. Casein, the positive control, gave results similar to those expected for an experiment carried out under these conditions. Of the three dry-heated groundnut flours tested, only the one most severely heated (121°. 4 h) gave significantly different performance from that obtained with the unheated flour X.428; its *NPU* was only 69% of that for X.428. As in the previous experiment there were some differences between food intake on the various treatments but they showed no consistent pattern.

The values obtained in experiment 3 are not directly comparable with those obtained in the first two experiments as the protein level was raised from 10% to 15% and the feeding period extended to 2 weeks. It is seen from Table IV that dry heat at 121° for 0·75 h has given results that were not significantly lower than those obtained with the corresponding unheated flour X.742. Nor were the values obtained with the two expeller flours significantly different.

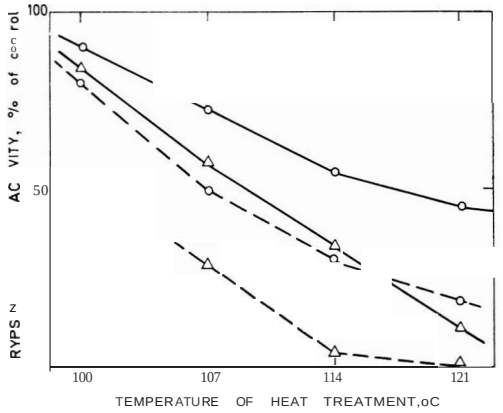

FIG. I. *Trypsin inhibitory activity of heated groundnut preparations relative to the III/heated flour (X.428) from the same batch of cotyledol/s*

O O'5 h heating; △ 1·0 h heating; — — dry heating; - - - wet heating

TABLE II

Weight gain and nitrogen retention in 10 days of rats receiving diets containing 'wet-heat' treated groundnut flour samples

Experiment I

Wet-heat treatment of test groundnut samples	(Protein -free)	Control (X.428)	100°e 0·5 h	107°e 0·5 h	121°e 0·5 h	S.E. of treatment means (23 d.f.)
Relative trypsin inhibitory activity		100	78	49	18	
Nitrogen in diet, %	0·07	1·62	1·63	1·54	1·55	
Gain in weight/rat, g	- 6,7	II ·5	11 ·9	9·4	11·2	± I·2
Nitrogen in carcasses, %	3·17	2·70	2·79	2·80	2·83	N.D.
Food intake/rat, g	N.D.	51 ·5	56·0	49·3	58·0	± 3'1
Net protein utilisation *(NPU)*		39·5	43·0	43·7	44·3	± 2·0
Protein efficiency ratio *(PER)*		2·09	2·07	1·96	2·00	± 0·12
Net protein ratio *(NPR)*		3·47	3·26	3·40	3·20	± 0'09
Appetite quotient		0·155	0·168	0·152	0·176	±*D·008*

TABLE iII

Nitrogen retention (10 days) on diets containing 'dry-heat' treated groundnut flour samples

Experiment 2

Dry-heat treatment of test groundnut samples	(Protein -free)	(Casein X.413)	Control (X.428)	107°e 0·5 h	121°e 0·5 h	121°C 4 h	S.E. of treatment means (35 d.f.)
Relative trypsin inhibitory activity			100	71	45	0	
Nitrogen in diet, %	0·07	1·69	1·70	1·68	1·67	1·63	
Gain in weight/rat, g	- 10,1	25·3	12·6	12·0	14·2	2·4	± 1·12
Nitrogen in carcasses, %	3·04	2·67	2·58	2·62	2·65	2·74	N.D.
Food intake/rat, g	N.D.	73·4	59·2	57·4	69·5	49·0	± 3·30
Net protein utilisation *(NPU)*		64·2	40·3	42·2	42·7	27·8	± 2·10
Protein efficiency ratio *(PER)*		3·25	2·00	1·94	1·96	0·44	± 0·14
Net protein ratio *(NPR)*		4·56	3·62	3·68	3·36	2·45	± 0·12
Appetite quotient		0·183	0·162	0·156	0·184	0·144	± 0·007

TABLE IV

Weight gain and protein efficiency over 2 weeks on diets containing 15% protein from different groundnut flour samples

Experiment 3

Preparation of groundnut sample	(Protein -free)	Control (X.742)	121°e 0·75 h (dry) (X.769)	De-skinned expeller flour (X.367)	Expeller meal containing skins (X.210)	S.E. of treatment means (23 d.f.)
Relative trypsin inhibitory activity (X.428 = 100)		100	0	99	0	
Protein (N x 6·25) in diet, %	0·41	15·1	15·0	15·1	15·1	
Gain in weight/rat, g	- 13,0	35·7	37·8	32·5	39·5	± 2·63
Food intake/rat, g	N.D.	97·2	106·4	92·5	107·3	± 4·56
Protein efficiency ratio *(PER)*		2·45	2·36	2·30	2·46	± 0·12
Net protein ratio *(NPR)*		3·35	3·18	3·26	3·28	± 0'12
Appetite quotient		0'168	0·188	0·158	0·181	± 0·007
Pancreas g/100 g body wl.	N.D.	0·161	0·103	0·171	0·117	± 0·012

Table IV also records the pancreas weights of the rats and it is interesting that the two samples containing trypsin-inhibitory activity also gave significantly ($P < 0,01$) higher pancreas weights. These were also the samples on which rats ate less food in proportion to their size, so that they had significantly lower appetite Quotients.

Lysine and methionine

The results of the laboratory estimates of available lysine and methionine in six heated preparations are set out in Table V. The only value considerably reduced from those for the control flour was that of FONB-available lysine, for the sample dry-heated at 121° for 4 h; it was nearly 30% reduced. This was also the only sample that suffered a significant reduction in *NPU*.

TABLE V

Lysine and methionine determinations in groundnut preparations in relation to their *NPU* **value** for rats

Groundnut samples	FDNB-available lysine, *8/168* N	S. *zymogenes*-available **melhionine**, *8/168* N	*NPU'*
X.428 Control, unheated flour	3·27	1·18	40
Wet-heat treatments:			
100°c, 0·5 h	3·10	1·22	43
107°c, 0·5 h	3·20	1·08	44
121°c, O5h	3·20	1·23	44
Dry-heat treatments:			
107°c, 0·5 h	3·25	1·23	42
121°c, 0·5 h	3·12	1·20	43
121°c, 4 h	2·35	1·\2	28

• Rounded-off data from Tables II and UI.

Discussion

Trypsin inhibitory activity

The inhibitory activity of the unheated cotyledons was low in these assays, i.e. about 20% of the corresponding value for raw soya meal,[29] when compared with a value 87% that of soya meal reported elsewhere.[11] Nevertheless, in experiment 3 two samples when fed as approximately 27% of the diet produced a significant pancreatic hypertrophy as compared with samples having no inhibitory activity *in vitro*. Pancreatic hypertrophy is believed to be a specific response to the presence of trypsin inhibitor in the diet,[30,31] so that the level of activity in groundnut cotyledons does seem to have some effect on animals consuming them. At the same time the effect has not been sufficient to cause a severe depression of growth, nor have others found a correlation between inhibitor activity and nutritional value in a series of groundnut meals.[9,11]

The trypsin inhibitor(s) in these materials appears rather more resistant to heat than is the inhibitor(s) in soya.[31,32] However, Woodham & Oawson[11] found a greater sensitivity to heat in their work with cotyledons, all activity being lost apparently as a result of moist-heat at 108° for 15 min. Assay values record, of course, only the activity brought into solution by the extraction procedure used.

In the present authors' experience[29] (though not in that of another group[11]), inhibitor activity is considerably more concentrated in the skin than in the cotyledon of groundnuts. This emphasises that the present growth results must not be taken automatically to apply to groundnut preparations that still contain the skins. Nor, on the other hand, can it be assumed that plant materials with low inhibitory activity will have optimum nutritional value without cooking.[9]

Possible nutritional improvement from heating

The first interest in this work has been in whether or not a heat treatment is necessary to obtain the maximum nutritional value from groundnut cotyledons. As seen in Table II, the *NPU* values of the three wet-heated samples were similar, with a mean of 43·7. This is just over 10% greater than the value of 39·5 obtained with the unheated flour, X.428. Because of the variability of individual rats, the fiducial limits ($P < 0,05$) of this response are from - I to +21%. These results are therefore just not significantly distinguishable either from those who have claimed a response of approximately 20% to wet-heating[6-8] or from those who have found no improvement.[9]

If the results were to be judged just from *PER* and *NPR*, i.e. the measures based on live weight gain, there is no suggestion of a response to wet heat - the mean value for the three heated samples being slightly below that for the control flour in each case.

Damage from severe heating

The most severe heat treatment used (121° for 4 h) was only run under dry conditions; it resulted in a fall of 30% in *NPU* value for rats and a similar decline in FONB-available lysine. This is in line with previous reports[11,33,34] and confirms the relative resistance to heat damage of groundnut as compared with materials rich in reducing sugars. The specific effect of severe heat processing in reducing the value of available lysine and not of methionine in these laboratory tests, is also in line with published work using rats[10] and has been confirmed in further work with chicks[35] which will be considered further in a later paper.

Mauron & Mollu[36] reported FONB-available lysine values of 2'7-3'0 g/16 g N in carefully prepared samples of groundnut flour and concluded that samples giving 2·5 g/16 g N or less could be considered to be significantly damaged. These were the first determinations reported for groundnut flours and were obtained by a procedure which did not include a special correction factor for hydrolytic losses. This could well result in a 10-15% under-estimation, and an adjustment of this magnitude would give values agreeing well with the estimate of at least 3·0 g/16 g N for good Quality samples. It appears that varietal differences in the lysine content (g/16 g N) of groundnuts is relatively small.[34]

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CONSTITUTION OF LEGUMINOUS SEEDS
VII.*-Ease of cooking field peas *(Pisum sativum* L.) in relation to phytic acid content and calcium diffusion

By T. M. ROSENBAUM and B. E. BAKER

Cooking tests (single pea puncture), phytic acid analyses, water absorption and 45Ca diffusion experiments have been performed on individual peas selected from two samples of field peas *(Pisum sativum* L.). One sample (Avion brand) was judged by the supplier to have good cooking qualities and the other sample (3CW brand) to have poor cooking qualities.

The rapid rate of cooking of the interior of the pea, compared with that of the peripheral region was not associated with a higher phytic acid content. There was no consistent difference between the distribution of 45Ca in Avion and 3CW peas that had been soaked in water containing 45Ca. There was a significant (I %level) difference between the average loss of solids of Avion and 3CW peas that had been soaked a125'.

Pea plants were injected with 45Ca and the peas were harvested. Autoradiograms of the radioactive peas demonstrated the movement of calcium in the seed coats and in the cotyledons during cooking.

Introduction

Several workers have studied the effects of the phytic acid content of peas on their 'cookability'.1-5 Rosenbaum *et al.*[6] made a detailed study of the effects of the phytic acid contents of peas and calcium ions in the cooking water on the cookability of two different samples of peas. The experiments showed that there was a significant correlation between the cookability of individual peas (seed coats removed) and their phytic acid contents when the peas were cooked in distilled water. There was no such correlation between cookability and calcium content.

The present paper deals with the distribution of phytic acid in the pea cotyledon and the movement of calcium ions in individual peas during cooking.

Experimental

Materials

The peas were obtained from the B.C. Pea Growers Ltd., Portage La Prairie, Manitoba. One sample (Avion Brand, Sterling variety) was judged by the suppliers to have good cooking qualities and the other sample (OW, Sterling Variety) to have poor cooking qualities.

Methods

Measurement of cookability of different sections of the cotyledon

The apparatus and procedure for the measurement of cookability were described in a previous paper.[6] To measure the cookability of different sections of a pea cotyledon, a pen was attached to the weight arm C of the apparatus described previously. As the pea softened and the weight arm moved downward, the pen traced out a curve on a kymograph. The slope of the curve at any point was taken as a measure of the cookability of a particular section of the pea cotyledon.

Determination of phytic acid

Phytic acid was determined by a modification[6] of the method of Holt.?

Location of 45Ca in pea cotyledons by autoradiography

Peas that had been soaked or cooked in water containing 45Ca were sectioned (200 μm thick; hand microtome, Reichert Model OM2) by slicing the cotyledon in a plane that was perpendicular to the flat surface of the cotyledon. Slicing was begun at a point farthest away from the micropile. Each section was mounted on a glass slide which was then wrapped in Saran Wrap (Dow Chemical Company, Midland, Michigan). The slide was placed in a cassette and was covered with a sheet of *X-ray* film (Kodak Medical *X-ray* film, Blue Brand). The film was exposed for 7 days and was then developed.

Results

Effect of calcium ions on the cookability of different sections of the pea cotyledon

Peas of approximately the same size (400 mg $\pm$ 20 mg) were selected from two different samples (Avion and 3CW). The seed coats were removed and only those peas having cotyledons of approximately the same size were kept for analysis. One cotyledon from each pea was cooked in distilled water and the other cotyledon was cooked in water containing calcium chloride (100 ppm Ca^{2+}). Cookability was estimated by puncture of a single pea. Fig. I shows typical curves that were traced out on the kymograph. The lower portion of the curves represents the movement of the prongs into the flat portion of the cotyledon. The curves show that the convex peripheral region of the cotyledon cooked less readily than the interior of the pea. They also show that the cookability of Avion peas is less affected by calcium ions than that of the 3CW peas.

Distribution of phytic acid in peas

Peas were selected at random from the two samples (Avion and 3CW) and the seed coats were removed. Thin sections were cut uniformly from the surface of each pea by means of a scalpel, until an amount equal to 15-20% of the weight of the pea had been collected. The phytic acid contents of the two sections (exterior and interior) of each pea were determined. The results reported in Table I show that the exterior portion of the peas contained a higher concentration of phytic acid than did the interior region.

• Part VI: *J. Sci. Fd Agric.*, 1966, 17, 237

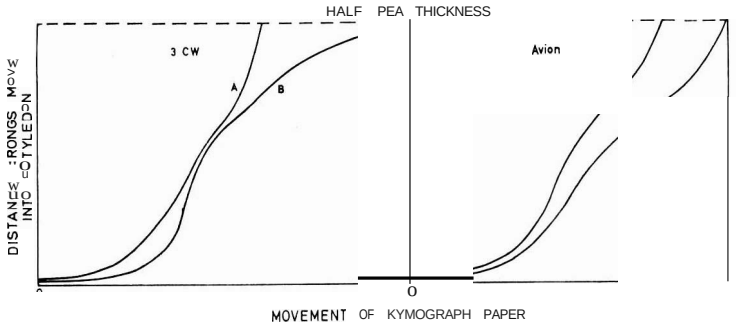

FIG. 1. *Cookability of different sections of pea cotyledolls*

Curves A and B were obtained using distilled water and calcium chloride solution (100 ppm Ca^{2+}) respectively

TABLE 1

Distribution of phytic acid in peas

Pea No.	Exterior region		Interior region	
	Sample taken, % of total wt. of pea	Phytic acid, %	Sample taken, % of total wt. of pea	Phytic acid, %
Avian				
1	19·0	1·65	81·0	1·23
2	16·0	2·26	84·0	1·79
3	16·2	2·06	83·8	1·49
3eW				
1	19·0	1·53	81·0	0·98
2	20·7	1·33	79·3	0'83
3	15·0	1·08	85·0	0·70
4	16·9	1·38	83·1	1·00

Diffusion of calcium ions from the cooking water into the cotyledon

A previous experiment showed that the peripheral region of the pea cooked less readily (the prongs moved more slowly; see Fig. 1) in distilled water than the interior, despite the lower concentration of phytic acid in the latter. The experiment also showed that calcium ions in the cooking water adversely affected the cooking quality of the peripheral region more than they did the interior. It was suspected that the rates of diffusion of calcium into the two regions of the cotyledon were different and this might account for the differences in cookability of the peripheral and interior regions. The present experiment was designed to gain information on the diffusion of calcium ions into the cotyledon during soaking and cooking.

Peas (Avion and 3CW) were selected at random and their seed coats were removed. One cotyledon from each pea was soaked in calcium chloride solution (100 ppm Ca^{2+}; 0'1 μCi 45Ca/loo ml) at 25° for 18 h. The other cotyledon of the same pea was cooked in the same solution at 100° for 1 h. Autoradiograms of the cotyledons showed that the calcium was uniformly distributed throughout the cotyledons when the peas were soaked at 25° for 18 h but was present mainly in the peripheral regions when the peas were cooked at 100° for 1 h. There was no consistent difference between the distribution of calcium in the Avion and in the OW peas.

Absorption of water and loss of solids by peas during soaking and cooking

Adam & Siddappa[8] noted that peas of good cooking quality absorbed more water during cooking than did peas of poor cooking quality. Snyder[9] stated that any treatment which reduced the amount of water that was absorbed by a pea would reduce its cookability. Previous experiments showed that calcium ions in the cooking water affected the cookability of 3CW peas more than they did the Avion peas. The purpose of this experiment was to see whether calcium ions in the cooking water affected the absorption of water by peas.

Peas (Avion, 3CW) were selected at random and their seed coats were removed. The individual cotyledons were weighed and one from each pea was soaked (25°) in distilled water and the other in calcium chloride solution (100 ppm Ca^{2+}). They were removed from the water at 30 min intervals, their surfaces were freed of excess moisture by use of paper tissue and the cotyledons were weighed.

A second set of experiments was performed in which the temperature of the water was 100° and the cotyledons were removed at lO-min intervals. The surfaces of the peas were intact after a I h treatment at 100° as were those of the peas that were treated for 6 h at 25°. Fig. 2 shows {he results of these experiments. Calculations based on these results showed that there was no significant difference (10% level) between the average weight gain of the Avion cotyledons that were soaked (25°) for 6 h in distilled water and the average weight gain of cotyledons that were soaked (25°) for the same interval in water containing calcium ions. The 3CW cotyledons under the same conditions however did show a significant (5% level) difference. At 100° both the Avion and the 3CW absorbed more water when soaked in distilled water than in water containing calcium ions. The differences that were observed after a soaking period of I h were significant at the 5% level.

In another experiment peas (Avion and 3CW) were selected at random and their seed coats were removed. The moisture content of the individual cotyledons was approximately 10%. One cotyledon from each pea was placed in calcium chloride solution (100 ppm Ca^{2+}) at 25° and the other in the same solution at 100°. The cotyledons were removed from the solutions at 25° and at 100° after 12 h and I h intervals, respectively. The surfaces of the cotyledons were freed of

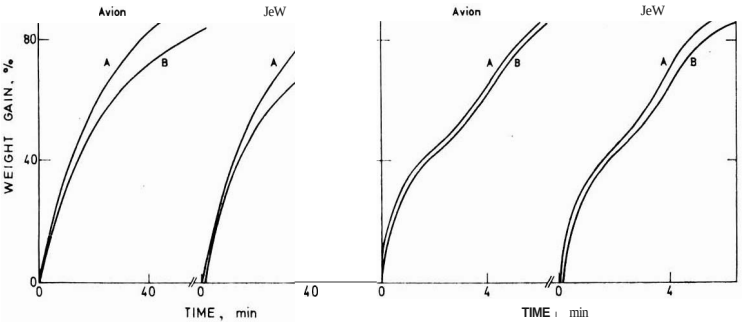

FIG. 2. *Absorption of waler by peas durillg soaking*

Curves A and B were obtained using distilled water and calcium chloride solution (**100** ppm Ca^{2+}) respecti vely

TABLE II

Absorption of water by peas during soaking

Pea No.	Weight gain, % Cotyledon A Soaking time- 12 h 25'e	 Cotyledon B Soaking time- I h l00'e
3CW		
I	92·1	93·0
2	95·5	93·8
3	94·9	90·8
4	93·7	93·6
5	99·4	100·6
6	91·4	89·0
	average 94·5	average 93·4
Avion		
I	102·0	99·5
2	90·3	91·3
3	94·0	95·3
4	III.I	105·6
5	83·6	87·0
6	92·2	92·0
	average 95·5	average 95·1

Difference between averages not significant at 10% level

TABLE III

Loss of solids by peas during soaking

Pea No.	Loss of solids, % Cotyledon A Soaking time-12 h 25'e	 Cotyledon B Soaking time-1 h 100'e
3CW		
I	4·24	11·7
2	2·48	10·5
3	2·50	10·6
4	1·63	12·2
5	2·73	11·9
6	3·28	11·4
	average 2·87	average 11·4
Avion		
I	2·97	10·0
2	3·51	\3·1
3	4·40	12·1
4	5·58	10·5
5	8·87	12·0
6	6·13	14·0
	average 5·08	average 12·0

Difference between averages significant at 1% level

excess moisture by the use of paper tissue, and the cotyledons were weighed and then dried to constant weight at 110'. Table 11 shows the percentage gain in weight of the individual cotyledons due to soaking and Table 111 shows the loss of weight of the same individual cotyledons after they had been soaked and then dried to constant weight.

There was no significant difference (10% level) between the average gain in weight of peas that were soaked at 25' and 100'. There was a significant difference (1% level) between the average loss of weight of the peas that were soaked at 25' and 100° and then dried to constant weight. As the surface of the cotyledons was intact at the end of the 12 h treatment at 25° and the I h treatment at 100' it may be assumed that more soluble components diffused through the surface layers of the cotyledons during the 100° treatment than during the 25' treatment. The gain in weight of the peas that were soaked at 100' was, however, approximately the same as that of the peas which were soaked at 25'. It is evident therefore that the degree of hydration of the solids that remained in the peas after the 100° treatment was greater than that of the solids that remained after the 25° treatment. There was a significant (1% level) difference between the average loss of solids of 3CW and Avion peas that were soaked at 25' but no significant (10% level) difference when the peas were soaked at 100'.

Movement of calcium in the seed coats and cotyledons during soaking and cooking

Pea plants (Sterling) were injected with radioactive calcium (0'001 N hydrochloric acid solution of calcium chloride neutralised to pH 3·5 with sodium hydroxide; 0·01 101; *20* μCi 4SCa) at a point in the internode which was just below the node carrying the blossom. The injection was made five days after full flowering. The peas were harvested after an additional period of 40-50 days and were stored at room temperature.

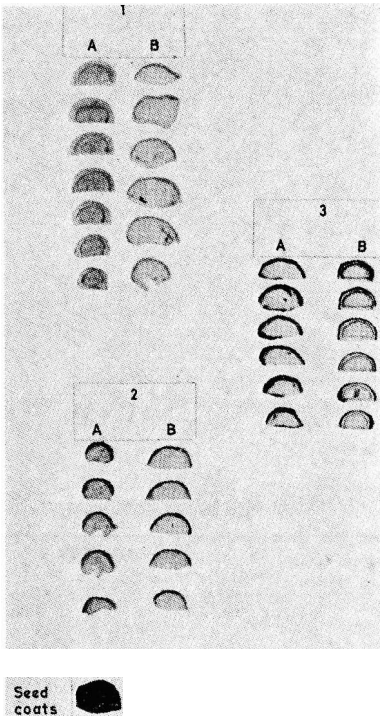

FIG. 3. *All/oradiograllls of pea co/yledolls*

Group I, peas from plants injected with **45Ca.** Seed coats removed. A-soaked at 25°c. B-soaked at 100°c. Group 2, peas from plants injected with **45Ca.** Seed coats not removed. A-soaked at 25°c. B-soaked at 100°c. Group 3, peas from the same pod, soaked in solution containing **45Ca** (plants not injected with **45Ca).** A-not blanched. B-blanched before soaking

Peas from the same pod were either soaked (25°) for 6 h or soaked (25°) for 6 h and then cooked (100°) for 30 min in calcium chloride solution (100 ppm Ca^{2+}), and autoradiograms of cotyledon slices were prepared. Fig. 3 shows typical autoradiograms that were obtained. Group 1 shows peas that had been either soaked or cooked with their seed coats removed and Group 2 shows peas that had been either soaked or cooked with their seed coats intact. Autoradiograms of the seed coats after soaking or cooking are included in this latter group.

Group 3 shows autoradiograms of non-radioactive peas from the same pod that had been soaked (18 h, 25°) in calcium chloride solution (100 ppm Ca^{2+}, 0·1 ,,0 45CajlOO 011), without previous blanching (subgroup A) and after blanching (100°) for 1 min in distilled water (subgroup B).

Radioactive peas that had been soaked with their seed coats removed contained less calcium at the periphery and more calcium in the interior of the cotyledon than did those that had been cooked. The peas that had been soaked also contained a region of high calcium concentration which was located at a distance of 1-2 0101 from the outer surface of the cotyledon.

Peas that had been soaked with their seed coats on contained more calcium near the convex surface of the cotyledon than they did in the interior region. Peas that had been cooked with their seed coats on contained less calcium in the interior region than did the peas that were soaked. The seed coats that had been cooked contained a much lower concentration of calcium than did the seed coats that were soaked.

When non-radioactive peas were soaked in calcium chloride solution containing $45Ca^{2+}$, there was a high concentration of calcium in the convex peripheral region of the pea and a fairly uniform distribution of calcium in the interior of the cotyledon. When the peas were first blanched and then soaked in radioactive calcium chloride solution there were two layers of high calcium concentration, one at the periphery and the other at a distance of about I mOl from the periphery.

Discussion

The peripheral region of the cotyledon of single peas cooked less readily than the interior and contained more phytic acid. Previous experiments" showed that Avion peas of high phytic acid content tended to cook faster in water containing calcium ions than did those of lower phytic acid content. The present findings indicate that the higher cookability of the interior of the pea is not associated with a higher phytic acid content.

Radioactive (45Ca) peas that were soaked with their seed coats intact, contained more calcium at the periphery of the cotyledon than did those which were soaked with their seed coats removed. This suggests one or both of the following: the seed coats prevented the diffusion of calcium from the cotyledon into the surrounding medium; calcium diffused from the seed into the cotyledon.

The present work suggests that several factors such as the diffusion of calcium from the seed coat into the cotyledon and the ease of hydration and solubility of certain pea components may determine the cookability of peas. It will be necessary to gain more precise information on the constitution of the various pea components before a satisfactory explanation can be offered for the observed variations in pea cookability.

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ANALYTICAL EVALUATION OF COCOA CURING IN THE IVORY COAST

U. BRACCO, N. GRAILHE, W. ROSTAGNO and R. H. EGLI

During an experimental cocoa curing on the Ivory Coast, samples of cocoa beans were collected every day, freeze-dried and analytically checked.

Some of the analytical results are discussed in relation (o the possibility of using chemical indices to assess the degree of fermentation of commercial cocoas.

Introduction

Cocoa curing (harvesting, fermentation and drying) in tropical countries involves biochemical, chemical and physical changes in the beans and is responsible for the development of cocoa flavour precursors.

Several authors have described the changes of some basic parameters (pH, temperature, moisture), t the presence and activity of micro-organisms (yeasts and bacteria)2.3 the transformation of flavonoid compounds4.5 and proteins,6 their interaction,4 changes in the structure and the appearance of the cotyledon cells.8

However, commercial cocoa is still judged by visual assessment, merely by the presence or absence of defects, since a quick analytical evaluation of the qualities of fermented beans is not available at present.

The visual assessment (cut test) depends on a subjective appreciation, and therefore gives a wide disparity in the results.

The present work deals with the analytical measurement of the quality of fermented beans. Several chemical indices have been determined and correlated with the duration of the fermentation. Some of them may be used for an objective evaluation of cocoa fermentation.

Experimental

During the main crop season in the Ivory Coast, ripe fruits (pods) of the variety 'Forastero' (Amelonado) were collected and seeds and pulp were laid in a special box with a perforated bottom to allow the juice from the fermenting mass to 'sweat out'.

The fermentation box (60 x 60 x 60 cm), made from local wood, was fitted with 150 lb of wet freshly harvested beans; a wooden lid was provided to give better insulation for the fermenting mass.

Every day the mass was thoroughly mixed and a sample was taken; this was immediately frozen, dispatched to laboratories in Switzerland and freeze-dried (Fig. I).

At the end of fermentation (6th day), the beans were sun-dried and transformed into plain chocolate for organoleptic evaluation. The course of the fermentation was followed by a number of measurements and analyses. Temperature, moisture, pH and dry weight of 100 beans were measured daily on fermenting beans. The following measurements were made on freeze-dried beans: fat content, by a hydrolytic method;9 fatty acid composition, by gas-liquid chromatography of the methyl esters;10 unsaponifiable matter and its sterol composition, by preparative thin-layer and gas-liquid chromatography;II total nitrogen;'2 soluble nitrogen, by the method of de Witt[13] on 70% alcoholic extracts; purins, by gravimetry;14 phenolic compounds-catechins (3,3',4',5,7-flavanpentols) and non-catechinic soluble tannins by the precipitation method of Duthie & StiansY,15 oxidisable tannins by the NaOCI titration method described by Sauda,16 and pigments by Rohan's colorimetric method;17 and sucrose, glucose and fructose by enzymic conversion into 6-phosphogluconic acid and spectrophotometric evaluation.[18]

Some individual carbohydrates were determined by gas-liquid chromatography of their trimethylsilyl esters.[19]

Results and Discussion

pH

Fig. 2 shows the pH variation in pulp and cotyledons using a 'flat surface' electrode (Beckmann Model No. 39182) on the external and internal bean surface.

It should be noted that the pH on the internal surface of the cotyledon decreased more slowly than on the external surface; this seems to be due to the organic acids, which developed in the pulp, entering progressively into the bean.

Within 70 h, the cotyledons exhibited homogeneous pH on the whole surface, and a value of 5·4 was reached after 6 days.

The pH in the pulp juice gradually rose. The citric acid present at the beginning of the fermentation was metabolised and replaced by the less dissociated lactic and acetic acids.[20]

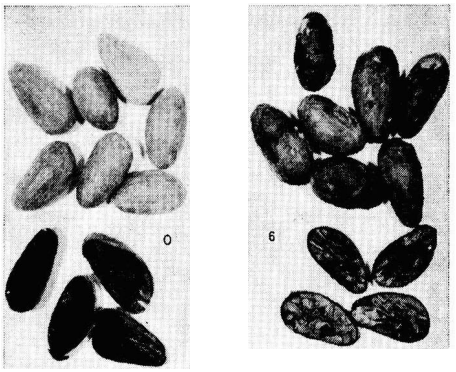


FIG. I. *Freeze-dried cocoa beans after 0 and* 6 *days offermentation*

Dry weight of 100 beans

The variation in dry weight of 100 dried, shell-free beans is shown in Fig. 3. The dry weight decreased during the first 3 days and then increased again without reaching the original value. The total loss of dry matter was about 5%. The initial decrease can be explained by the diffusion of soluble substances into the shell and the pulp during the first hydrolytic phase. The slight increase observed after the third day is more difficult to explain.

Fatty components

Fat content on a dry weight basis rose during fermentation; an increase of 2·2% was found, in agreement with the results of another author.[21]

During the fermentation, the composition of cocoa fat did not change significantly in its main components, namely fatty acids and sterols. Table I shows the values found for the several fermentation steps.

Nitrogen content

Total nitrogen and soluble nitrogen contents varied during the fermentation. Table II and Fig. 4 give the ratio between soluble and total nitrogen contents for different days of fermentation.

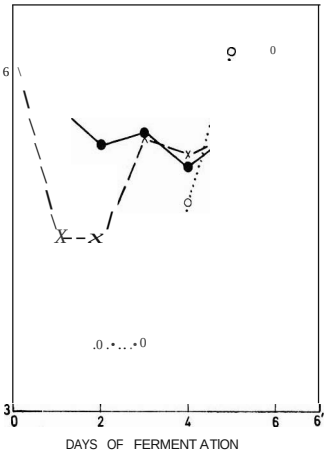


FIG. 2. *pH evolution during the cocoajerme11larion*
- Internal cotyledon surface; — — — external cotyledon surface; . . . pulp: 6' sun-dried: 0

This value could be used as a fermentation index, as previously suggested by Rohan.[21]

The contents of the a-amino nitrogen, which represents 4% of the total nitrogen, and the purinic nitrogen (50% of total nitrogen) did not show significant variations.

Phenolic compounds

Table III shows the results of the gravimetric determination of catechins and non-catechinic soluble tannins; colorimetric evaluation of pigments and oxidisable tannins are also reported. Pigment values are expressed as % of cyanidin chloride (used for the calibration curve).

The content of catechins decreased significantly during the fermentation and chiefly during the sun-drying of the beans, whereas soluble and non-catechinic tannin contents showed small variations. The ratio catechins/soluble tannins decreased regularly and may be used as an index of fermentation; Fig. 5 shows this correlation.

It was also possible to measure the catechin content by spectrophotometry at 203 or 212 nm;[23] the results agreed with those from the gravimetric determination.

The variations in pigments and oxidisable tannins are shown in Figs 6 and 7.

Soluble carbohydrates

Table IV gives the glucose, fructose and sucrose contents. These values, which were also confirmed by gas-liquid chromatography, indicate that glycolysis occurs from the

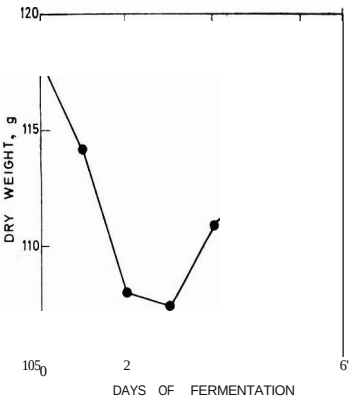


FIG. 3. *Variation in dry weight oj 100 beans*
6' sun-dried: 0

TABLE I

Fatty acid and sterol composition in cocoa fats

Days of fermentation	% on weight of total fatty acids				% on weight of total slerols		
	Palmitic	Stearic	Oleic	Linoleic	,a·Sitosterol	Stigmasterol	Campesterol
O (freeze-dried)	26'5	35·4	34·7	3'4	55·5	34	10·5
I	25·5	33·6	36·6	4·3			
2	26·8	33·2	36·2	3·9			
3	26·4	34·4	35·6	3·8			
4	26·3	34·1	36·2	3·4			
5	25·6	34·8	35·1	6·5			
6 ,, ,,	25·3	34·9	35·6	4·2	49·6	38	12·5
6' (sun-dried)	25·8	34·8	35·3	4·1	50·3	39·5	11·2

third day of fermentation. Therefore, it is possible to correlate the ratio glucose + fructose/sucrose with the course of the fermentation, as shown in Fig. 8.

Organoleptic evaluation

Beans fermented for 0, 2, 4 and 6 days were evaluated organoleptically using the cut test method; plain chocolates were also manufactured with these beans, according to methods and formulae proposed by the O.l.c.c. Commiltee.[24] Table V shows the results of the organoleptic assessment and correlates them with the analytical values.

TABLE II

Total and soluble nitrogen contents, % on dry shell-free **bean**

Days of fermentation	Total N	Soluble N	$\frac{\text{Soluble N}}{\text{Total N}} \times 100$
0 (freeze-dried)	2·27	0·70	30
I	2'15	0·76	35
2	2·06	0·93	45
3	1·99	0·87	44
4	1·77	0·87	50
5	1·97	0·96	49
6 ,, ,,	2·04	0·81	40
6′ (sun-dried)	2·18	0·73	33

From this Table, it appears that a well fermented cocoa, giving a good chocolate flavour, can be characterised by chemical indices which are different from the values found for the non-fermented or underfermented beans.

More work is planned to check the statistical validity of these results on further Ivory Coast samples as well as on samples from other botanical and geographical sources.

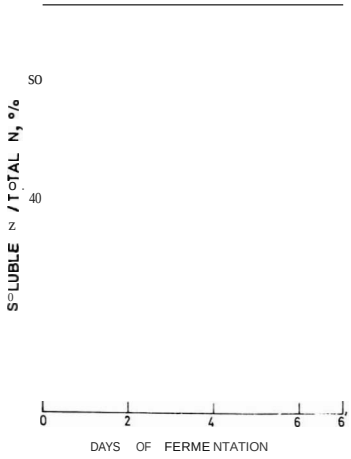
Conclusions

During the cocoa fermentation, hydrolytic phenomena occur in the bean which may explain the variations in the contents of nitrogen compounds, phenolic substances and carbohydrates.

The systematic analytical control of these substances allows several indices to be proposed for the control of the fermentation.

The absolute values of residual pigments, catechins and oxidisable tannins describe the course of the fermentation but their variations are smaller than those for the three indices proposed.

A nitrogen index of about 33, a catechin index of about O'20 and a carbohydrates index of I'6 seem to correspond to well fermented cocoa from the Ivory Coast.



FtG.4. *Ratio of soluble N/total N for differelll days o[fermentation*
6' sun-dried: 0

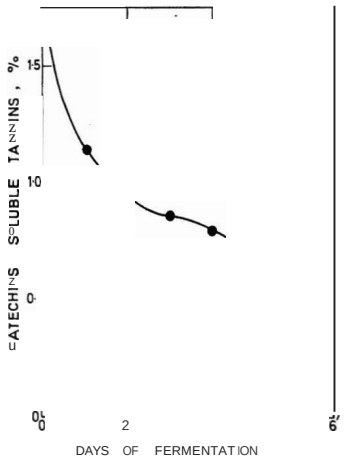


FIG. 5. *Ratio o[catechins/soluble tannins for different days of [ermelllation*
6' sun-dried: 0

TABLE III

Variation in contents of phenolic compounds during the cocoa curing, %, on dry, shell-free **bean**

Days of fermentation	Catechins	Soluble tannin	$\frac{\text{Catechins}}{\text{Soluble tannins}}$	Pigments	Oxidisable tannins
0 (freeze-dried)	5·97	3·55	1·7	0·444	1·67
1	4·70	4·18	1·15	0·412	1·41
2	3·82	4·71	0·81	0·398	1·38
3	3·73	4·29	0·87	0·296	1·02
4	3·16	3·94	0·81	0·216	0·99
5	2·75	3·94	0·7	0·174	0·94
6 ,, ,,	2·26	4·39	0·5	0·137	0·84
6′ (sun-dried)	1·32	5·58	0·23	0·07	0·89

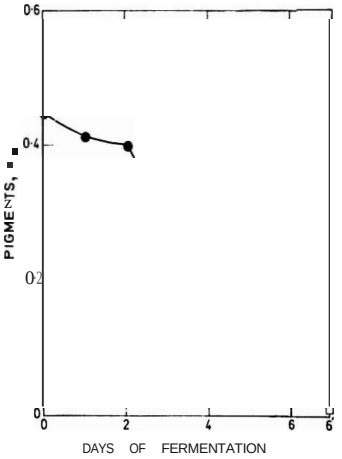


FIG. 6. *Variation in pigments for different days of fermentation*
6' sun-dried: 0

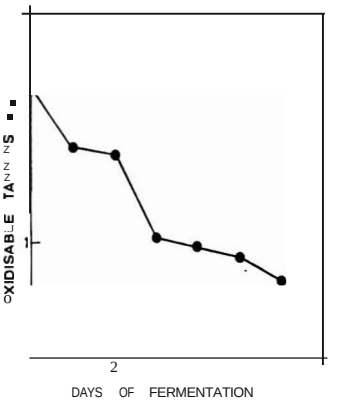


FIG. 7. *Variation in oxidisable tannins for different days of fermentation*
6' sun-dried: 0

TABLE **IV**

Soluble carbohydrates, % on dry, sbell.free bean

Days of fermentation	Glucose	Fructose	Sucrose	Glucose + Fructose / Sucrose
0 **(freeze-dried)**	0·303	0'102	1·65	0·245
2	0·309	0·124	1·61	0·262
4	0·669	0·397	1·16	0·915
6 " "	0'643	0·386	0·7	1·47
6' **(sun-dried)**	0'441	0·464	0·545	1·66

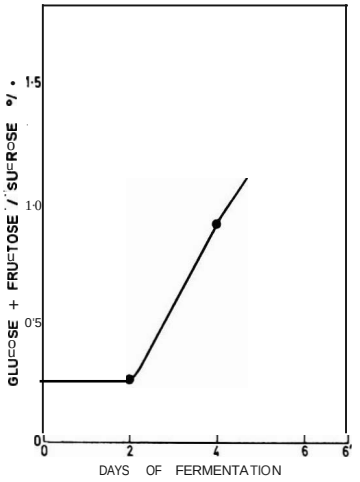


FIG. 8. *Ratio of glucose + fructose/sucrose for different days of fermentation*
6' sun-dried: 0

TABLE V

Organoleptic and analytical evaluation of cocoa beans

Days of fermentation	Cocoa beans (100)			Chocolate flavour	Analytical values		
	Brown	Purple	Slaty		Nitrogen index'	Catechin index"	Carbohydrates index···
O		94	6	No cocoa aroma-beany, astringent	30	1·7	0·245
2		80	20	No cocoa aroma-acid, astringent	35	0'81	0·262
4	42	54	4	Very weak cocoa aroma-bitter, astringent	50	0'81	0·915
6′	88	10	2	Strong cocoa flavour-normal bitterness, low levels of acidity	33	0·23	1'66

• Soluble N / Total N x 100; •• Catechins / Soluble tannins' .··· Glucose + Fructose / Sucrose

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COMPARISON OF METHODS FOR ASSESSING THE AVAILABILITY OF POTASSIUM AND PHOSPHORUS IN FIELD SOILS TO BLACK CURRANTS AND STRAWBERRIES

By W. J. REDMOND

The validity of activity ratio measurements for potassium, and ion potential measurements for phosphate as criteria for the availability of these nutrient elements to black currants and strawberries growing in the field was compared with the results obtained by determinations of exchangeable potassium and acetic acid-soluble phosphorus for three different soils. The correlations between soil nutrient values and uptake by the plants, as evidenced by leaf nutrient content, were similar for each element irrespective of which method was used, so that for the particular field experiments concerned there would appear to be no advantage in replacing the straightforward extraction determinations by ion activity determinations for diagnostic purposes.

Introduction

It has been shown! that for certain soil/crop combinations, measurements of the activity ratio of potassium ions in equilibrium soil solutions provide a more valid assessment of the potassium-supplying power of a soil, as evidem;ed by the removal of potassium by growing crops, than is obtainable from determinations of exchangeable soil potassium. The factor measured is the ratio existing between the activity of the potassium ions and the square root of the activity of the divalent ions (calcium and magnesium) in an equilibrium dilute aqueous extract. The equilibrium value of this ratio $aK/[a(Ca + Mg)]^{\frac{1}{2}}$ found by Schofield[2] to be independent of the total concentration of ions present and designated by the symbol *AR:*,[3] represents the intensity or energy of exchange of potassium compared with that of the divalent ions.

Similarly, the concept of the phosphate potential, expressed by the value $\frac{1}{2}\text{pCa} + p\text{H}_2\text{PO}_4^{4-6}$ and corresponding essentially to the activity of mono-calcium phosphate, has been recommended as a possibly better indicator of the phosphate status of soils than the conventional soil-test methods.[7],s

In order to compare the usefulness of ion potential measurements and traditional extraction methods in assessing the availability of soil potassium and phosphorus in field conditions, studies were made of the correlations between the soil values found by both procedures and the corresponding leaf contents of these elements in strawberry and black currant plants grown in long-term nutritional field experiments at the N.A.A.S. Experimental Horticulture Stations at Elford and Luddington and at Long Ashton.

Experimental

Sites and soils

Efford

The soil was a silty loam of the brick earth type, on a site with an average annual rainfall of 31 in. The layout and fertiliser treatments used for the NPK factorial experiment with Royal Sovereign strawberries and with two black currant varieties have been described.[9-11] Soil and leaf samples were taken in 1963.

Luddington

This was a rather exposed site with southern aspect, of elevation about 150 ft. The soil was 'Pershore' series on Lias clay with an average rainfall of 24 in. The soil was sampled in 1966 and the leaves were sampled in 1965.

Long Ashton (Plot 14)

This was an imperfectly drained site of elevation about 100 ft. Soil series (unnamed) were loam over heavy loam with an annual rainfall of 36 in. Soil and leaves were sampled in 1966.

Analytical methods

Potassium

Exchangeable potassium was determined by extraction of the soil with N ammonium acetate at pH 7·0. To obtain a value for the K activity ratio *AR:*, a 109 sample of air-dried soil was first of all shaken for 10 min with 20 ml of dilute (0'01 M) calcium chloride solution at a constant temperature (24°) and the potassium content of the resultant extract determined after filtration, using an EEL (Evans Electroselenium Ltd) flame photometer. Further samples were then extracted with calcium chloride solutions containing amounts of potassium chloride giving initial potassium concentrations slightly higher and slightly lower than that originally found. If these concentrations span the true equilibrium value, the more dilute should gain potassium from the soil and the more concentrated should lose potassium to it. The calcium and magnesium concentrations of the extracts were also determined, using an atomic absorption spectrophotometer. After calculating the activity ratios *aK/(aCa)!* for the initial solutions and $aK/[a(Ca + Mg)]^{\frac{1}{2}}$ for the extracts, the value of $aK/[a(Ca + Mg)]^{\frac{1}{2}}$ for a solution whose potassium concentration would remain unchanged in contact with the soil was determined by graphic interpolation as described by Taylor[12]

Phosphorus

'Available' phosphorus was assessed by extracting a 10 g sample of air-dried soil in a mechanical shaker for 2 h with 200 ml 0·5 N acetic acid, filtering, and determining the phosphorus in solution by a molybdenum blue method.[13] Phosphate potentials were determined by initially shaking a 2·5 g sample of air-dried soil with 25 ml 0·01 M calcium chloride solution for I h, filtering, and determining the phosphorus in solution. Further extractions were then carried out with calcium chloride solutions containing varying amounts of $Ca(H_2PO_4)_2$ to establish the concentration at which the soil

phosphate was in equilibrium with the phosphate ions in solution. From this value, and those of the calcium concentration and pH of the extract, the factor $pH_2PO_4^- + \frac{1}{2}pCa$ could be determined for the given soil: solution ratio.[4]

Leaves were sampled and analysed for P and K as described by Bould.[10,11]

Results

In a preliminary series of determinations carried out on soil and leaf samples from the Efford strawberry experiment, the soil samples were bulked, each final sample representing nine plots, which had received applications of three nitrogen levels and three phosphorus levels for each K level.

To keep the number of determinations down to a convenient figure, the soil samples from the Efford and Luddington field experiments were also bulked, the three different N levels for each combination of P and K being mixed to give a single sample, thus reducing the 81 treatments to 27 bulked samples. Corresponding mean leaf values were calculated. The sampling from Plot 14 at Long Ashton was on a limited scale, the twelve individual plots studied being selected to show the effects of differential K and Mg treatments only.

Potassium

Table I shows the mean values for the changes in 'available' soil potassium, as determined by the two methods being investigated, and in leaf potassium resulting from the application of different amounts of K fertiliser to the various trial plots. Table II shows the corresponding correlations between soil and leaf values at each site. For strawberries and black currants at Efford, and for black currants at Luddington, there was little difference in the significance of the correlations between either of the sets of soil values and the leaf content, whether the soil depth considered was 0-6 in or 6–12 in. Only for the black currants on Plot 14 at Long Ashton (0-8 in) did the activity ratio method show a slight superiority in significance of correlation.

Phosphorus

Table III shows the mean response of both soil and leaf phosphorus to varying levels of applied superphosphate at Efford and Luddington, with soil phosphorus determined either as acetic acid-soluble P or by the phosphate potential method. Since the latter values are expressed in terms of negative logarithms, higher numerical values were obtained for lower equilibrium concentrations of P, and hence the correlations appear negative unless corrected. The phosphorus status of the Efford soil was so low (hat it was not practical to obtain values for the 6–12 in layer in either of the experiments. In the 0-6 in layer, both sets of soil P values showed a high degree of correlation with leaf P content

TABLE 1

Changes in soil and leaf potassium caused by graded applications of potassium fertiliser

Site	Crop	K_2SO_4, cwt/acre	Exchangeable K, ppm		$K(AR_e)$		Mean leaf K, %DM
			0–6 in	6-12 in	0–6 in	6–12 in	
Elford	Strawberries	0	85	84	0·0045	0·0031	0·78
		1	107	94	0·0064	0·0035	1·05
		2	139	109	0·0097	0·0045	1·19
Elford	Black currants	0	82	67	0·0038	0·0025	0·63
		1	138	82	0·0092	0·0038	1·08
		2	184	105	0·0135	0·0050	1·42
Luddinglon	Black currants	0	329	234	0·0125	0·0050	1·49
		1	392	271	0·0182	0·0069	1·66
		2	481	310	0·0254	0·0087	1·71
			(0-8 in)		(0-8 in)		
Long Ashton	Black currants	0	203		0·0089		1·62
		1	247		0·0139		1·65

DM, dry matter

TABLE II

Correlations between soil K contents, determined by two different methods, and leaf K

Site	Crop	Soil depth, in	Values of r	
			Exchangeable soil K/leaf K	(*A* R:)/leaf K
Elford	Strawberries	0–6	0·8420··	0·8037··
		6–12	0·9132···	0·8941··
Elford	Black currants	0–6	0·8585···	0·8574···
		6–12	0·8144···	0·7629···
Luddington	Black currants	0–6	0·7197···	0·7456···
		6- 12	0·7082···	0·6019···
Long Ashton	Black currants	0–8	0·3829 (n.s.)	0·6537·

n.s., not significant

TABLE III

Changes in soil and leaf P contents caused by graded applications of superphosphate

Site	Crop	Superphosphate, cwl/acre	Mean soil P values: O'5 N acetic acid-soluble P, ppm		Mean soil P values: Phosphate potential		Mean leaf P, % *DM*
			0–6 in	6-12 in	0-6 in	6-12 in	
Elford	Black currants	0	5·2		7·26		0·255
		3	18·6		6·60		0·290
		6	53·4		5·97		0·343
Luddington	Black currants	0	148	111	6'50	7·56	0·266
		3	217	129	6·00	7·39	0·334
		6	272	155	5·68	7·16	0·434
Elford	Strawberries	0	5·5				0·207
		2	7·8				0·214
		4	12·0				0·214

DM, dry matter

TABLE IV

Correlations between soil P contents, determined by two different methods, and leaf P

Site	Crop	Soil depth, in	Values of r: Acetic acid-soluble P/leaf P	Values of r: Phosphate potential/leaf P
Efford	Black curranls	0-6	0·7825'"	0·8004'"
Luddington	Black curranls	0–6	0,8602'"	0·8130'"
		6-12	O'5349"	0·4994·'
Elford	Strawberries		No response to phosphate fertiliser	

(Table IV). At Luddington, the correlation between soil P and leaf P was significant at the 0·1% level for the 0-6 in values and at the I% level for the 6-12 in values; at neither depth was there any marked difference in significance between the two methods.

In the strawberry experiment at Efford, soil phosphate was so low that it was not possible to carry out phosphate potential investigations, since the greater dilution of the extracts entailed in this method gave concentrations below the limits of determination. On these plots there was no increase in leaf phosphorus content in response to added phosphate fertiliser (Table III), so that no correlation was obtained between leaf phosphorus and that found in the soil by acetic acid extraction.

Conclusions

Thus, for the particular combinations of soils and crops being studied in these nutritional experiments on black currants and strawberries, neither the potassium activity ratio nor the phosphate potential methods of determining available soil nutrient levels seems to have any advantage over the older-established chemical extraction methods, which require much less time to carry out, though under other soil and ecological conditions they might be found to be a more useful means of predicting fertiliser requirements.

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VOLATILE HOP CONSTITUENTS: CHARACTERISATION OF β-COPAENE AND PENTADEC-6-ENE-2-0NE

By R. G. BUTIERY, R. TERANISHI, T. R. MON and L. C. LING

Evidence has been found for the identity of two previously uncharacterised CIS volatile constituents of hops as ,B-copaene and pentadec-6-ene-2-one. Some inconclusive evidence was also found for the identity of another.

Introduction

In 1967 the characterisation of a number of CI5 constituents of hops was reported.I More recently Naya *et al.*[2] reported the characterisation of several additional CIS components. There were a few CI5 components of hops, however, that still remained to be characterised. The present paper is concerned with three of these.

Experimental

Hop oil

This was obtained from Bullion hops by a steam distillation procedure.[1]

β-Copaene

The hop oil was separated into its hydrocarbon and oxygenated fractions by chromatography on silica gel as described previously.I

The hydrocarbon fraction was fractionally distilled under vacuum to separate the sesquiterpene fraction from the monoterpenes. The sesquiterpenes were then resolved into a number of fractions by chromatography on silica gel.[3] Components were separated roughly according to their degree of unsaturation. Sesquiterpenes such as a-copaene and a-ylangene with only one double bond were eluted in the first fractions, and those such as humulene and farnesene with 3 and 4 double bonds were eluted in the last. Several of the earlier fractions contained a component corresponding to that named peak 48 in some previous publications.! Such fractions were combined, and peak 48 and another which was named peak 47a were isolated from these combined fractions by gas chromatography using a 100 ft x 0·2 in Ld. column packed with 100-120 mesh Chromosorb G* coated with 1% Carbowax 20M (air particle selected).[4] These components isolated on a packed column were further purified by separation from a 1000 ft x 0·03 in stainless-steel capillary gas-liquid chromatography column coated with Carbowax 20M.[4]

The infra-red (i.r.) absorption spectra of these fractions were measured as thin films using a Perkin-Elmer 237 double beam grating instrument.

Pentadec-6-ene-2-one

The CI5 mono-unsaturated ketone of hop oil was isolated from the hop oil oxygenated fraction as previously described.[1] It was ozonised in acetic acid, and the ozonide was reduced by addition of zinc dust and water. The aldehyde fragments so formed were converted to their 2,4-dinitrophenylhydrazones (2,4-DNPs) by treatment with a saturated solution of 2,4-dinitrophenylhydrazine in 6 N hydrochloric acid. After being filtered, washed and dried, the 2,4-DNPs were chromatographed on a column of silica gel giving two main bands. The 2,4-DNP in the less adsorbed band which was eluted with benzene, was recrystallised twice from heptane. Its rate of movement (paper chromatography:heptanej2-phenoxyethanol system)5 was identical to that of nonanal 2,4-DNP. It had a melting point (uncorrected) of 102-103° which was not depressed when it was mixed with an authentic sample of nonanal 2,4-DNP of m.p. 101-102°.

Results and Discussion

Figs 1 and 2 show the Lr. spectra of the two sesquiterpene peaks 48 and 47a isolated from hop oil as described above.

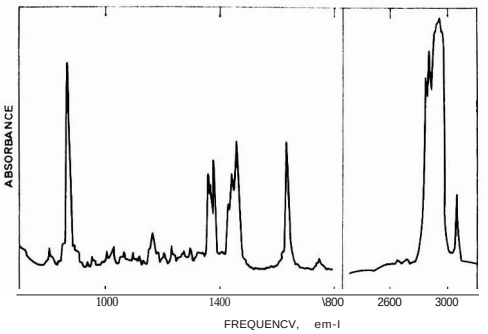

FIG. I. *Infra-red absorption spectrum of thin film of sesquiterpene hydrocarbon peak 48*

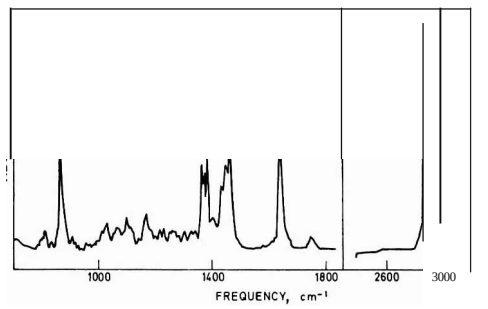

FIG. 2. *Infra-red absorption spectrum of thin film of sesquiterpene hydrocarbon peak 47a*

• Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

The i.r. spectrum of peak 48 is consistent with the spectrum of **β-copaene** (2-isopropyl-5-methyl-9-methylene tricyclo [4,4,-0,0^{5,10}] decane) published by Westfield.[6] It is also consistent with that published by Hunter *et al.*[7,8] Hunter & Brogden? had first reported their spectrum as that of {J-ylangene but later Veldhuis & Hunter[8] corrected this to {J-copaene.

The i.r. spectrum of peak 47a shows some similarity to that of {J-copaene and to that of a-ylangene and is possibly {J-ylangene although there is no i.r. spectrum of authentic {J-ylangene available in the literature for comparison.

a-Copaene and a-ylangene had been previously identified in hops by Naya *et al.*[2] and independently by some of the present authors.',4

As outlined above the ozonisation of the C_{15} mono-unsaturated ketone in hops gave nonanal, which would locate the position of the double bond nine carbon atoms from the saturated end. Proton magnetic resonance, i.r. and mass spectra had previously established, that it was a straight-chain methyl ketone with the double bond somewhere towards the middle of the molecule. The combined evidence from spectra and ozonisation then indicates that the major C_{15} mono-unsaturated ketone is pentadec-6-ene-2-one with the double bond probably *cis*, because of the absence of any band in the i.r. spectrum in the region of 965 cm^{-1}. This ketone bears a relationship to the previously identified di-unsaturated ketone in hops, pentadec-6,9-dien-2-one. The two ketones are also somewhat related to oleic and linoleic acids in regard to the position of the double bonds measured from the satured end of the chain.

Although the pentadec-6-ene-2-one is the major C_{15} mono-unsaturated methyl ketone of hops some tentative evidence (from ozonisation) was also obtained for the presence of lesser amounts of other isomers, one with the double bond in the 12-position and another with the double bond in the 9-position These isomers would be extremely difficult to separate from the major 6-isomer by conventional methods.

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ISOLATION AND CHARACTERISATION OF A WATER-SOLUBLE WHEAT-FLOUR PROTEIN

By W. W. FISH· and DONALD C. ABBOn

The main component of the water-soluble proteins of wheat flour has been isolated in suflicient quantity for chemical and physical characterisation. This was achieved by a combination of ion-exchange chromatography on carboxymethyl cellulose and gel filtration through Sephadex G75. The protein isolated was judged to be 90% pure by zone electrophoresis. Sedimentation analysis yielded a single symmetrical peak with an $s_{20,w}$ value of 2·45. The molecular weight was found to be 19,300 by gel filtration, and the diffusion coeflicient estimated by the same method was $10{\cdot}47 \times 10^{-7}$. A molecular weight of 16,000 was calculated from sedimentation equilibrium analysis in a dissociating medium. The ultra·violet spectrum of the isolated albumin exhibited a maximum at 278 nm and from this an $E^{1\%}_{278}$ value of 13·1 was calculated. No free sulphydryl groups could be detected. Amino acid analysis showed that this protein has a composition similar to those of other soluble wheat·flour preparations. A molecular weight of 16,300 was calculated from the amino acid analysis. End-group analysis of the protein showed that serine is the N-terminal amino acid. The C·terminal amino acid was found to be resistant to release by both carboxypeptidase A and carboxypeptidase B.

Introduction

In early work with wheat proteins, fractions isolated by solubility properties were used, and all such fractions have been shown to be grossly heterogeneous. Heterogeneity of the flour proteins soluble in water and dilute salt solution has been demonstrated by a number of workers.[1-5] Ion-exchange chromatography on DEAE-cellulose has been used for the isolation of several components from the dilute·salt extracts of flour by a number of investigators[5,7] while a few components of the water-soluble fraction of flour have been isolated by small-scale preparative starch-gel electrophoresis[8] or by a combination of gel filtration and starch-gel electrophoresis.[9] In most cases, the amount of homogeneous protein isolated was insufficient for extensive characterisation studies, although amino acid analysis and molecular weight determinations[7] and N-terminal amino acid analysis[10] have been reported for some.

In earlier work,[9] the major protein component of the water-soluble fraction of flour was isolated as an electrophoretically homogeneous component by a combination of gel filtration through Sephadex G 100 and small-scale preparative starch·gel electrophoresis. However, only small quantities could be isolated by this procedure and means of effecting the isolation of this protein in larger amounts were sought. Since separation by charge differences as effected by starch-gel electrophoresis was an essential step in the isolation, it appeared that other methods of separation based on charge differences might be effective.

The present paper describes the application of ion·exchange chromatography on carboxymethyl (CM) cellulose in combination with gel filtration through Sephadex G75 for the isolation of a major albumin of the water-soluble fraction of wheat flour. Sufficient quantities were isolated to permit physical and chemical characterisation of the protein.

Experimental

Flour and protein solutions

Straight-grade flour experimentally milled from a 1963 crop composite of hard red winter wheat was the source of water·soluble flour proteins. Protein (N x 5·7) and ash contents were 12'4% and 0'42%, respectively. Solutions of the water-soluble flour proteins for ion-exchange chromatography experiments were prepared by stirring flour with water at a flour: water ratio of 1 : 1·5 (wt.fvol.) at room temperature. The thick slurry was stirred at 5 min intervals for 30 min and then centrifuged at 13,000 x *g* for 30 min at 2°. The clear supernatant was dialysed for 24 h against two changes of ten volumes of the starting eluant. The protein content of the extracts was determined by a micro-Kjeldahl procedure or by a biuret method.[11]

Electrophoresis apparatus and procedures

The apparatus and procedures for starch-gel electrophoresis were the same as those reported earlier by Abbott & Johnson.[9] Thin gels on glass plates were used exclusively in this investigation. Polyacrylamide gels for electrophoresis contained 7·5 g of Cyanogum 41 (American Cyanamid Co., New York) per 100 ml of buffer and gelation was catalysed by the addition of ammonium persulphate and dimethylaminopropionitrile.

Three buffer systems were utilised for electrophoresis. An 8 mM aluminium lactate-3 M urea buffer, pH 3·3, was used for analysis of column fractions by starch-gel electrophoresis. The protein fractions isolated were analysed electrophoretically in a 20 mM sodium cacodylate-HCl buffer, pH 6'0, and in 15 mM Tris-3 mM citric acid-8 mM boric acid buffer containing 0'5 M urea with sufficient sodium hydroxide added to give a pH of 8·6.

The electrophoretic purity of the wheat albumin isolated was estimated by a modification of the procedure for electrophoresis on cellulose acetate strips.[12] The strips (Sepraphore III, Gelman Instrument Co., Ann Arbor, Mich.) were soaked for 4 h in the buffer of pH 8'6; the excess buffer was blotted off and protein solutions were applied with a Gelman applicator. Samples containing 25-100 μg of protein were subjected to electrophoresis for 3 h at 20 V/cm in a Buchler Universal Electrophoresis Cell using a Buchler d.c. power supply (Buchler Instruments, Fort Lee, N. J.). The protein bands were then stained for 15 min with Ponceau S stain and the strips were cleared in 5% acetic acid. Each stained band was cut from the strip and dissolved in a chloroform–ethanol

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solvent (9 : I by vol.). The absorbance of each solution was measured against an appropriate blank and the percentage of each protein band was determined by dividing its absorbance by the sum of the absorbances of the three protein bands. The absorbance of the dye-protein complex of each band was a linear function of the amount of samples applied to the strip.

Ion-excbange column chromatography

CM-eellulose (Mann Research Labs, New York) was washed with 0·05 N-NaOH for 5 min followed by 0·5 N-HCl for 15 min, then washed with de-ionised water until no acid remained, and finally equilibriated with the starting buffer, 0·05 M sodium acetate, pH 4·6. Columns were poured from this slurry. Preliminary experiments were conducted with columns having bed dimensions of 2'2 x 15 cm while columns with bed dimensions 4·4 x 15 cm were used for preparatory work.

40 ml of dialysed extract containing about 250 mg of protein were applied to the CM-cellulose column in the preliminary experiments; these quantities were increased fourfold for preparatory experiments. In the latter experiments, the walls of the column were washed twice with 20 ml of buffer, then I I of starting buffer was passed through the column to eluate unadsorbed material. Elution of adsorbed proteins were then carried out using 6 I of 0'15 M-NaCl, 2 I of O'28 M-NaCl and II of 0·4 M-NaCI. Effluent was collected in 10 ml fractions from the small columns and in 20 ml fractions from the preparatory columns.

Gels and columns for gel filtration experiments.

Commercial crosslinked dextran gel, Sephadex G75 (Pharmacia, Uppsala, Sweden), was used. The preparatory column gel bed dimensions were 2·0 x 70 cm. Protein solutions were applied to the gel by layering under the eluanl. Eluants used were 0·5 M lactic acid or 0·02 M Tris buffer, pH 7'4, containing 5 mM-$MgCl_2$; 3 ml fractions of effluent were collected. Column void volume was determined from the elution of Blue Dextran 2000, mol. wI. 2 x 10^6 (Pharmacia Uppsala, Sweden).

The method of Andrews[13,14] was used for the estimation of the molecular weight and the diffusion coefficient of the isolated albumin. A 1·3 x 100 cm column of Sephadex G75 was used. The gel column was calibrated with the following proteins: ovalbumin, mol. wI. 45,000, $D_{020,w}$ 7·76 x 10^{-7}; a-chymotrypsinogen, mol. wI. 25,000, $D_{020,w}$ 9'5 x 10^{-7}; cytochrome c, mol. wI. 12,400, $D_{020,w}$ 13,0 x 10^{-7} (aU from Sigma Chemical Co" SI. Louis, Mo.); p-Iactoglobulin, mol. wI. 35,000; a-lactalbumin, mol. wI. 14,440; papain, mol. wI. 20,700, $D_{020,w}$ 10'23 x 10^{-7} (Difco Laboratories, Detroit, Mich,); bovine pancreatic ribonuclease, mol. wI. 13,700, $D_{'20,w}$ 11·9 x 10^{-7} (Mann Research Laboratories, New York). Each protein standard was crystalline and was eluted as a single peak under the experimental conditions used.

Analysis of fractions from eM-cellulose and Sephadex columns

Proteins in eluates were detected by measurements of absorbance at 280 and 260 nm on each tube. Absorbance was read in a Beckman Model DU spectrophotometer equipped with a Gilson Medical Electronics transferator. In some experiments with CM-cellulose, the agreement of absorbance at 280 nm with protein concentration was checked by analysis of the fractions by the method of Lowry *el al.*[15] Carbohydrate in the fractions was determined by the anthrone procedure.[16] The ionic strength gradient in effluent from CM-cellulose columns was followed by measurement of conductivity on a Radiometer conductivity meter.

Physical measurements

Sedimentation measurements were made in a Beckman Model E ultra-centrifuge using a single-sector, synthetic boundary cell. For sedimentation velocity experiments, the solvent used was 0·1 M-NaCI in I mM sodium phosphate buffer, pH 6·95. Protein concentrations were determined spectrophotometrically. Sedimentation coefficients were determined with a rotor speed of 59,780 rev/min and were corrected to values in water at 20°. Sedimentation equilibrium experiments were performed according to the method of Yphantis[17] in two solvent systems: 0'I M-NaCI in I mM sodium phosphate, pH 6'95, and 6 Mguanidine hydrochloride containing 0, I Mmercaptoethanol. Rotor speeds used with these systems were 44,770 and 42,040 rev/min, respectively. Solution column heights of 3 or 4 mm were used. It was assumed that the partial specific volume of the protein did not change in 6 M guanidine hydrochloride. Plates were analysed on a Nikon Model 60 microcomparator.

The ultra-violet absorption spectrum of the isolated protein was determined on a Cary Model 14 recording spectrophotometer,

Chemical studies

Amino acid analyses were conducted on a Beckman Model 120C automatic amino acid analyser according to the method of Moore & Stein.[18] Samples were hydrolysed in 6 N-HCI for 12, 24, 48 and 72 h at 110° in sealed, evacuated tubes. Nitrogen recovery after chromatography of the hydrolysates was 99 ± I%. Hydrolysis was incomplete after 12 hand low values for all amino acids were obtained. The values for threonine and serine were determined by extrapolation of the data to zero time of hydrolysis. Little change with time in the values for the other amino acids was observed. Half-cystine was determined as cysteic acid after performic acid oxidation[19] and hydrolysis of the protein. Tryptophan and the ratio of tyrosine to tryptophan were determined spectrophotometrically,[20] The adjusted ammonia value was obtained by subtracting from the observed ammonia value at 24 h the losses in serine, threonine, and half-eystine after 24 h hydrolysis together with twice the tryptophan value,

Free sulphydryl groups were determined by the spectrophotometric method of Boyer[21] according to the procedure of Fraenkel-Conral.[22] Ovalbumin was run as a check. Samples were assayed in buffer with and without O'6% sodium lauryl sulphate.

N-terminal and C-terminal amino acid determinations

Proteins used for end-group analyses were suspended in cold, 5% trichloroacetic acid (TCA) and centrifuged to eliminate low molecular weight impurities. The precipitated protein was then treated with the appropriate reagents. The dinitrophenylation method for amino end-group analysis was carried out according to the method of Fraenkel-Conrat *el al.*[23] The Edman degradation was done by the paper strip technique.[24] Simultaneously, egg white lysozyme (Nutritional Biochemical Corp., Cleveland, Ohio) was degraded as a check on techniques. Two-dimensional paper chromatography was used for identification of dinitrophenyl amino acids, while one-dimensional paper chromatography was used for identification of the phenylthiohydantion amino

acids.[25] Synthesised standards were employed in both cases.

For carboxyl-terminal studies, DFP-carboxypeptidase A and DFP-carboxypeptidase B were used (Sigma Chemical Co., St. Louis, Mo.). The TCA-precipitated protein was treated with enzyme in 0'1 M-NH4HC03 at pH 8·0 and 25°. I : 15 and I : 50 molar ratios of enzyme to substrate were used for carboxypeptidase A and carboxypeptidase B, respectively. Aliquots of the incubation mixtures were removed at intervals during an 8 h period and TCA was added to a final concentration of 5% to terminate the reaction and precipitate residual protein. After centrifugation, the supernatants were subjected to paper chromatographic analysis. Carboxyl-terminal analytical procedures were checked by the treatment of lysozyme with carboxypeptidase A and trypsin (Mann Research Lab., Inc., New York) with carboxypeptidase B.

Results and Discussion

CM-cellulose chromatography of flour water-solubles

Preliminary experiments on the fractionation of the water-soluble flour proteins by CM-cellulose chromatography demonstrated that the major water-soluble component was eluted at a salt concentration of about 0·28 M-NaCl regardless of the type of elution gradient used. A typical starch-gel electrophoretic pattern for the protein eluted by 0·28 M-NaCi is shown in Fig. I together with the pattern for the aqueous extract. It is apparent that the mobility of the major component in this fraction corresponds to that of the major component in the unfractionated extract. Further work showed that a series of stepwise changes of salt concentration permitted the easiest and purest preparation of this major component. Therefore, the CM-cellulose fractionation procedure using a stepwise elution system was scaled up for use in preparatory work.

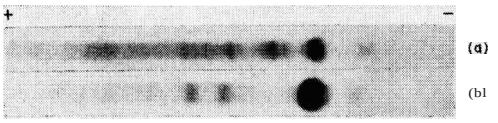

FIG. I. *Electrophoretic comparison of* (a) *the aqueous flour extract and* (b) *the major albumin component eluted from CM-cellulose by 0·28 M-NaCI*

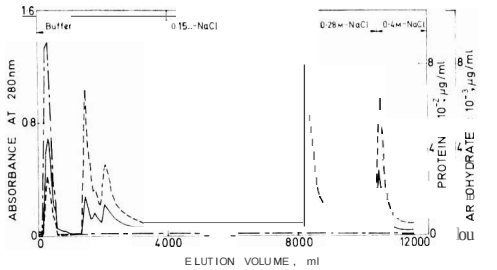

FIG. 2. *Preparative CM-cellulose chromatography of water extract of flour*

I g of protein (N × 5'1) in 190 ml of extract was dialysed overnight against 0'05 M acetate buffer, pH 4,6, then applied to a 4·4 x 15 cm CM-cellulose column. Unabsorbed materials were eluted from the column with t 1 of the buffer. - Absorbance at 280 om; – - - protein measured by Lowry procedure; —- - carbohydrate measured by anthrone procedure

After analysis for carbohydrate and protein, tubes from the preparatory column were pooled into appropriate fractions, dialysed to remove the salt, and lyophilised. The column elution pattern is shown in Fig. 2 and the starch-gel electrophoretic patterns of the fractions are shown in Fig. 3. Both Amido-Black and Ponceau S stains revealed the same protein bands in a given fraction and with corresponding relative intensities.

The elution pattern from the large column was identical to that of the smaller columns as measured by the absorbance at 280 nm. The material in the first peak, eluted by buffer only, contained about 40 mg of carbohydrate/mg of protein, and it gave a positive Bial's test for pentose. Thus, it appears to correspond to a similar fraction described earlier by Coates & Simmonds.[26] Satisfactory starch-gel patterns could not be obtained because of the high carbohydrate : protein ratio in this fraction.

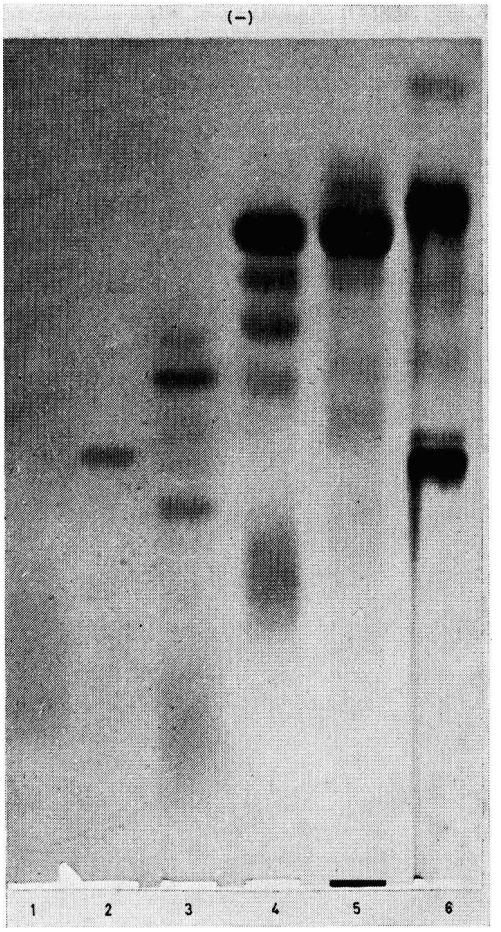

FIG. 3. *Starch-gel electrophoresis of pooled fractions from preparative CM-cellulose column*

300-800 µg of sample were applied to wicks. Electrophoresis was for 20 h at 5 V/cm in aluminium lactate-urea buffer, pH 3·3.
Pooled fractions and elution volumes were as follows: (1) 1400-1460 ml; (2) 170<1-1760 ml; (3) **2000–2080** ml; (4) 5340-5520 ml; (5) 8160-8900 ml; (6) 10,460-10.800 ml

The peaks eluted by various concentrations of salt contained large amounts of protein and essentially no carbohydrate. Nearly all the major albumin component of the water-soluble protein was found in the fraction eluted by 0·28 M-NaCl (elution volume 8160–8900 ml), although small amounts of this protein were present in the fractions eluted by 0·15 M- and 0·40 M-NaCl. When 1 g of protein (N × 5·7) was applied to the column, about 150 mg of material (essentially 100% protein by Lowry assay) were obtained from the fraction eluted with 0·28 M-NaCl. Re-chromatography of this fraction using various types of gradients between 0·15 M- and 0·28 M-NaCl produced no further separation of the major albumin protein from the other proteins in the fraction.

Fractionation by gel filtration of protein eluted from CM-cellulose by 0·28 M-NaCl

Earlier research[9,27] indicated that gel filtration in Sephadex G75 did not effect a clean separation of albumin and gluten proteins present in a water extract. It appeared, however, that such a separation might be possible by use of a less complex starting material. Therefore, the CM-cellulose fraction containing the major albumin protein was subjected to gel filtration on Sephadex G75. The elution profile for a typical experiment is shown in Fig. 4 and the starch-gel electrophoresis patterns of the appropriately pooled fractions are shown in Fig. 5. Proteins in the first peak were eluted from the column in the void volume. Their migration rates in starch gel were similar to those of gliadin proteins. The second peak contained the protein of interest which appeared to be nearly homogeneous electrophoretically. One or two faint protein bands were occasionally observed when large amounts of some preparations were subjected to starch-gel electrophoresis (Fig. 5, protein sample 4). Lowry protein assays on the fractions from a Sephadex G75 column showed that two-thirds of the protein placed on the column was in the second peak. The same results were obtained when a 5 mM Tris buffer, pH 7,4, was used as the eluant instead of 0·5 N lactic acid.

Reversible aggregation of wheat protein fractions has been reported by several investigators.[28-30] Therefore, the possibility that some of the proteins in the first peak from the G75 column were an aggregate of the protein in the second peak was investigated. When the material in the second peak from a G75 column was re-chromatographed on the same column, no protein, as measured by the absorbance at 280 nm, was observed in the elution volume corresponding to the first peak (Fig. 4). Thus, it appears that the isolated albumin is not a monomer unit of some of the larger proteins in the first peak. This observation is not conclusive, however, since the experiment was not repeated at a higher protein concentration.

Test of purity of the isolated protein by electrophoresis

The isolated albumin was subjected to electrophoresis in various buffer systems of different pH and in both starch and polyacrylamide gels as the supporting media. Fig. 6 shows the results of these experiments. The protein, as revealed by three different protein stains, usually migrated as a single band at pH 3·3 in both types of support. As shown with gel 6,

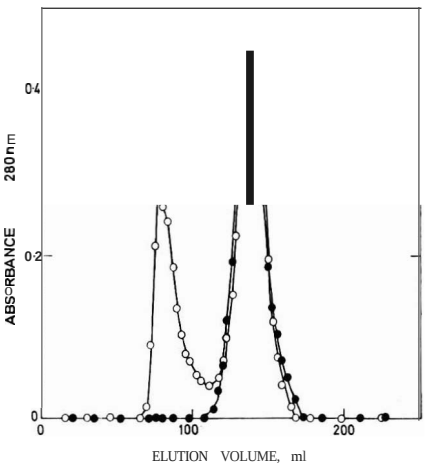

FIG. 4. *Gel/iltration chromatography alld re-chromatography* 011 *Sephadex G75 of proteill eluted from CM-cellulose by 0·28 M-NaCI*

IS rng of protein from the 0·28 M-NaCI peak were dissolved in 1·5 011 of 0·5 N lactic acid and placed on a 2·0 x 71·0 em G7S column. Elution was carried out with 0·5 N lactic acid. The second peak from the gel filtration fun was dialysed, Iyophilised. and re-dissolved in I·S mt of 0·5 N lactic acid and fe-rUD tbrough the same G7S column.
a Protein after first pass through G7S; • protein after re-run of second peak through G7S

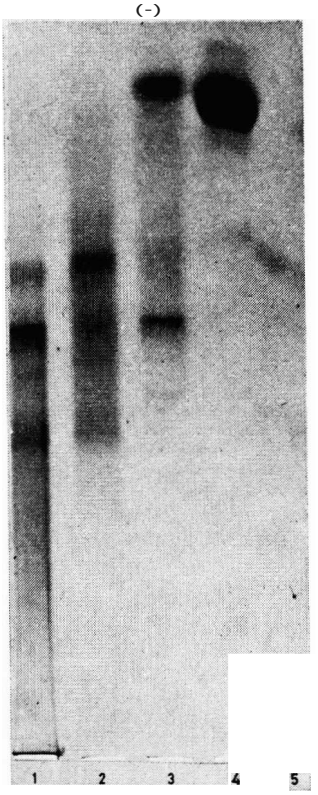

FIG. 5. *Starch-gel electrophoresis of fractions from gel/iltration experimelll shown* ill *Fig. 7 (sillgle pass through G75)*

About 800 μg of sample **were applied to each wick. Electrophoresis was for 20 b at 5 V/cm in aluminium lactate-urea buffer, pH** 3·3.
Pooled fractions and elution volumes were as follows: (1) 66-90 ml; (2) 91-108 ml; (3) 109-123 ml; (4) 124-156 ml; (5) 157- 180 ml

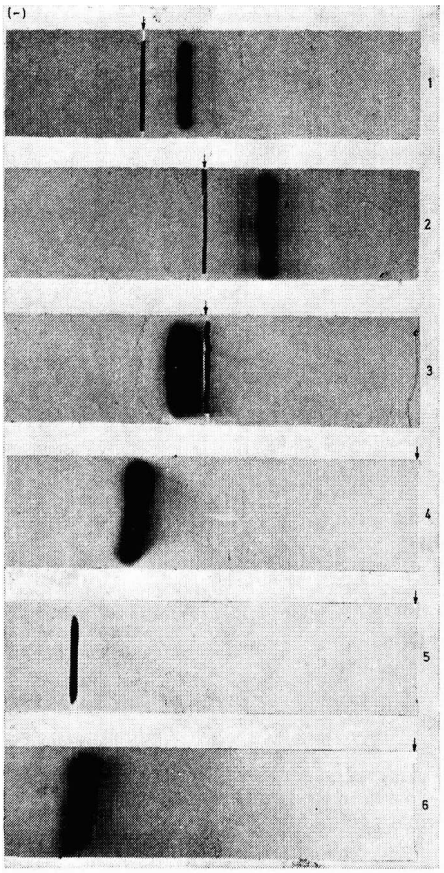

FIG. 6. *Electrophoresis of isolated protein in starch and polyacrylamide at various pH*

Gels (1)-(S) stained with Amido·Black lOB, gel (6) stained with nigrosine. The cathode is to the left in the figure with origins marked by arrows.
(I) starch gel, pH 8·6,5 V/cro for 18 h, 500 μg of protein; (2) polyacrylamide gel, pH 8·6, 5 *V/cm* for 18 h, 500 μg of protein; (3) polyacrylamide gel, pH 6'0, 4 V*lem* for 14 h, 700 μg of protein; (4) starch gel, pH 3·3. 5 Y*lem* for 17 h. 700 μg of protein; (5) polyacrylamide gel. pH 3·3, 5 *V/cm* for 21 h, 300 μg of protein; (6) starch gel, pH 3·3,5 *V/cm* for 14 h, 700 μg of protein

electrophoresis of greater amounts of the preparation revealed a trailing shadow area behind the major protein band when the gel was stained by the sensitive dye nigrosine. At pH 6'0, the protein migrated very slowly toward the cathode and a diffuse area appeared behind the main component. At pH 8·6, three protein bands, two faint and one heavy, were clearly seen in both starch gel and polyacrylamide gel. The number of protein bands appearing at this pH was the same regardless of the protein stain used.

Electrophoresis of the isolated protein fraction on cellulose acetate strips at pH 8·6 and staining with Ponceau S also revealed three protein bands. When the percentage of each protein band was determined as described by Scherr,12 the heavy band constituted at least 90% of the isolated fraction, while the slower migrating protein made up about 2% and the faster migrating component about 8% of the fraction.

Absorption spectra

The ultra-violet absorption spectrum of the isolated albumin in dilute acid was similar to those of most proteins and exhibited an absorption maximum at 278 nm. The extinction coefficient of a 1% solution of the protein, $E_{278}^{1\%}$, was 13·1. The tyrosine : tryptophan ratio was 2·1 when calculated from the absorption spectra in 0·1 N-NaOH by the method of Goodwin & Morton[31] as described by Beaven & Holiday.20

Physical studies using gel filtration

The semi-log plot of elution volume from Sephadex G75 vs. molecular weight for eight protein standards and the isolated wheat albumin is shown in Fig. 7. The isolated protein eluted in the same volume over the concentration range 1-10 mgjml. A molecular weight of 19,300 ± 2000 (95% level) was calculated from the regression equation. This value is in general agreement with the range of molecular weights reported for wheat-flour albumins (16,000[32]-28,000[1]).

The method suggested by Andrews[14] was used to estimate the diffusion coefficient of the isolated albumin from the gel filtration data. The linear plot of elution volumes of standard proteins vs. the reciprocal of the diffusion coefficients is shown in Fig. 8. A diffusion coefficient for the isolated albumin of $10{\cdot}47 \pm 0{\cdot}09 \times 10^{-7}$ cm^2/sec (95% confidence level) was calculated from the regression equation.

The above value for molecular weight is probably slightly higher than the true value and that for the diffusion coefficient somewhat lower than it should be, owing to the

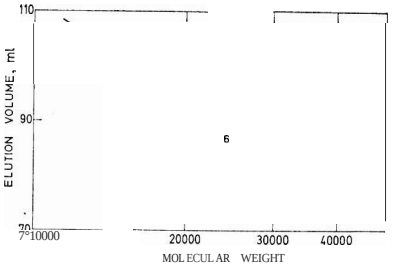

FIG. 7. *Semi-logarithmic plot of elution volume vs. molecular weight*

Molecular weight orthe isolated albumin based on its elution volume (96,5 ml) was 19,300. V_o = 60ml
(I) Cytochrome *c* monomer; (2) a-lactalbumin; (3) ribonuclease; (4) papain; (5) a-chymotrypsinogen; (6) cytochrome c of dimer; (7)p-lactoglobulin; (S)oval·bumin. 0 Isolated protein

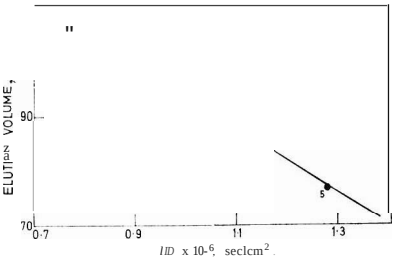

FIG. 8. *Elution volume vs. reciprocal of the diffusion coefficient*

The diffusion coefficient of the isolated albumin based on its elution volume (96-5 ml) was $10{\cdot}47 \times 10^{-7}$ cm^2/sec. V_O=60ml
(I) Cytochrome *c;* (2) ribonuclease; (3) papain; (4) a-ehymotrypsinogen; (5) ovalbumin. 0 Isolated protein.

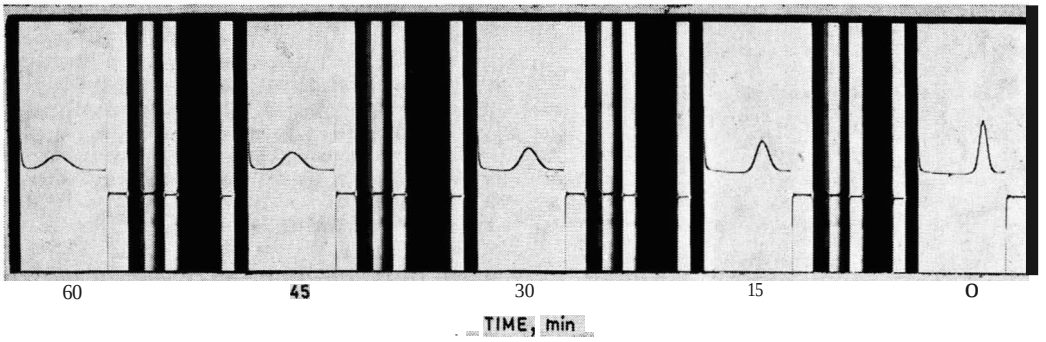

FIG. 9. *Sedimentation pal/ern oJ the isolated albumin in a sYll/hetic boundary cell*

Temperature 20°c, phase-plate angle 70". Sedimentation from right to left. Photographs were taken at the times indicated after full rotor speed of 59,780 rev/min was attained. Isolated albumin: 6'5 mg/ml in 0·1 M-NaCI, pH 6'95.

probable existence of an associating-dissociating system. This phenomenon is discussed further in the following section.

Analytical ultra-centrifuge analysis

The isolated albumin sedimented as a single symmetrical peak in O' I M-NaCI, as shown in Fig. 9. The dependence of the sedimentation coefficient *(S20.W)* on protein concentration is apparent in Fig. 10. A sedimentation coefficient of 2·45 was obtained by extrapolation of the data to infinite dilution. The increase in the sedimentation coefficient with increasing protein concentration is not typical of the behaviour of most proteins. Schachmann,33 however, has reported that associating-dissociating, single component systems involving rapidly attained equilibria do exhibit this phenomenon. A single almost symmetrical boundary is usually observed, and S increases with concentration in dilute solutions. The increase in S with concentration is presumed to be the result of a shift in the equilibrium toward the higher aggregates as the concentration increases. Several other wheat protein preparations have been shown to exhibit the same behaviour.[7,28,29]

The molecular weight of the wheat albumin in 0·1 M-NaCI, pH 7'0, was determined by sedimentation equilibrium at only one speed, The plot of fringe displacement vs. the square of the distance from the centre of rotation showed only slight curvature at the extremities. A weight-average molecular weight of 21,000 was calculated from this plot. Since the sedimentation velocity experiments had indicated that this was a rapidly associating-dissociating system in the solvent system used, sedimentation equilibrium experiments were performed in 6 Mguanidine hydrochloride in the presence and absence of mercaptoethanol. Under such conditions, the molecular weight of the monomer species can be calculated. Fig. I I shows the plot of fringe displacement vs. the square of the distance from the centre of rotation for a typical experiment of this type. From the slope of this plot, a molecular weight of 16,000 was calculated. The results of these experiments in a dissociating medium support the hypothesis that a rapid **monomer–dimer** association-dissociation system exists for this protein in dilute salt solutions.

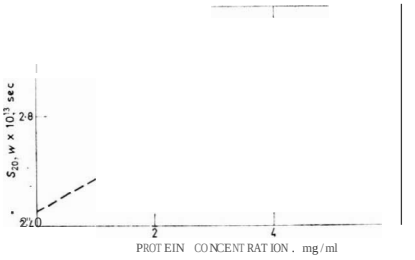

FIG. 10. *Effect oJ proteill concell/ration on the sedimell/atioll coefficient* (S20,w) *oJ the isolated protein*

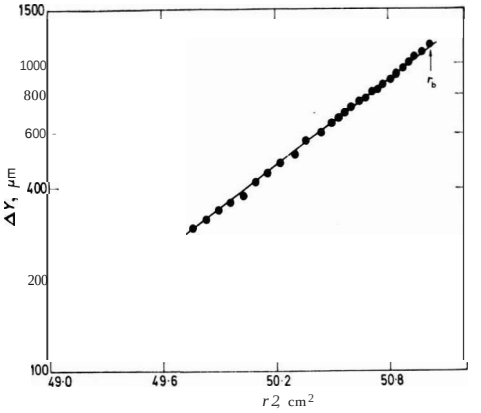

FIG. II. *Semi-logarithmic plot oJ Jringe displacement* (△Y) *vs. the square oJ the distance Jrom the cell/re oJ rotation* (r)

Sedimentation equilibrium for a solution of isolated wheat albumin (0·02% in 6 **M** guanidine hydrochloride-O·I **M** mercaptoethanol, pH 7·0). The experiment was conducted for 36 h at 20°e and 42,040 rev/min. r: = 45·499 em', r_b^2 = 51·032 em'

TABLE 1

Amino acid composition of the major albumin from *Tri/icum aes/ivum*

Values are in residues jl6,000 g

Amino acid	Hydrolysis time, h					Nearest integer
	24	24	24	48	70	
Lys	3·96	4·46	4·25	4·23	4·24	4
His	1·98	2·23	1·98	2·06	2'12	2
NH3						12"
Arg	8·90	8·92	8·79	8·90	8·78	9
Asp	8·90	8·92	8·50	8·90	8·78	9
Thr	4·61	3·50	3·69	3·50	3·33	4"
Ser	8·24	7·96	8·22	7·50	6·96	9"
Glu	15·83	15·92	16·44	15·90	16·65	16
Pro	10·88	11·14	10·77	10·80	11 ·80	11
Gly	12·53	12·74	13·04	13 ·04	13·02	13
Ala	18·80	20·70	19·84	19·12	19·07	19
½ Cys						10[c]
Val	11·21	10·5/	11·06	10·78	12·41	11
Met	3·96	3·50	3·68	3·67	3'63	4
lie	3·63	3·50	3·40	3·48	3·63	4
Leu	11·54	11·46	11 ·91	11·86	11·80	12
Tyr	5·94	5·73	5·67	5·88	5·75	6
Phe	2·31	2·55	2'55	2'46	2·42	3
Trp						3[d]

• Estimated by correcting for losses in Ser, Thr, Trp, Cys
" Extrapolated to zero time
c Determined as cysteic acid
d From spectrophotometric estimation[20]

Amino acid analysis

The amino acid composition of the isolated wheat albumin is given in Table 1. The minimum molecular weight of the protein, based on its histidine content, was 16,300, which is in good agreement with the value obtained by sedimentation equilibrium in the dissociating medium.

Comparison of the amino acid composition of this wheat albumin with the composition of several flour protein preparations reported in the literature indicates that its composition is not unlike that of the 'solubles' class. The similar compositions and electrophoretic properties suggest that this albumin was the major component in the unrefined fraction described by Nimmo *et al.*[31]

The apparent specific volume of the isolated albumin was estimated from its amino acid composition by the method described by McMeekin & Marshall.[35] The partial specific volume calculated in this manner was 0·727 cm^3/g.

Sulphydryl group determination

No free-SH groups were detected in the protein in either the presence or absence of detergent.

Amino-terminal residue

Both dinitrophenylation and Edman degradation showed that serine was the only N-terminal amino acid in the isolated albumin. It may be noted that serine was one of the N-terminal amino acids observed in the water-soluble proteins by Simmonds.[10]

Carboxy terminal residue

Attempts to determine the carboxyl-terminal residue were unsuccessful. No amino acids were released from the protein by digestion with carboxypeptidase A or carboxypeptidase B under any of the experimental conditions used. These results suggest[36] that either the carboxyl of the C-terminal amino acid is substituted or that proline is the C-terminal or penultimate amino acid. The presence of a cyclic peptide unit at the C-terminal end of the chain would also account for these results.

Conclusions

The present work has resulted in the isolation and partial characterisation of the major albumin component of flour proteins. The biological or functional roles of this protein in the wheat kernel or in flour have not yet been established. This protein may have an enzymic function in the maturation of the kernel or in the degradation of storage constituents during germination. Alternatively, its role may be that of a minor but necessary part of the total flour protein complex which gives a wheat-flour dough its characteristic properties. The study of these and related problems as well as the isolation and characterisation of other water-soluble flour proteins should be facilitated by the techniques used and the information obtained in this investigation.

Acknowledgment

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ISOLATION AND CHARACTERISATION OF A WHEAT ALBUMIN

By J. A. D. EWART

A major albumin from Cappelle-Desprez wheat flour has been isolated by carboxymethyl-cellulose chromatography in a sufficiently pure state for characterisation. The water-soluble protein is free from phenylalanine and histidine, and this enables the purity to be estimated as > 95%. It appears to consist of a single chain with a mol. wI. of 26,000. The absorbance coefficient at 280 nm is $1{\cdot}9 \times 10^3$, the high value being due to 8 tryptophan and 8 tyrosine residues per molecule. There are 10 S. S bonds but no sulphydryl groups or sugar residues in the molecule. Sedimentation studies indicate that the molecules tend to aggregate in 0·2 M-NaCI. No protease or amylase activity was detectable.

Introduction

Baudet & Mossé[1,2] have shown that 45-50% of the protein of wheat flour can be obtained in clear solution by repeated extraction with water. Nevertheless the term albumin is usually restricted to those flour proteins which dissolve easily in water, the so-called 'water-soluble proteins', comprising about 10% of the total. Finney[3] produced evidence that albumins could influence the baking behaviour of flour and this was supported by Pence *et al.*[4] Later a salt fractionation scheme was devised for separating the water-soluble proteins into albumins and globulins.[5] In the ultra-centrifuge the albumins appeared fairly homogeneous with a mol. wI. of at least 20,000. Tiselius electrophoresis[5] indicated heterogeneity which was confirmed by resolution into 6 components by paper electrophoresis. Subsequent work has revealed increasing complexity among the albumins.[6-9] Some of the numerous attempts which have been made to separate them have been described.[10-16] Kelley" prepared a salt-soluble protein from flour which gave a major peak in the Tiselius apparatus and had a mol. wI. of 76,500. It was not, however, subjected to gel electrophoresis. Amino acid analyses have been reported on albumins, both fractionated'[0] and un·fractionated.'[B-20] Recently Feillet & Bourdet[21,22] have isolated a Cappelle albumin which ran as a single band on starch-gel electrophoresis. This paper describes concurrent work on what is probably a similar protein to this,

Experimental

Albumin preparation

Flour was milled from Cappelle-Desprez wheat. At first the method of Pence & Elder[5] was used but later a O'5 M-NaCI extraction followed by 2 Mammonium sulphate precipitation (substantially that of Feillet & Bourdet[21]) was adopted to obtain crude albumin.

Column chromatography

A column (75 × 3 cm) was packed with Whatman micro-granular CM 52 carboxymethyl-cellulose (500 g), which had been pre-cycled according to the manufacturer's instructions and equilibriated first with Msodium acetate buffer, pH 5·2, and finally with 0·1 Mbuffer solution. The column was loaded with I·2 g of crude albumin taken up in 20 ml of the 0·1 M acetate buffer. In early work 0·1 M acetate buffer was used for elution with a pH gradient from 5,2 to 6·2. Later the pH was maintained at 5·2 but the second flask was made O'34 M in NaCI to give a linear ionic strength gradient. Fractions were passed through a Uvicord ultra-violet absorptiometer (253·7 nm) into a Beaumaris collector, dialysed vs. water and freeze-dried. They were examined by starch-gel electrophoresis at a concentration of ~ 10 mg/ml.

Starch-gel electrophoresis

This was carried out as described by Ewart[23] using 0·0083 M aluminium lactate buffer, pH 3·3 or 0·0023 M borate buffer, pH 9·1. Voltage gradients of 18 V/cm were used for runs of 2-2'5 h. Water was circulated underneath the gel platform and a water-cooled copper tank insulated with l.e.l. Melinex film lay on the top surface of the gel. This apparatus was a slight modification of that of Graham.[13]

Analyses

An accurately weighed quantity of protein (~ I mg) was hydrolysed with I ml of constant-boiling HCl for 24 or 72 h. The Technicon amino acid analyser was used as previously described.[24] Two runs were made on both hydrolysates. In the case of valine the 72 h values were averaged but otherwise apart from cystine (see below) there was no evidence for slow liberation or unusually rapid destruction of the other amino acids and the mean of four runs was calculated. Tests for sulphydryl groups were made by the Ellman method.[25] In a further experiment the protein was treated with iodoacetic acid· in 7 M guanidine hydrochloride by a modification of the method of Crestfield *el al.*[26] and hydrolysed in an evacuated sealed tuhe for estimation of cysteine as the carboxymethyl derivative. Spectrophotometric determination of the ratio of tyrosine to tryptophan as described earlier[27] enabled the content of the latter acid to be estimated from the analyser value for tyrosine. The protein was oxidised with performate as recorded earlier.[28] Cystine was estimated as cysteic acid and related to the analyses on unoxidised protein by reference to the quantities of stable amino acids.

Trypsin digestion of performate-oxidised protein took place in 0·1 M triethylamine acetate buffer, pH 8 for 64 h at *37°* and fingerprints were obtained as previously described.[29]

Stegemann's amide method[30] was applied to both oxidised and untreated material.

Reduction took place for I h in 8 M urea, 0·2 M in ammonium bicarbonate, by addition of 10% by vol. of 2-mercaptoethanol under nitrogen. An equal volume of acrylonitrile was added, and after standing for a further I h, the solution was acidified, dialysed and freeze-dried. Excess of cyanogen bromide[31] was allowed to react with a portion of the reduced and blocked protein in 0·0\ M-HCl for 64 h and the mixture was freeze-dried.

Mol. wt. determinations were carried out in the Oxford University Biochemistry Dept. by Dr. l. O. Walker as previously related.[27]

Sugars were identified in acid hydrolysates of protein by their positions compared with standards on paper chromatograms, developed for 24 h with ethyl acetate-pyridine-water (8:2:1 by voL) and revealed with the alkaline silver nitrate reagents of Trevelyan *el al.*[32]

Results and Discussion

Purification

Chromatography gave partial resolution of the albumin complex. By combining corresponding fractions, subjecting them to further chromatography, and rejecting the flanks of the peak, eventually small quantities of a substantially pure major component of Cappelle-Desprez albumin were obtained. At least three passages through a column were necessary to achieve the desired degree of purity. Losses of material due to partial insolubility after freeze-drying and incomplete recovery from the column necessitated the use of several grammes of starting material to prepare ~ 100 mg of the protein.

Electrophoresis

Fig. '1 shows a pattern of a Cappelle-Desprez gliadin preparation; bands in the slower group are prolamins whereas the faster group of bands consists of albumins. The albumin isolated, which was easily soluble in water, gave a single band on starch-gel electrophoresis as illustrated in Fig. 1 at acid pH, and also at alkaline pH. At lower resolution the isolated protein and that corresponding to the band marked with the arrow in Fig. I were not separated, as is seen in the photographs of an earlier paper.[8] At pH 9·1 the molecule was negatively charged. After reduction and cyanoethylation the protein still migrated as a single band suggesting that it consisted of a single polypeptide chain.

Composition and molecular weight

The amino acid composition is displayed in Table l. The protein is lacking in phenylalanine and histidine, which gives a useful test for its purity. Making the reasonable assumption that contaminating proteins will be albumins, and in both cases using the most unfavourable of three published analyses of unfractionated wheat albumins,[18-20] the maximum levels of impurity suggested by the phenylalanine and histidine figures are 5% and 1% respectively.

According to the Ellman method[25] there were 0,15 moles of SH per mole of protein, while 0·39 moles of S-carboxymethylcysteine were found after treatment with iodoacetate. These low values for SH are most probably due to alkaline

• The dangers of using iodoacetic acid are not sufficiently stressed in the literature. On no account should this substance be allowed to have even brief contact with the skin. By its widespread inactivation of enzymes it causes deep and slow-healing burns of a similar nature to those induced by mustard gas. The fact that these may not manifest themselves until some hours after contact is an additional hazard.

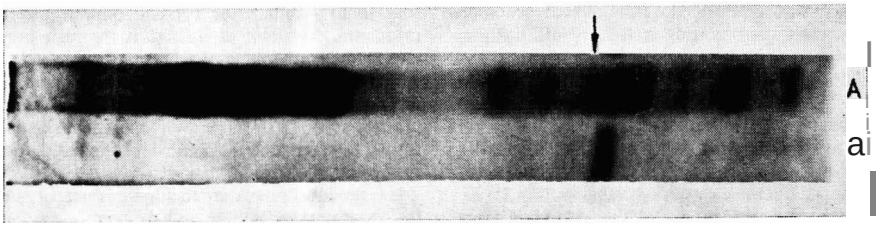

FIG. I. *Starch gel electrophoresis* ill *alumilliumlactate buffer pH* 3·3
A, Cappelle–Desprez gliadin preparation; B, purified Cappelle-Desprez albumin. Starting slot on left. Migration towards cathode.

TABLE I
Amino acid composition of Cappelle-Desprez albumin

Amino acid	Residues per molecule	
	Found	Integers
Asp	18·50	18
Thr	5·89	6
Ser	15·60	16
Glu	25·97	26
Pro	18·15	18
Gly	19·94	20
Ala	20·25	20
Val	27·32	27
CySj2	19·35	20
Met	6·21	6
lie	4·18	4
Leu	18·40	18
Tyr	8·04	8
Phe	0·36	O
Lys	12·12	12
His	0·05	O
Arg	13·65	14
Trp	7'42	8
	241·40	241

Calculated mol. wI. = 26,250

fission of S. S bonds and indicate that the protein is free from SH groups. (The last-mentioned analysis gave a value of 18 for aspartic acid; hence 18·5 was adjusted to 18 in column 3 of Table I.)

By inspection of the amino acid results the integral values were estimated by assuming 2 residues per molecule for isoleucine. The experimental results were then multiplied by the factor which converted their total (without His and Phe) to the sum of the estimated integral values putting His = Phe = 0. All amino acids had integral values within the experimental error of automatic analysers. The minimum mol. wI. was calculated to be 13,000.

Sedimentation studies gave M_z 31,000 and M_w 30,200. These values suggest that the true value is twice the minimum mol. wI. Accordingly, the experimental results were doubled and are shown in column 2 of Table I. Only one alteration of the doubled integral values was necessary; valine was changed from 28 to 27. For cystine, 20 was chosen because the number of half cystine residues must be even in a molecule free from cysteine. Tryptophan is usually underestimated by the spectrophotometric method[33] and hence the value of 7·4 was made up to 8. From the total of 241 residues, a mol. wI. of 26,250 was calculated. This was lower than that obtained on the ultra-centrifuge but the difference is probably within the error of the methods used. The value of 26,000 is of a similar order to that found for the whole albumin fraction by Pence & Elder.[5] There was some evidence from the sedimentation data that association was occurring in 0·2 M-NaCI solution and this would tend to increase the observed mol. wI.

The calculated partial specific volume[34] was 0·729.

Since Lys + Arg = 26 it would be expected that 27 peptides would be formed by tryptic digestion assuming no consecutive runs of these acids. In practice the boundaries between spots of fingerprints were not always easily discernible, but estimates from three gave totals ranging from 25-27, which is in good agreement with the analysis based on twice the minimum mol. wI. Cyanogen bromide can split polypeptide chains at methionine and may be used to find the number of such residues per molecule. Incomplete reaction could, however, diminish the reliability of the results by producing extra subsidiary bands. The albumin gave 9 bands on electrophoresis after treatment but two were very faint. The theoretical number is 7 and so this result agrees best with an analysis based on twice the minimum mol. wI. No hexosamines were detected on the amino acid analyser, nor could any sugars be found in the protein hydrolysate after paper chromatography. The methods used were sensitive enough to reveal one sugar residue per protein molecule unless destruction of carbohydrate had been very severe. It may be concluded that the preparation isolated is not a glycoprotein.

Amide determinations are not satisfactory when only small quantities of protein are available and no reliable figure could be obtained. Stegemann's method on unoxidised protein gave 22, on oxidised protein 31, whereas extrapolation of the ammonia peaks on the analyser to zero time gave only 13 moles of amide ammonia per mole. The lack of a reliable amide value prevented an estimate of total nitrogen recovery from being made. Of the nitrogen loaded 85% was accounted for by recovered amino acids.

$E^{1\%}_{1\,cm}$ at 280 nm is 19 which is in accordance with the high content of tyrosine and tryptophan. The fast electrophoretic mobility at acid pH is due to the increased levels of basic amino acids compared with the gliadins.[24] It is interesting that the ratio of ionic + polar groups to non-polar is only 0·71 and yet the protein is easily soluble in water. From earlier data[24] the ratio is 1·09 for gliadin and for glutenin, both of which have low solubilities in water. The reason may be that the highly crosslinked albumin is stabilised by a hydrophobic core and the molecular surface carries most of the polar and ionic groups.

Function

It is evident that a major component of Cappelle-Desprez wheat albumin has been isolated. The protein was devoid of proteolytic activity asjudged by a modified Kunitz[35] procedure at pH 7·6 on casein, even after addition of cysteine, and at pH 4·3 on haemoglobin. Cereal fi-amylases reveal themselves during electrophoresis by attacking the starch gel.[8] No such activity was observed with this albumin nor was any sign of a-amylase activity detected when soluble starch was used as a substrate. A small amount of acid phosphatase activity manifested itself when phenolphthalein diphosphate was used as a substrate but this was presumably due to traces of the enzyme rather than to a weak activity of the wheat albumin itself.

Some trypsin inhibitor activity was detected (Skupin, J. S., personal communication). It is unlikely that such a major component has enzymic activity. Its purpose in the endosperm is possibly analogous to that of serum albumin, namely to help to maintain the pH and osmotic pressure of the cytoplasm. A point of interest is the high proportion of even numbers of amino acids in the molecule. Even if cystine/2 and tryptophan are counted as odd because the nearest integers to the experimental values are odd, the probability of 13 or more even values in 16 is only I in 94. It may be speculated that gene doubling has played a part in the evolution of this protein.

It is probably premature to adopt permanent nomenclature for wheat proteins until more have been isolated and characterised and their relationships to one another have been established.

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TOXICITY OF RAPESEED MEAL AND ITS USE AS A PROTEIN SUPPLEMENT IN THE DIET OF TWO HYBRID STRAINS OF CAGED LAYING HENS

By N. JACKSON

An experiment is described in which extracted Algerian rapeseed meal was included at levels of 4, 8, 12, 16 and 20% in the diet of two hybrid strains of caged laying hens.

The rapeseed meal was thyrotoxic and this effect, when assessed by thyroid weight per kg of body weight, was more marked for the light-weight hybrid than for the medium-weight hybrid birds.

The light-weight hybrids exhibited a high mortality when fed rapeseed meal at a level of 8% or above in the diet but inclusion of rapeseed meal did not cause increased mortality in the medium-weight hybrids.

The medium-weight hybrids gave satisfactory egg production when fed up to 16% of dietary rapeseed meal; food conversion efficiency was best with 8% of dietary rapeseed meal, and metabolisable energy conversion (Mcal/kg eggs) and the efficiency of utilisation of protein were satisfactory at all levels of rapeseed meal inclusion. The rapeseed meal used had a standard metabolisable energy content of 1820 kcal/kg at a dry matter content of 89%.

Introduction

Brassica napus L. (rape) and *B. campestris* L. (turnip rape) produce seeds, the oil of which is used in human foodstuffs. The meal left after the oil has been expelled or solvent-extracted (rapeseed meal) is used in animal feedstuffs. The chemical compositions of rapeseed meals and their use in animal feeding have been reviewed up to 1964 in a publication of the Canada Department of Agriculture[1] and in several other publications.[2-4] The latter emphasised European studies dealing mainly with *B. napus* L. The meal left after solvent extraction contains 32–44% crude protein. Compared with soyabean meal, rapeseed meal is slightly lower in lysine content but higher in methionine. The availability of the lysine in rapeseed meal (71%) is lower than that in soyabean meal (90%).[5]

One of the principal drawbacks to the use of rapeseed meal in animal feedstuffs is the fact that it contains goitrogenic factors, believed to be derived mainly from thioglucosides, which yield isothiocyanates and oxazolidinethione on hydrolysis in the presence of the enzyme myrosinase. Oxazolidinethione is believed to be responsible for the thyroid enlargment which has been observed when rapeseed meal was fed to non-ruminant species. The toxic effect of a high level (20%) of rapeseed meal in a chick diet was observed by Pettit *et al.*[6] and the goitrogenic nature of rapeseed meal for the chick was reported by Turner.[7,8] The histology of the thyroid glands of growing chickens and laying hens fed rapeseed meal has been described by Clandinin & Bayly.[9] Clandinin[10] has reported that 10-15% rapeseed meal may be used in chick starter rations and that 10% rapeseed meal in the rations of laying and breeding chickens and turkeys will result in production, feed conversion, fertility and hatchability as satisfactory as those given by corresponding amounts of protein from soyabean. Yogt, Schubert & Stute[4] found that 17·5 and 35% rapeseed meal in broiler rations resulted in lower weight gains and feed conversion in the seventh and eighth week compared with a diet containing no rapeseed meal and that the 35% level resulted in enlargement of the thyroids to twice the normal size. They recommended that the rapeseed meal content of broiler rations should not exceed 10-12%. They have also pointed out that although a considerable amount of work has been carried out on the feeding of rapeseed meal to chickens and broilers relatively few experiments have investigated the value of feeding rapeseed meal to laying hens.

Fangauf & Haensel[11] recommended that not more than 10% of rapeseed meal should be used in the diets of layers, while Friilich[12] found that the performance of layers decreased with 5 and 10% rapeseed meal in the feed which was fed with 60 g of mixed cereals, so that the actual concentration of rapeseed meal in the diets was probably rather more than half these values. O'Neill[13] carried out three experiments in which oil-expelled rapeseed meal was used to replace various amounts of soyabean meal in laying rations which also contained some animal protein. Even when all the soyabean meal protein was replaced by rapeseed meal protein, there were no significant differences between egg production and the feed required per dozen eggs produced.

During the past few years, an interest has been shown both in the possibility of rapeseed meal production in the United Kingdom and in the use of imported rapeseed meal in animal feedstuffs. Because of the lack of available data, it was decided to initiate an experiment to assess the value for egg production of rapeseed meal available in Northern Ireland.

The experiment described in this paper was designed to investigate and compare the effects of including rapeseed meal up to a level of 20% in the diets of two modern hybrid strains of caged laying hens. It was not possible to ascertain the species of rape from which the meal was derived, but since the meal was imported from Algeria it is most likely that it was prepared from the seeds of *B. napus.*

Experimental

The experiment was started in August 1967 and continued for nine 28-day periods until April 1968. The birds were housed in individual cages at 20 weeks of age and recording was begun at 24 weeks of age.

204 birds comprising 109 Hyline (light-weight) and 95 Hybrid 4 (medium-weight) pullets were housed in individual cages equipped with individual feed troughs and communal drinkers, and were maintained on a lighting programme of 17 h of light and 7 h of darkness. The pullets of each breed were randomly divided into six treatment groups and each group was fed one of six experimental diets containing 0, 4,

8, 12, 16 or 20% solvent-extracted Algerian rapeseed meal, this replacing mainly fishmeal which was the other supplementary protein source in the diets. The first 28-day period was used as a pre-experimental period. During the remaining eight 28-day periods, individual records were kept of egg weight, egg numbers and feed intake for each hen. Egg production was recorded daily and egg weight was recorded twice a week. Body weight was recorded at the beginning of the first experimental period and at the end of each of the 8 experimental periods.

The Algerian rapeseed meal contained 36·6% crude protein, 0·76% Ca, 0'89% P, 2'78% oil and 12'1% crude fibre at a dry maller content of 89'1%. The isothiocyanate and oxazolidinethione contents of the rapeseed meal were determined using the method of Appelqvist & Josefsson.[14]

The composition, metabolisable energy *(ME)* and crude protein content of the diets are given in Table I together with the calcium and phosphorus contents. *ME* determinations were carried out for each diet by the total collection method using two hens of each hybrid strain, taken from the appropriate dietary treatment at the end of the experimental period. The *ME* of the rapeseed meal was also determined in this way. The calculated and determined contents of lysine, methionine and cystine of the diets are also presented in Table I. The amino acid contents of acid hydrolysates of the diets were determined using a Technicon Auto-Analyzer. The cystine content was corrected to allow for a 25% breakdown on hydrolysis, and the methionine content was corrected to allow for a 10% degradation at this stage of the analysis.

At the end of the experiment, five birds of each breed on each treatment were slaughtered by decapitation. Blood haemoglobin (Hb) and packed cell volumes *(PCV)* were determined. The livers were removed and weighed and the lipid content was determined by the method of Folch *et aU*[5] The total Fe content of the livers was determined by atomic absorption spectroscopy after dry ashing and dissolution in dilute HCI. The thyroid glands were removed and weighed. A histological examination was carried out on both the liver and thyroid glands.

Results

The oxazolidinethione content of the rapeseed meal used in the experiment was O'648% and the isothiocyanate content was 0·147%. The value for oxazolidinethione was not very different from the value of O'534% obtained by C1andinin[16] for a solvent-processed commercial sample of *B. 1/0pUS,* and the isothiocyanate content was lower than the value of 0.312% reported by Clandinin[16] for the same meal. The oxazolidinethione content was, however, considerably greater than the values of 0·161% and 0·193% reported by Clandinin[3] for prepress-solvent-extracted and solvent-extracted rapeseed meal respectively. Vogt *et al.*[4] cited values of **0·15–0·42%** and 0'75-1'05% for the 5-vinyl-2-oxazolidinethione contents of *B. llapus* (summer type) and *B. llapus* (winter type) respectively. Appelqvist & Josefsson1[4] reported values of 0'95% and 1·41% of oxazolidinethione in dry summer and winter rapeseed meal respectively and O'36% and 0·46% for the corresponding isothiocyanate contents. Values for oxazolidinethione obtained using their method, which was used in the present experiment also, were about 50% higher than those obtained by the method of **Wetter.**[17]

An unexpected result was the marked breed difference in the mortality of the two hybrid strains of pullets that were fed the rapeseed meal-containing diets. The mortality data were

TABLE 1

Composition of the basal diet and the rapeseed meal diets

A vitamin-mineral supplement was added to all the diets to supply 8·0)(IO'I.V. vitamin A, 2·0 x 10^6 I.V. vitamin 02, 4·0 g vitamin 82. 6·8 rng vitamin Bl2, 8·0 g vitamin E, 1·0 g vitamin K, 20·0 g nicotinic acid, 8·0 g pantothenic acid, 0·5 g folic acid, 100·0 g choline chloride, 20·0 g Fe, 3·0 g Co, 100·0 g Mo, 5·0 g I, tOO g Zn and 8,0 g Cu per 1000 kg of diet

	Treatment group					
		2		4		6
Maize meal	78·0	75·6	73·4	71·2	68·9	66·6
Soyabean meal, 45·1% crude protein	3'0	3·0	3·0	3·0	3·0	3'0
Fishmeal, 67·7% crude protein	10·0	8·0	6·0	4·0	2·0	
Rapeseed meal, 36'6% crude protein		4·0	8·0	12·0	16·0	20·0
Dried grass meal, 14'4% crude protein	2·0	2·0	2·0	2·0	2·0	2·0
Ground limestone	5·0	5·2	5·2	5·2	5·3	5·4
Steamed bone flour	1·5	1·7	1·9	2·1	2·3	2'5
Common salt	0·5	0·5	0·5	0·5	0·5	0·5
Total	100·00	100·00	100·00	100·00	100·00	100·00
Standard *ME,* kcaljkg	3000	2910	2820	2760	2710	2680
N-corrected *ME,* kcaljkg	2920	2830	2760	2690	2630	2590
Crude protein, %	15·6	15·3	15·3	15·3	15·3	15·4
Calorie: protein ratio	187	185	180	178	172	168
Calculated lysine, o %	0·73	0·72	0·71	0·70	0·69	0·68
Calculated methionine,o %	0·34	0·32	0·31	0·30	0·28	0·27
Calculated cystine,o %	0·24	0·23	0·23	0·23	0·24	0·24
Determined lysine, %	0·50	0·68	0·49	0·63	0·69	0·62
Determined methionine, %	0·20	0·32	0·31	0·30	0·33	0·28
Determined cystine, %	0·11	0·09	0·09	0·13	0·11	0·11
Calcium, %	3·03	3·02	3·36	2'88	3'06	2·92
Phosphorus, %	0·60	0·64	0·70	0·62	0·70	0·66
Dry matter, %	87·0	87·9	87·5	88·0	88·4	88·5

• The amino acid contents were calculated using the following values for the dietary constituents: maize: 0-27% lysine, 0·20% methionine and 0-19% cystine; soyabean meal: 3-08% lysine, 0-69% methionine and 0'70% cystine; fishmeal: 4'07% lysine, 1'56% methionine and 0-60% cystine; rapeseed meal: 1-94% lysine, 0-55% methionine and 0-43% cystine; dried grass meal: 1-06% lysine, 0-31 % methionine and 0·19% methionine

analysed statistically on time of death and percentage mortality, and the results are presented in Table II. There appeared to be no significant differences between the overall mean survival times of the two breeds, but there was a highly significant difference ($P < 0.001$) between the percentage of deaths in the two breeds, the Hyline strain having a significantly higher death rate than the Hybrid 4. Dietary treatment did not affect the death rate of the Hybrid 4 birds, but death rates of the Hyline pullets differed significantly with treatment, treatments I and 2 giving lower death rates than 3-6. There were no significant differences in death rate between diets I and 2 or between any of the diets 3-6.

The thyroid, liver and blood analysis data for both breeds are presented in Table III.

The blood haemoglobin *(Hb)* level was significantly affected by diet, mainly because treatment I of the Hybrid 4 birds gave a considerably higher mean value than did the remainder. The packed cell volume *(PCV)* was unaffected by treatment.

Total liver weight, percentage of dry matter in the liver and the percentage of lipids in the liver dry matter were not affected by diet, but this last figure was very high (~ 40%) with all treatments for both breeds. Values of 10-15% of lipids in liver dry matter have been obtained in other experiments in this laboratory using laying Hyline birds.

Dietary treatment had a highly significant effect on thyroid weight, the thyroid weight increasing with the amount of dietary rapeseed meal. There was no breed difference for this effect. A random sample was taken of the thyroids from a Hyline bird on each dietary treatment, and these are presented in Fig. 1. This shows the marked effect of the dietary rapeseed meal on thyroid size. The heaviest total weight of the two glands was 6·27 g for the Hybrid 4 birds and 7·81 g for the Hyline birds, these being obtained on the 20% rapeseed meal diet. The mean thyroid weight was 0·14 g for the Hybrid 4 control group and 0·20 g for the Hyline control group. When thyroid weight was expressed as kg of body weight, there was a significant difference ($P < 0.05$) between the thyroid weights of the two breeds, the mean for the Hyline birds being over 50% greater than that for the Hybrid 4 birds.

The main cause of death was haemorrhage of the liver. The livers of birds on all the dietary treatments showed fatty changes. The Fe content of the liver dry matter was significantly affected by diet, the trend being for Fe content to increase as the level of dietary rapeseed meal increased. The breed mean values showed that more Fe was present in the livers of the Hyline birds fed rapeseed meal than in the livers of the Hybrid 4 birds. The mean level of liver Fe for the control birds was identical for both breeds (127 ppm in the dry matter). Histological examination of the thyroids showed considerable enlargement of the follicles and the evidence indicated that this was due to follicular hypertrophy rather than to an increase in the number of follicles. There was no evidence of cellular infiltration into any of the glands examined. Table IV gives the weights and the number of follicles observed in the microscope field under a standard magnification for a thyroid from a bird of each breed on each

TABLE II

Mortality during the experimental period

Breed	Diet	Rapeseed meal in diet, %	Total number of birds	Number dead	Number surviving	%Dead
Hyline	1	0	18	I	17	5·56
	2	4	18	0	18	0·00
	3	8	19	5	14	26·32
	4	12	18	8	10	44'44
	5	16	18	6	12	33·33
	6	20	18	9	9	50·00
	Total		109	29	80	26·61
Hybrid 4	I	0	16	1	15	6·25
	2	4	16	1	15	6·25
	3	8	16	3	13	18·75
	4	12	15	0	15	0·00
	5	16	16	I	15	6·25
	6	20	16	1	15	6·25
	Total		95	7	88	7·36

Survival time for bird(which died

Breed	Survival time (28-day periods)						Total dead
	2		4		6		
Hyline	1	13	5	4	2	0	29
Hybrid 4	0	I	4	0	0	0	7

Chi-squared tests:
1. For correlation between breed and death, $\chi_1^2 = 12'9$ (0'1 %highly significant)
2. Hylin.e correlation between treatment and death, $\chi_5^2 = 19{\cdot}0$ (I %significant)
3. Hybrid 4 correlation between treatment and death, $\chi_5^2 = 4{\cdot}35$ (non-significant)
4. Hyhne: treatments (I and 2) and treatments (3-6) vs. death, $\chi_1^2 = 15{\cdot}6$ (0'1 %highly significant)
5. Hyhne: treatments 3–6 vs. death, $\chi_3^2 = 2{\cdot}68$ (non-significant)

TABLE III

Thyroid and liver weight and blood data for botb hybrids

Diet no.	2		4		6	Approximate s.e. of a treatment mean	Breed		Approximate s.e. of a breed mean mean
Rapeseed meal in diet, %	0	4	12	16	20		Hybrid 4	Hyline	
Hb, mg/100 ml	10·7	9·6	9·9	9·0	9'8	8·6	**0·400**	9·5	9·7	0·231
PCV, %	30	30	31	28	30	26	1·44	29	30	0'632
Thyroid weight, g	0·17	0·80	1·19	2·22	2·30	2·94	0·396	1·50	1·70	0·229
Thyroid weight, *g/kg* body weight	0·081	0·396	0·574	1·197	1·237	1·555	0·212	0·666	1·014	0·122
Total liver weight, g	44·8	42·7	38·8	45·3	41·4	47·0	3·65	48·9	37·8	**2·10**
Liver lipid content, % in dry matter	43·5	37·5	35·5	36'8	32'4	32·0	**3·64**	38·9	33·7	2·10
Liver Fe content ± s.e.m., ppm in dry matter	127 ± 12	199 ± 14	247 ± 40	227 ± 39	**305 ± 40**	287 ± 42		224 ± 18	251 ± 26	
Liver dry matter, %	36·8	34'0	32'6	33·0	32·6	32·2	1·81	35'0	32·1	1'04

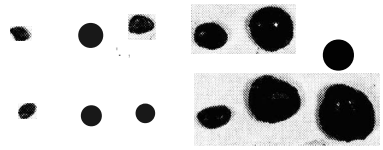

FIG. 1. *Thyroid glands from Hyline birds fed different levels of rapeseed meal ill the diet*

Half actual size
Left 10 right; 0, 4,8, 12, 16 and 20% rapeseed meal in diet

TABLE IV

Thyroid weight and number of follicles in a standard microscope field

Diet no.		2		4		6
Rapeseed meal in diet, %		0	4	12	16	20
Hybrid 4:	thyroid weight, g	0·21	O·51	1·26	1·94	**1·81**	1·40
	no. of follicles in microscopic field	26	11			
Hyline:	thyroid weight, g	0'16	1·40	0·66	2'04	4·78	3'01
	no. of follicles in microscope field	25	8	18		2	4

of the dietary treatments, taken at random, and this again illustrates the degree of enlargment of the thyroid follicles.

The amino acid analyses showed that the dietary contents of those amino acids for which requirements are known, other than lysine, methionine and cystine, were satisfactory. The percentage of lysine in the diets was adequate by the Agricultural Research Council's standard of O·5% for layer diets. The determined values were all below the calculated values. The determined methionine levels were in good agreement with the calculated values, but the determined cystine values in most cases were only about half the calculated values. In the case of the diets containing 8% or more of rapeseed meal, the calculated methionine + cystine values fell slightly short of the A.R.C.[18] recommendation of O·55%, while the determined methionine + cystine value for all the diets was well below the recommended value. The determined methionine levels, except for the control diet, were above O·25% and the determined cystine level was well below 0·18%, these being the values recommended for the dietary content of these amino acids by Combs[19] for light hybrid layers at an 80% level of production.

The results for egg production, egg weight, food intake and food conversion, expressed as bird means for the various treatments, are given in Table V. Since there was a very high mortality rate in the Hyline hybrid strain, it was possible to statistically analyse only the Hybrid 4 strain data for production and efficiency of feed utilisation. For the Hybrid 4 birds, analysis for production was carried out over all periods for each bird. The mean data for the Hyline strain are also tabulated, but discussion of the results refers to the Hybrid 4 birds unless otherwise indicated.

Daily food intake by the Hybrid 4 birds was not affected by dietary treatment. Dietary treatment did have an effect ($P < 0.05$) On egg production, the percentage productions obtained on the control, 4, 8 and 16% rapeseed meal diets being significantly greater than that obtained at the 20% level of rapeseed meal in the diet. The 12 and 20% levels of rapeseed meal in the diet gave the lowest egg production. The results for total egg weight and egg number showed basically the same patterns. Dietary treatment did not affect mean egg weight, although the mean egg weight was greater for four of the five rapeseed meal diets than for the control.

The best food conversion efficiency was obtained with diet 3, which contained 8% of rapeseed meal. Treatments I, 2 and 3 had better conversions than had treatment 6 ($P < 0.05$). There were nO other significant differences in food conversion efficiency between treatments.

TABLE V

Egg production, egg weight, food **intake** and food conversion **data**

Diet no.	Hybrid 4							Hyline					
	2		4	5				2		4			6
Rapeseed meal in diet, %	0	4	8	12	16	20		0	4	8	12	16	20
No. of observations	15	15	13	15	15	15		17	18	14	10	12	9
Mean eggs per bird	181	182	179	170	180	165	5·718	179	179	160	162	168	150
Mean egg production, %	80·80	81·25	79·91	75·89	80'80	73·7	2-553	79·91	79·91	71·43	72·32	75·00	66·96
Mean total egg weight, kg	10·17	10'26	10·50	9·69	10·09	9'26	0·348	10·32	10·60	9·20	9·29	9·60	8·54
Mean egg weight, g	56·15	56-63	57·84	57·43	56·14	56-32	0·870	57·64	59·23	57·31	56·50	57·08	56·99
Mean total food intake, kg	24'86	24·67	25·10	25·37	26·24	26·12	0'566	24·23	23·63	23-08	24·11	24·19	**22·97**
Mean daily food intake, g	111	110	112	113	117	116	2'53	108	105	103	108	108	103
Mean food conversion, kg meal/kg eggs	2·510	**2·450**	2'435	2·722	2·630	2·833	0-111	2-360	2-246	2-665	2-684	2'522	2-752

TABLE VI

Mean body weight, *ME* **intake** and *ME* conversion **data**

Diet no.	Hybrid 4						Appropriate s.e. of a mean	Hyline					
	2		4		6			2		4			6
Rapeseed meal in diet, %	0	4	12		16	20		0	4	12		16	20
Body weight, kg													
initial (all birds)	2·02	2'01	2·08	2·10	2·09	2·09	0'050	1'56	1-61	1·51	I-58	1·59	1·52
final (survivors)	2·38	2'25	2·29	2·32	2·21	2-08	0-065	1-78	1·80	1·70	1'68	1·71	1·64
Mean body weight increase of survivors, g	298	253	227	213	120	-19	51'8	208	191	163	122	115	104
Body weight increase, %	11'5	10·3	9·6	8·6	5-2	-1,0	2·281	13·2	11·9	10·6	7-9	7-2	6-8
Average body weight (survivors) over the experimental period, kg	2'20	2'14	2-16	2-21	2-15	2-06		1-70	1·73	1·66	1·68	1·68	1-63
Mean daily *ME* intake, keal	333	320	316	313	317	312	7·27	324	307	291	297	293	275
Daily crude protein intake, gl	7·32	16·83	17·14	17·29	17·90	17'86		16·85	16·06	15·76	16·52	16·52	15·86
Mean *ME* conversion, Meal/kg eggs	7·331	7·000	6·739	7-221	7-044	7·556		7·040	6·484	7-077	7'166	6-826	7·208

The mean initial and final body weight, *ME* intake, crude protein intake and *ME* conversion data are presented in Table VI together with the average body weights over the experimental period. This last figure is the arithmetic mean of nine weighings made at 28-day intervals over the experimental period.

The daily *ME* intake of the control birds was significantly higher ($P < 0'05$) than those of the other birds on treatments 2-6 inclusive. The best *ME* conversion, expressed as Meal/kg eggs, was obtained for the Hybrid 4 birds at the 8% level and for the Hyline birds at the 4% level of rapeseed meal in the diet. The *ME* conversion was slightly poorer at the higher levels of dietary rapeseed meal although it was better than the control value, except at the 20% level. The rapeseed meal was found to have a standard *ME* value of 1820 kcal/kg at a dry matter content of 89.1%.

Discussion

The Hybrid 4 birds were more tolerant of the rapeseed meal diet than were the Hyline birds. One possible reason for this is that the Hyline strain could have a greater myrosinase activity in the gut than the Hybrid 4 strain, thereby releasing more of the thyrotoxins in the gut. Myrosinase activity has been found in the gut contents of the rat[20] and a number of bacteria which are normal inhabitants of the gut have been shown to have the property of converting the precursors to the toxic compounds.[21] Another possibility is that the Hybrid 4 birds may have a more effective mechanism than the Hyline birds for detoxifying or eliminating the active substances.

Although the average daily food intake and hence the daily rapeseed meal intake, expressed as intake per kg of average body weight during the experimental period, show that the Hyline birds took in a larger amount of rapeseed meal per unit of body weight on anyone diet than did the Hybrid 4 birds on the same diet, the data presented in Table VII do not suggest that the greater intake of the potentially toxic substances per unit of body weight could alone account for the much higher toxicity of the diet towards the Hyline birds. The mean intake for the Hyline birds was over 20% greater than that for the Hybrid 4 birds.

Both strains showed considerable thyroid enlargement but it is not certain that the substances which caused this enlargement were responsible for the high mortality in the Hyline strain. There was no significant difference in the thyroid weights between breeds, but when these are considered on a body weight basis it is evident that the Hyline birds were suffering from a greater thyroid enlargement than were the Hybrid 4 birds, possibly as a result of the greater intake of rapeseed meal per unit of body weight. The weight of the thyroid glands in the Hybrid 4 birds increased 14-fold at the 20% level of dietary rapeseed meal, while this level caused a 22-fold increase in the thyroid weight of the Hyline birds. This again illustrates the more severe thyrotoxic effect in the Hyline birds.

TABLE YII
Daily food and **rapeseed** meal intakes, g/kg body weight

Breed	Rapeseed meal in the diet, %					
	0	4	8	12	16	20
Hybrid 4: food intake, g/kg body wt.	50·5	51·4	51·8	51·2	54·3	56·4
rapeseed meal intake, g/kg body wt.	0	2·06	4·14	6'14	8'69	11·28
Hyline: food intake, g/kg body wt.	**63·4**	60·8	62·0	66'8	64·4	63·3
rapeseed meal intake, g/kg body wt.	0	2·43	4·96	8·02	10·30	12·66

The degree of thyroid enlargement which has been found in this experiment is much greater than that reported by other workers. Turner[8] found that by three weeks of age, chicks fed a diet containing 40% rapeseed meal had thyroids about 8 times the weight of those in control birds, when expressed on a body weight basis. Klain *et 01.*[22] found that 11,22 and 31.5% oil-expelled rapeseed meal in the diet of chickens resulted in a thyroid weight increase of 2·2, 3·0, and 4'1 times the control, respectively. Renner *et 01.*[23] found that 5% oil-expelled rapeseed meal from *B. napus* in the diet doubled the thyroid weight on a body weight basis, while 20% oil-expelled rapeseed meal from *B. campestris* was needed for this effect. Clandinin *et 01.*[24] found that 28% rapeseed meal caused about a 4-fold increase in thyroid weight. Clandinin[25] found that thyroid weight on a body weight basis was doubled when 39% rapeseed meal was fed to chicks of up to 4 weeks of age. Yogt *et 01.*[4] found that 35% rapeseed meal in the diet caused an enlargement of thyroids to twice the normal size. **Frölich**[12] found no significant differences in thyroid weight between control laying hens and those fed 5 and 10% rapeseed meal in the meal portion of the feed for a period of 112 days.

In these experiments, with the exception of that of **Frölich,**[12] feeding was for fairly short periods and to less mature **birds,** while in the present experiment the diet was fed for 252 **days** to birds which were being subjected not only to dietary stress but also to the normal physiological stresses which accompany the egg-laying process. The histological picture of the thyroids was not identical with that obtained by Clandinin & BaylY,[9] since in the present experiment no evidence was obtained of the cellular infiltration described by these authors.

The experiment did not give any indication as to when or over what period the thyroid enlargement took place. Clandinin & Robblee[2] cite a report by New Zealand workers which showed that thyroid changes in the rat are at a maximum at the end of three weeks on diets which contain rapeseed meal, the thyroid reaching a physiological equilibrium at an increased thyroid : body weight ratio.

The liver lipids content was high in both breeds and on all treatments, and the reason for this is not obvious. It is possible, nevertheless, that the fatty livers did render the birds more susceptible to the toxic effect of the rapeseed meal, the main cause of death being haemorrhage of the liver. At autopsy, many of the birds on the rapeseed meal diets showed evidence of liver haemorrhages which had not proved fatal. The present author has no evidence that rapeseed meal causes liver haemorrhage in the fowl, although there is evidence[26] of increased liver weight in pigs fed a ration containing rapeseed meal. The data for the Fe content do not suggest any massive build-up of Fe in the liver, although they do indicate that some substance present in the rapeseed meal causes an increased Fe content of the liver. It is interesting to note that Greenhalgh *et 01.*[27] have recently shown that when kale (a member of the Brassicae, also known to contain goitrogens) was fed to cattle, it caused haemolytic anaemia and in the one *post mortem* case examined it was found that the liver had accumulated a great deal of Fe and that free Fe was associated with necrotic areas of the liver.

Those birds which survived the time of highest mortality (period 3) continued to lay fairly satisfactorily, there being a 75% production by the Hyline survivors which received 16% dietary rapeseed meal. Of the 60 birds examined at the end of the experiment, all had oviducts and ovaries in a condition that indicated normal laying activity. The mortality data (Table II) indicate that even 8% of Algerian rapeseed meal in the diet of Hyline birds may cause mortality.

The best food conversion figure for the Hybrid 4 birds was obtained on the diet containing 8% rapeseed meal, and this is similar to the result obtained at the University of Manitoba,[28] where birds on 10% rapeseed meal were more efficient in feed utilisation than were either control birds or those fed higher levels of rapeseed meal. Slinger[5] has suggested that the *ME* of rapeseed meal-containing diets is affected by the length of time the meal is fed prior to the *ME* determinations.]n the present work, the *ME* values of the six diets were determined on birds which had been on the diets throughout the experiment, so that there was no problem of acclimatisation. The *ME* of the rapeseed meal was determined by feeding the meal to birds which had previously been on the 20% level of rapeseed meal and were thus acclimatised to a relatively high level of dietary rapeseed meal.

The fact that dietary treatment had no effect on mean egg weight is contrary to observations by workers at the University of Manitoba[28] who found that diets containing 10, 12 and 14% rapeseed meal in the diet caused a significant decrease in egg size. These workers also found that egg production was significantly lower with hens fed rapeseed meal as compared to control diets, while in the present experiment, for the surviving Hybrid 4 birds, there was no difference in production between the control birds and those receiving 4, 8 and 16% rapeseed meal in the diet. For the surviving Hyline birds, the controls and those receiving the diet containing 4% rapeseed meal had exactly the same percentage production, but at the higher levels, the rapeseed meal did result in a much lower production. The Manitoba results[28] also showed that there was a sudden decrease in production following the introduction of rapeseed meal into the diet.]n the present experiment, egg production was recorded from the first egg layed up to the beginning of the pre-experimental period, as well as over the nine 28-day periods. These observations showed that the birds fed the rapeseed meal-containing diets came into lay at the same rate as the birds fed the control diet.

Although the lysine in rapeseed meal is believed to have a low availability[3] and the determined methionine + cystine levels in the diets were low, the percentages of these amino acids in the diets appear to have been adequate since the surviving Hybrid 4 birds were capable of about 80% production on several of the diets, with a minimum of 73·7%

production at the 20% level of dietary rapeseed meal. This fact and a comparison of the determined and calculated methionine and cystine values suggest that the correction of 25% applied to the cystine value may not be adequate to compensate for the cystine destroyed in the acid hydrolysis of the protein.

For the Hybrid 4 strain, the data suggest that satisfactory egg production with no mortality may be obtained with up to 16% rapeseed meal in the diet, while the Hyline survivors receiving 8% rapeseed meal in the diet showed a marked fall in egg production. The results of the experiment support the view that the initial effect of the rapeseed thyrotoxins on the thyroid gland are compensated for by thyroid enlargement, and that those birds which do survive the initial phase are then capable of normal physiological function with regard to the egg-laying process.

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DETERMINATION OF MOISTURE AND OIL IN THE SEED OF WINTER RAPE *(Brassica napus)*

I.-Comparison of oven methods for the determination of moisture

By M. HUGHES

The method for estimating moisture in winter rape seed *(Brassica /lapus)* by drying at 130° for 1 h gives results SIgnificantly higher than methods of drying at 103° and 105°. For the routine estimation of moisture in large numbers of samples a convenient method is to dry 10 g of whole seed for 16 h. This method compares favourably with results obtained on milled samples dried at 103° for 5 h or 105° for 16 h.

Introduction

Until very recently in this country, the methods of analysis of oilseeds have been of interest mainly to the oil extraction and processing industries. However in the last 2-3 years the growing of oilseed rape *(Brassica llaplls)* has aroused considerable interest, and methods of moisture and oil evaluation have become of concern to agriculture as well as to the processing industries. B.S. 4289' includes methods of analysis for moisture and oil, but a further standard procedure for the estimation of moisture is given in the rules of the International Seed Testing Association (I.S.T.A.).2 Temperatures of 103° and 130° are recommended by the respective bodies; whilst 103° might be considered satisfactory for drying oil seeds, the higher temperature of 130° might lead to loss of volatile oil constituents. Use of 103° requires that the drying time be experimentally determined after an initial period of 3 h, but 130° specifically requires a drying period of I h. Neither method requires that the seed be ground, but in many routine procedures for analysing oil, it is customary to subsample the ground seed using one part for oil and another for moisture evaluation. These moisture levels are usually low, but in the study of harvesting and drying techniques, high moisture contents are dealt with; for moist seed, grinding is difficult and is often associated with loss of moisture.

It was therefore decided to compare the oven methods of drying at 103° and 130° to include ground and unground seed. The object of the experiments was to indicate the values which might be obtained by each of the methods and to conclude which method was most suited to the routine analysis of large numbers of samples over a wide range of moisture contents. A distillation method and also field moisture meters were compared in a preliminary investigation by Hughes.[3]

Previous work on cereal moisture determination has helped in the planning of these experiments and many of the important aspects of technique; as with cereals, interaction of varietal and other differences can be factors which influence results[4,5] The consideration of methods not requiring milling of the seed have been discussed by Matthews[6] and by Warner & Browne.[7] There is a further attendant possibility that in seeds where the dry matter is composed of approximately 40% oil, any loss of the oil fraction to the mill, or loss of volatile products, can influence the result for moisture content.

Experimental

Experiment I

In Experiment I samples of freshly harvested seed of winter rape were roughly cleaned, and dried to give a range of moisture levels from 6 to 20% on a wet basis. The three methods investigated were as follows: (a) 130° oven for I h (I.S.T.A. for brassicas), (b) 103° oven for $3 + xh$ (B.S.4289), and (c) 105° oven for 16 h (I.S.T.A. for seeds with volatile oils).

From the bulk sample of ground seed, 3 replicates of approximately 5 g each, were weighed to the nearest mg into aluminium tins (3 in dia. x ½ in high). Two Townson & Mercer ovens Type E 522 with a temperature fluctuation of approximately O'5° were used for the experiments. In order to minimise the effects of shelf height and position on shelf, the order of positioning was random. For method (b) the samples were dried for 3 h at 103° then taken out, cooled in a desiccator and weighed. They were then replaced in the oven and heated for a further I h, cooled and re-weighed. The drying was repeated on an hourly basis until the attainment of constant weight (within 5 mg variation). It was found that after 5 h drying at 103° the difference in weight between the final and previous weight was less than 5 mg.

Experiment II

In Experiment II, the same bulk samples of seed, over the range 6-20% moisture, were used to determine whether any significant difference could be shown between samples of milled and unmilled seed. Any difference in moisture between these two conditions of seed would be reflected most in the method which required the shortest drying time demonstrating incomplete drying of unmilled seed. On this assumption, the 130° method was further examined.

Three 5 g samples of milled and unmilled seed were weighed into aluminium tins and loaded into random places on shelves in an oven preheated to 130°. After being dried for I h, the samples were cooled in a desiccator and weighed. The calculated moisture content values were expressed on a wet basis.

Experiment III

The relationship of moisture content of milled and unmilled seed was further examined only at temperatures of 103° and 105°, for appropriate times. Milled and unmilled

samples of seed of 5 g weight were tested at drying times of 5 hand 16 h and an additional 16 h drying treatment was added using a 109 sample of unmilled seed.

Results

Experiment I

The mean values at each moisture range are given in Table I. Analysis of variance of these results shows that the methods are significantly different at the 1% level and this difference is attributed to the higher results obtained by drying at 130°. There is no significant difference in moisture content between the B.S. method of 103° for 5 hand 105° for 16 h. Fig. 1 shows the deviations of each method from the mean at each moisture level of all the methods.

Experiment II

The mean results of three replicates at each moisture level are shown in Table II. An analysis of variance of these results shows that differences between milled and unmilled samples were not statistically significant at the 5% level. A difference does occur at the 10% level and in order to establish whether this is of any importance the experiment would have to be repeated to include more samples and increased numbers of replicates in each sample.

Experiment III

The mean results by each oven method are given in Table III. An analysis of variance of these results shows that there is a significant difference between the five methods examined and this difference is attributable to the method of drying whole seed at 103' for 5 h. The results obtained by this method, which is a B.S. method, are significantly lower than

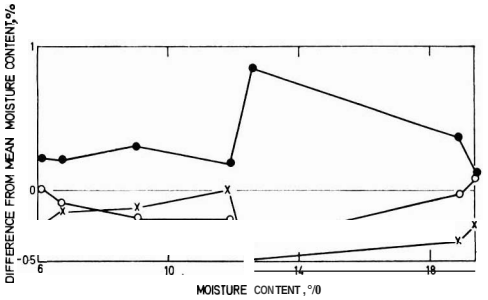

FIG. 1. *Deviations of the methods from the mean at all moisture levels for milled seed*

• 130'c (I h); x IOI'c (5 h); 0 105'c (16 h)

TABLE I

Moisture content' of seed for three oven methods

Sample	Moisture content, %		
	130'c (1 h)	I03'c (5 h)	105'c (16 h)
1	6·91	6·53	6·59
2	6·39	5·90	6'17
3	9'33	8·89	8·81
4	12·05	11·86	11·65
5	13·37	12·04	12'16
6	19·23	18'49	18·81
7	19·53	19·15	19·49
$\bar{x}$	12·40	11·84	12'00

•All moisture contents are presented on a wet basis
S.E. of the mean = 0·0502
L.S.D. at 5% for sample 2 = 0·154 and for sample 3 = 0·189

TABLE II

Moisture content of milled and unmilled seed dried at 130'c for I h

Sample	Moisture content, %	
	Milled	Unmilled
1	6·93	7·09
2	6·31	6'44
3	9·41	9·72
4	12·19	12·30
5	12·30	12·32
6	19·17	19'09
7	20·05	20·16
$\bar{x}$	12·83	12'90

TABLE III

Moisture content of mined and unmilled seed for two oven methods

Sample	Moisture content, %				
	Milled seed		Unmilled seed		
	5g	5g	5g	5g	109
	103°c (5 h)	105'c (16 h)	103'c (5 h)	105'c (16h)	105'c (16h)
1	4·69	4·76	4·42	4·67	4·70
2	5·80	5·70	5·43	5·69	5·67
3	6·47	6·48	6·34	6·50	6·52
4	7·24	7'31	7·17	7·31	7·29
5	8·40	8·42	8·39	8·45	8·54
6	9·39	9·40	9·26	9·43	9·42
7	12·05	12·12	12·08	12·24	12·19
$\bar{x}$	7·72	7·74	7'58	7·76	7'76

S.E. of the mean = 0·0228
L. S. D. at 5% for sample 2 = 0·067 and for sample 5 = 0·095

all the other methods, at the 0·1% level. Therefore for whole seed, the drying time of 5 h is too short a period. There is no significant difference between any of the other methods examined, which include the B.S. method in which a milled sample of seed is dried at 103° for 5 h.

Any method which does not require the seed to be milled has many advantages for the routine determination of large numbers of samples and if such a method is acceptable then an increased weight of sample for analysis will help in reducing sampling error. For this reason the 10 g sample, drying for 16 h was included and in Table IV the standard deviations of the sampling error of each of the methods are compared, indicating that the sampling error of the method using a 10 g sample is the smallest.

Fig. 2 shows the deviations of each method from the mean at each moisture level of all the methods. Figs 3 and 4 show the results of moisture content determined by drying 109 samples for 16 h at 105° plotted against the results obtained by the B.S. method using milled and unmilled seed respectively.

TABLE IV

Comparison of standard deviations of sampling error

Method of moisture determination	Standard deviation, σ
Milled seed:	
5 g at **103°c** (5 h)	0·047
5 g at 105°c (16 h)	0·046
Unmilled seed:	
5 g at 103°c (5 h)	0·055
5 g at **105°c** (16 h)	0'061
10 g at 105°c (16 h)	0·025

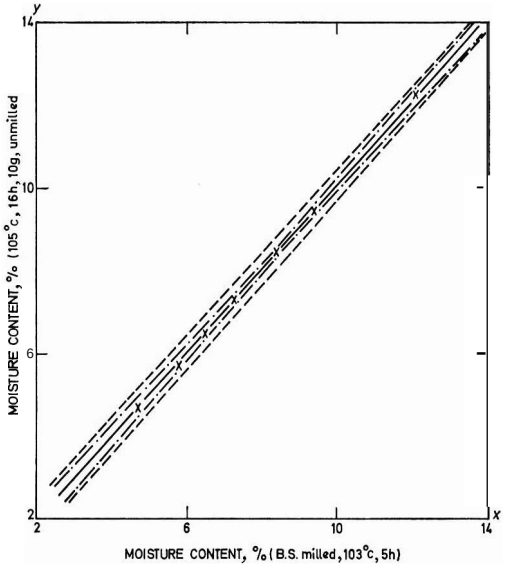

FIG. 3. *Comparison of the B.S. method (milled) and drying at 105°c for* 16 *h (10 g, unmilled)*

$y = 1{\cdot}0242x - 0{\cdot}4380$

95% confidence limits: – . – . $\bar{y}$; – – – $\hat{y}$

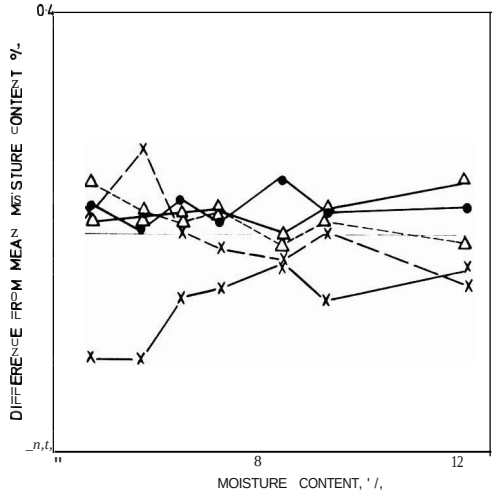

FIG. 2. *Deviations of the methods from the mean at 01/ moiSlllre levels for milled* (- - -) *and unmilled* (– – –) *seed*

x 10loe (5 h); Δ 105°e (16 h); • 105°e (t6 h. 10 g)

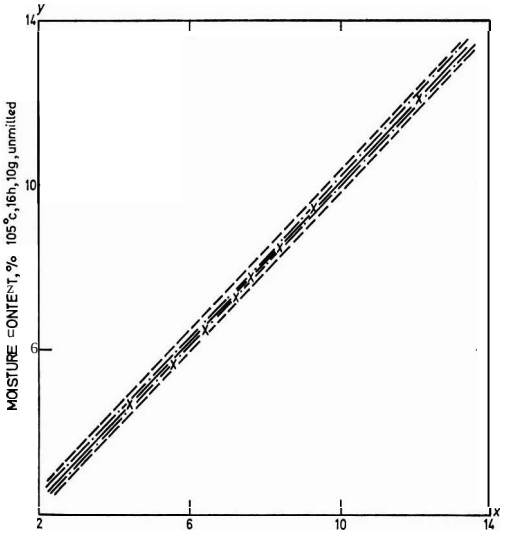

FIG. 4. *Comparison of the B.S. method (unmilled) and drying at 105°c for* 16 *h (10 g, unmilled)*

$y = 0{\cdot}98049x + 0{\cdot}9766$

95% confidence limits: – . – . $\bar{y}$; – – –;

The 95% confidence limits for predicting a value for each method are also included. The statistical analysis of the three experiments has shown that there is a highly significant variation in the sampling error of the groups of three repeat samples taken from each individual sample and this tends to be greatest at the high moisture levels. However, in practice, it would be extremely difficult to reduce the sampling error at high moisture content and it is not practical to carry out more than three repeat determinations -per sample. Thus for routine work a difference of 0·2% moisture content is suggested as an arbitrary limit outside which further repeat determinations should be made. The standard deviations of each method examined in Experiment III supports the acceptance of a 0·2% difference and compares favourably with the deviations quoted by Matthews.[6] Nevertheless, the presence of these variations in sampling error will modify the statistical conclusions reached in all the analysis of variance of the data which have been discussed.

Conclusions

The method which seed analysts would most probably use for the estimation of moisture in rape seed would be the recommended method of the I.S.T.A., viz. 130° for I h. However, the oil processor would restrict his method to that of B.S.4289, requiring the seed to be dried at 103° for a predetermined number of hours. The results of these experiments indicate that determinations made by using 130° give a higher moisture content than comparable analyses made by the B.S. method, by about O'5% at a sample moisture of 10%. These higher moisture values suggest that a fraction of volatile oil is being lost and it would be better to use a reduced temperature. The 103° method of the B.S. compares favourably with the second category method of the I.S.T.A., namely drying at 105° for 16 h, but this relationship is only maintained when the sample for the B.S. method is a milled sample. Because moisture content and oil content determinations are usually made simultaneously in the oil processing industry, a method using milled seed would be convenient so that from a bulk of milled seed, subsamples could be taken for oil and moisture evaluation. The B.S. of drying milled seed for 5 hat 103° is not significantly different from any of the 16 h drying methods at 105° and would therefore be the suggested method. For the estimation of moisture only, in large numbers of samples, the most convenient method is to dry the sample for 16 h at 105° using a 10 g sample of unmilled seed to reduce sampling error. Results obtained by this method would be comparable to values obtained for samples subsequently analysed for oil conten.! where moisture would most probably be obtained by drying a milled sample of 5 g for 5 h.

Acknowledgments

The author wishes to acknowledge the help of Miss J. Duncan who carried out most of the analyses, Mr. D. Higgs for the statistical results and Mr. M. E. Nellist for his interest in requesting the studies to be made.

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DETERMINATION OF MOISTURE AND OIL IN THE SEED OF WINTER RAPE *(Brassica napus)*

II.*-Comparison of extraction methods for the estimation of oil

By M. HUGHES

The rate of oil extraction from moist and dry rape seed was compared over an extraction time of 20 h and showed oil to be more readily extractable from oven-dried seed. Three methods of determining the oil content by extraction of single samples were compared with a method in which a number of samples contained in single satchets were extracted in one large extractor. No significant differences were shown between any of the methods used, and it is proposed where sample numbers are large, and oil content is the only analysis required, that the multiple extraction method of oven-dried seed is satisfactorily quick and accurate.

Introduction

The generally accepted methods for determining the oil content of rape seed are based on extraction of the oil with petroleum solvent and weighing the oil after evaporation of the solvent. These procedures involve lengthy extraction (minimum 8 h), and attempts have been made to adopt more rapid methods of estimation[1-4] (also Frederiksen, P. S., personal communication). Whilst many of these methods are adaptable for the rapid analysis of large numbers of samples, the object of the following experiments was to compare the results obtained by extraction using simple laboratory glassware and to show the effects of modifying any of these methods. Experiments I and II were designed to examine the extraction rate of oil from wet and dry seed. The conclusions of these experiments provided the basis for a comparison, in Experiment III, of four methods of analysis so that results obtained by one extraction process could be equated to any of the other methods, by the use of correction factors.

The basic extraction method common to all, has been specified previously in detail.' This B.S. specifies that a sample of ground or unground seed weighing approximately 10 g is extracted with n-hexane or light petroleum, distilling between 40 and 60°, for a minimum period of 8 h, made up' initially of 4 h then at successive 2 h intervals, until all the oil is extracted. The sample of oil is freed from solvent, dried for a period of at least 1 h and finally weighed. The oil content is expressed as a percentage of the wet sample, but when the seed has a moisture content above 10%, the sample contained in the extraction thimble is dried to below this level, in an oven at a temperature not higher than 80°. The difference between two determinations carried out simultaneously should not exceed 0·4 g oil/100 g sample.

A similar method, developed in Canada, is used by the Grain Research Laboratory.6 However, the sample for extraction is differently prepared, as ground seed is always dried in a vacuum oven at 100° for a minimum of 6 h. A comparison of this method with the B.S. one is of special interest because imported Canadian rape seed is the chief competitor of home-grown seed and is of graded and defined quality.

A third method (Ashley-Jones, J., personal communication) similar to the B.S. and the Canadian ones but requiring extraction tubes and not Soxhlet apparatus, is used by a large oil processing manufacturer in this country.

A fourth method of extraction which can be termed multiple extraction has been suggested by van Roon (personal communication). In this method, a large number of samples are extracted in a single extractor. The outline of the three experiments can be stated as follows: (I) a comparison of 5 h and 8 h extraction on the oil content of wet and dry seed; (II) to establish the rate of oil extraction at intervals from 4½ to 20 h of wet and dry seed; and (III) a comparison of extraction methods for wet and dry seed.

Experimental

A bulk sample of seed *(Brassica napus)* dried to approximately 6% moisture on an experimental low-temperature drier and subsequently stored in a sealed container at 10° was used for Experiments I and II. Seed from the following year (1968) was used for Experiment III, but these samples were harvested and dried over a wide range of conditions, so giving a wide range of residual moisture levels at which individual samples were sealed and stored at 10°. Extraction of the oil from the seed samples weighed into 22 x 80 mm Soxhlet thimbles was made using a 6 unit Electrothermal heater. Residual solvent removal and final drying of the oil were carried out using a forced-draught oven operating at 100°.

Experiment I

Samples of wet seed milled at its stored moisture content of 6% and dry seed milled after having been dried at 100° for 16 h, followed by cooling in a desiccator, were extracted for 5 hand 8 h. The degree of milling was defined as meal containing no whole or partly broken seed. The moisture content of the wet samples was determined by drying duplicate 5 g samples of seed at 105° for 16 h.' Samples (each 10g) were quickly transferred to Soxhlet thimbles and the oil was extracted into weighed flasks using petroleum ether (b.p. 40-60°) as solvent. After a period of 4 h extraction, the samples were allowed to cool, the seed was reground in a pestle and mortar to which 1 g of dry sand was added, and the extraction was continued for a further 1 h. Excess petroleum ether was distilled off by substituting distillation units and the flasks containing oil and residual solvent were dried at 100° for 1 h. After being cooled, the flasks were reweighed and the oil content was evaluated, the results being expressed on a dry weight basis. The extraction time was continued for a further 3 h for samples being analysed by the longer extraction method, to a total time of 8 h.

• Part I: Preceding paper

Experiment II

In this experiment the oil content of a sample of wet milled seed and a sample of dry milled seed was determined by extraction for a total time of 20 h. Duplicate analyses were also made at 4½, 5½, 6½, 8, 12 and 16 h. The preparation of samples by milling and extraction of the oil was as for Experiment I.

Experiment III

In this experiment, four modified methods for the determination of oil content were compared to include B.S. 4289. The methods were as follows: (a) B.S. 4289; (b) method used by a commercial oil processor using wet milled seed; (c) method used by a commercial oil processor using dry milled seed; and (d) a multiple extraction method using dry, milled seed. Petroleum ether (b.p. 40-60°) was used as solvent for all the methods.

In methods (b) and (c) the Soxhlet extractor was replaced by an extraction tube in which the sample of seed, contained in two thicknesses of filter paper, was supported by a constriction in the tube. The flasks for the collection of oil were approximately 150 ml capacity and pear-shaped to allow the oil and solvent to flow easily down the side of the vessel. The flasks were heated on 'black-heat' laboratory thermal extraction units, so designed that the flasks were held well recessed and kept warm throughout the extraction process. The routine method used by the processor was to grind, by a mechanical Whitney pestle and mortar, sufficient seed for duplicate determination of oil and moisture content. This process is common also to the B.S. method. An addition to the processor's method was to include a series of samples which had been previously milled and dried at 100° for 16 h, prior to extraction. By these three single extraction methods, approximately 3-5 g samples were extracted for 4 h with petroleum ether. After this time they were removed from the extractors, allowed to dry on a warm hot-plate and then ground by hand in a pestle and mortar, using approximately 1 g of dry silver sand, until a fine powder was obtained. The fine powder was returned to the filter paper or filter thimble and extracted for a further 2 h. Using distillation units, the major fraction of the solvent was distilled off on a water bath and finally the flasks were dried in a ventilated thermostatically controlled oven at 100° for 1 h. The oil content was expressed as a percentage of the dry matter in the sample.

For the rapid analysis of large numbers of samples the multiple extraction method has many attractions but has the limiting factor that it is not possible to carry out further analyses of a single oil sample for fatty acids, etc. The samples of approximately 5 g of dried milled seed were weighed into satchets made from laboratory-grade filter papers (18 em dia.) which were then stapled to prevent any loss of fine powder. Two 600 ml Soxhlet extractors (Quickfit & Quartz EX 5/75) with 3 1 flasks were used to extract 20 samples at one filling. Petroleum ether was used as solvent and the samples were extracted for 16 h after which time the satchets were removed, allowed to drain and dry on a warm hot-plate and finally dried in an oven at 100° for I h. The satchets were then opened, and the dry contents of milled seed were weighed in a tared vessel. To obtain the weight of oil, the final weight of seed was subtracted from the initial weight of seed sample, the oil evaluation being expressed as a percentage of the dry matter of the seed. Because by this method of analysis, the value for oil was determined by subtraction of sample before and after extraction, the varying moisture content of a sample could influence the results and therefore it was decided to use only the dried milled fraction of samples as used in one of the single extraction methods.

Results

Experiment I

Ten samples of wet and dried seed from a bulk sample of nominally 6% moisture were analysed by extraction for 5 and 8 h and the mean results are shown in Table I. Because no fixed period of time is given in any of the methods for the final evaporation of solvent, where possible some of the samples after the initial I h drying period were further dried overnight at 100°. The results for 16 h drying of the oil are given in parentheses in Table I. Statistical analysis of the mean values for each of the treatments shows the dry seed oil content to be significantly higher than the wet seed at both times. The difference between the 5 hand 8 h results on dry seed was not significant at the 5% level, but for wet seed the results obtained after 5 h extraction were significantly lower than for 8 h extraction. Further drying of the oil for 16 h at 100° did not markedly alter any of the results obtained.

Experiment II

The oil content at intervals of extraction from 4½ h to 20 h on samples of wet seed at 6·6% moisture content and comparable samples dried overnight prior to analysis, are given in Table II. These values were obtained after final drying of oil at 100° for I h.

It was clear that oil was more rapidly extracted from the dry seed than from the wet seed during the first 5 h extraction. For a sample of seed weighing 109, an extraction time between 8 and 12 h was necessary for wet seed, but with dry seed good reproducibility was obtained even when extraction time was reduced to 6 h. Drying the final oil sample for 16 h

TABLE I

Percentage oil in sample dry matter

Values in parentheses are for collected oil dried for 16 h

5 h extraction		8 h extraction	
Wet seed	Dry seed	Wet seed	Dry seed
34·5	40·6	38·4 (38'7)	41·5 (41'8)

TABLE II

Percentage oil in sample dry matter

Results in parentheses are for further drying of the oil at 100°c for 16 h

Time of extraction, h	Wet seed at 6·6% moisture content	Dry seed
4½	34·8 (32'9)	38'9(37'1)
5½	37·9 (35'4)	40·8 (38'7)
6½	38·4 (36'3)	41·3 (41'6)
8	39·7 (39'6)	40·7 (40'9)
12	40·9 (40'8)	40·6 (40'9)
16	40·3 (41'4)	42·3 (42'4)
20	44'1(44,1)	42·4 (42'4)

at 100° resulted in little alteration to the final oil content evaluation. When the extraction time was continued for 16 and 20 h, the extracted oil appeared contaminated with a fine white powder which was possibly degraded products of the seed. This fraction would account for the increased values obtained at these extraction times.

Experiment III

In this experiment, 20 samples of seed were extracted by four different methods. Each sample was analysed in dulpicate, the weight of seed for each extraction being between 3-5 g milled. Table 11\ gives the mean results of oil content as a percentage of the sample dry matter together with the moisture content of the seed at the time of milling, for each method. Analyses of variance showed that there was no significant difference (S.D.) between the four methods. Differences between samples has no relevance to this experiment, but it was shown that the within sample variation is smallest (S.D. = 0'3341) for the multiple extraction method and largest (S.D. = 0,4406) for the B.S. method. It should be pointed out that these standard deviations were obtained by methods of analysis designed to be acceptable as routine for the handling of large numbers of samples. To have applied the repeatability limit a difference of 0'4 g oil/100 g sample as in the B.S., would have necessitated repeating many of the analyses.

TABLE III

Percentage oil in sample dry matter

Sample	B.S.4289	Commercial method		M It' I u 'ip I e extraction	Moisture content, %
		Wet	Dry		
1-20	43·5	**43·4**	43·4	43·3	7·7

S.E. of the mean = 0,11814
(F value = 0·77 n.s.)

Conclusions

Whilst B.S. 4289 (Part Ill) defines the method by which the oil content of seeds can be determined, alternative methods are more suited to routine analysis of large numbers of samples. Field experiments designed to investigate factors of harvesting, drying and storage of seed require large numbers of samples to be analysed in order to give significant differences in the treatment effects. Furthermore the effects of prolonged storage of samples prior to analysis can sometimes invalidate field effects and therefore methods by which large numbers of samples can be analysed rapidly are worth consideration. It is for these reasons that a multiple extraction method has been compared with the more generally accepted methods, and the results show that good reproducibility can be obtained with a much increased rate of sample analysis. The preparation of the sample and the rate of oil extraction can be increased by prior drying of the whole seed at 100° for 16 h, without any significant loss of oil.

Reproducibility of results can also be affected by the procedure of grinding. A sample of seed with a residual moisture content greater than 9%, grinds very poorly in laboratory hand grinders. If high-speed electric laboratory grinders are used, grinding is easier but great care is necessary to avoid deposition of the oil in a separate phase on the upper parts of the mill chamber. The ideal sample for solvent extraction is obtained with a mechanical pestle and mortar. The multiple extraction method is well suited to the routine analysis of large numbers of samples requiring oil content only and compares favourably with results obtained 'by general methods of single sample extraction. Final drying for up to 16 h at 100° appears to have little effect on the final oil content.

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The author wishes to acknowledge the help of Miss J. Duncan who carried out most of the analyses, Mr. D. Higgs for the statistical results and Mr. M. E. Nellist for his interest in requesting the studies to be made.

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CHEMOTHERAPY OF FASCIOLIASIS
II.*-4-Cyano-2-iodo-6-nitrophenol (nitroxynil) and related compounds

By M. DAVIS, J. ROSENBAUM and D. E. WRIGHT

A series of halogenated nitro- and cyano-phenols has been synthesised for structure-activity studies against the liver fluke, *Fasciola "epatica*. The functional groups of the most effective compound, 4-cyano-2-iodo-6-nitrophenol (nitroxynil), have been modified in several ways.

Introduction

In view of the fasciolicidal activity found with certain phenyl ethers,1 and with 2,2'-methylene-bis-(3,4,6-trichlorophenol) (hexachlorophene),[2] other phenols were examined against the trematode, *Fasciola hepatica.* Some effect against mature flukes shown by the anthelmintic 2,6-di-iodo-4-nitropheno13 (disophenol) (1)1 led the authors to synthesise more than 100 substituted phenols, and their derivatives, many of which are now reported. Activity was found only among halogenated nitrophenols, of which 4-cyano-2-iodo-6-nitrophenol (nitroxynil) (lId) was outstanding in its properties.[4]

Derivatives of the biologically active phenols studied included esters, ethers and acetals (Tables 1lI and IV).

Modification, separately or together, of the functional groups OH, N02 and CN of nitroxynil was undertaken both for an investigation of structure-activity relationships and to provide reference compounds for metabolism studies. The sequence of reactions is indicated by compounds XII-XXV.

Most of the salts of nitroxynil (lId) with inorganic or organic bases were sparingly soluble in water, but with N-methyl-, N-ethyl-, N-propyl-, or N-butyl-glucamine, salts were obtained which had a water solubility of over 45% wt./vol. A sparingly soluble dimorphic form of the *N*-methylglucamine salt has already been recorded.[5]

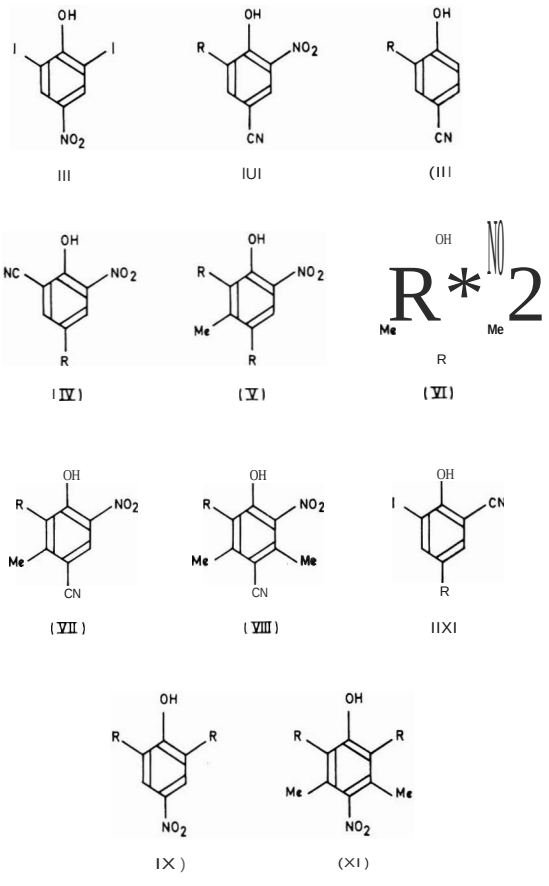
Experimental

Preparation of *o*- and p-nitrophenols

For the o-nitrophenols listed in Table I, either halogenation or nitration provided the final stage. 4-Cyano-2-iodo-6-nitrophenol (lId) was most conveniently obtained by mono-nitration of p-cyanophenol, and subsequent iodination of 4-cyano-2-nitrophenol *(110)*. The sequence was reversed for its chloro- (lIb) and bromo-analogues (lIe), obtained by nitration of 2-chloro- (IIIb) and 2-bromo-4-cyanophenol (IIIe), respectively, and was also preferred for the preparation of the nitroxynil isomer (IV*d*) from 2-cyano-6-nitrophenol *(IVa)*. Derivatives in which the 3- or 3,5-positions were substituted by methyl groups were made similarly. Thus 5-methyl- *(Va)* and 3,5-dimethYI-2-nitrophenol *(VIa)* were halogenated to (V*d*) and (VId) respectively, while the 4-cyano-analogues *(VIIa)* and *(VIIIa)* underwent iodination to (Vlld) and (VIIId) respectively. The orientation of the nitrophenol *(VIIa)*, obtained by nitration of 4-cyano-3-methylphenol, was confirmed by hydrolysis to the 4-carboxylic acid, which was identical with an authentic specimen prepared from 2-methyl-4,5-dinitrobenzoic acid.

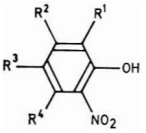
Similar methods provided the p-nitrophenols listed in Table II, including a further nitroxynil isomer (IX*e*), in which the cyano- and nitro-groups were interchanged, and the cyano-dinitrophenol (IVe).6 3-Methyl- *(Xa)* and 3,5-dimethyl-4-nitrophenol (XI*a*) afforded the di-iodo homologues *(Xd)* and (XI*d*) of disophenol (I).

The compounds in Tables I and II were prepared by the following general methods.



• Part I: *J. Sci. Fd Agrie.,* 1969, 20, 690

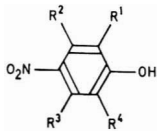
TABLE I
o-Nitrophenols



RI	R'	R3	R4	Method	Yield, %	Cryst. from	M.p., 'e	Formula	Found, %				Required, %			
									C	H	N	Hal	C	H	N	Hal
Cl	H	CN	H	Cb.•	96	Aq. EtOH	152-154	C,H3ClN,03			14·3	18·1			14·1	17·9
Br	H	CN	H	C·,,	68	EtOH	**166–169**	C,H3BrN,03			11·4	33·1			II·5	32·9
I	H	CN	H	A··r	93	Benzene	137- 138	**$C_7H_3IN_2O_3$**	28·9	1·0	9·5	43·7	29'0	1·0	9·7	43·8
CN	H	I	H	A	79	EtOH	127- 132	C,H31N,03			9·6	43·8			9·7	43·8
I	Me	I	H	Ag	44	Pet.' (60-80')	104-105	**$C_7H_5I_2NO_3$**	21·0	1·3	3·6	62·2	20·8	1·2	3·5	62·7
Br	Me	Br	Me	B0	72	Pet. **(80–100°)**	**140–142**	C,H,Br,NO,	30·0	2·2	4·4	49·3	29·6	2·2	4·3	49·2
I	Me	CN	H	AI	34	EtOH	147- 149	C,H,IN,O,			9·3	42·0			9·2	41·75
I	Me	CN	Me	AI	56	Benzene- Pet. (60-80')	164-165	C,H,IN,O,			8·7	40·0			8·8	39·9

• Pet. = light petroleum (b.p. in parentheses). b From 2-chloro-4-cyanophenol.' ' First prepared by Mr. W. G. Leeds. d From 2-bromo-4-cyanophenol.[10] · First prepared by Mr. D. R. Broad. I From 4-cyano-2-nitrophenol." g From 5-methyl-2-nitrophenol.' b From 3,5-dimethyl-2-nitrophenol.[12] J From 4-cyano-5-methyl-2-nitrophenol (see Experimental). J From 4-cyano-3,5-dimethyl-2-nitrophenol."

TABLE II
p-Nitrophenols



R'	R'	R3	R4	Method	Yield, %	Cryst. from	M.p., 'e	Formula	Found, %				Required, %			
									C	H	N	Hal	C	H	N	Hal
Br	Me	H	Br	B	64	Pet.' (60-80')	135- 140	C,H, Br,NO,	27·4	1·6		51·5	27·0	1·6		51·4
I	Me	H	I	Ab	50	Pet. **(40–60°)**	111- 112	**$C_7H_5I_2NO_3$**			3·1	62·5			3·5	62·7
I	Me	Me	I	A'	73	Pet. **(80–100°)**	**190–191**	C,H,hNO,	23·0	1·7	3·2	60·7	22·9	1·7	3·3	60·6
I	H	H	CN	Ad	71	EtOH	182- 185	C,H,IN,O,			9·7	43·6			9·7	3·8
NO,	H	H	CN	C	45	EtOH	**180–185°**	**$C_7H_3N_3O_5$**	40·3	1·6	20·3		40·2	1·4520·1		

• Pet. = light petroleum (b.p. in parentheses)
b From 3-methyl-4-nitrophenol'
e From 3,5-dimethyl-4-nitrophenol[12]
• From 2-cyano-4-nitrophenol'
• Reference,' m.p. 175- 176'

2-Cyano-4-iodo-6-nitrophenol (IVd) *(Method A)*

A filtered solution of 2-cyano-6-nitrophenol' (52 g), sodium hydroxide (12'5 g), potassium iodide (36'7 g) and potassium iodate (22'5 g) in water (315 ml) was added slowly to a stirred solution of ethanol (240 ml), sulphuric acid (25 ml) and water (50 ml). The temperature was maintained at 50' for 2 h, and at room temperature overnight. A small amount of sodium bisulphite was added to decolorise the solution, and the crystalline product was collected, washed with water and dried. Recrystallisation from ethanol afforded the iodophenol (79%), m.p. **127–132°.**

2,6-Dibromo-3-methyl-4-nitrophenol (Xc) *(Method B)*

A solution of bromine (6'1 ml) in glacial acetic acid (15'3 ml) was added slowly with stirring to 3-methyl-4-nitrophenol[8] (7 g) dissolved in acetic acid (25 ml). The reaction mixture was heated on a steam-bath for I h, treated with a small amount of sodium metabisulphite, and cooled. The solid product was filtered off, washed with water, dried and crystallised twice from light petroleum (b.p. 60-80') to give the dibromophenol (64%), m.p. 135- 140'.

2-Cyano-4,6-dinitrophenol (IV*e) (Method* C)

A solution of nitric acid (5 ml) in acetic acid (8 ml) was added to a solution of 2-cyano-6-nitrophenol' (10 g) in acetic acid (40 ml) and the mixture was heated on a steam-bath for I h. The solution was poured onto ice-water and the pale yellow product was filtered off, washed with water and crystallised from ethanol to yield the dinitrocyanophenol (45%), m.p. **180–185°.** (Reference,' m.p. 175- 176'.)

Preparation of esters, ethers and acetals of nitrophenols

Most of the esters were made from the isolated potassium salt of the phenol and the acid halide, with or without a solvent. For the ethers and acetals, the potassium salt was usually treated with the appropriate alkyl or substituted alkyl halide. The methyl ether of nitroxynil is described in the next section.

The compounds in Tables III and IV were prepared by the following general methods.

2,6-Di-iodo-6-nitrophenylehloroaeetate (Method D)

Chloroacetyl chloride (30 ml) was added cautiously with cooling to potassium-2,6-di-iodo-4-nitrophenoxide (15 g). The mixture was then heated on a steam-bath for I h, poured into water, and the precipitate was collected, washed and crystallised from aqueous acetone, to give the ester (15,3 g), m.p. 117_119°.

In some preparations (method E) acetone, 2-ethoxyethanol, toluene or sulpholane was used as a solvent.

1,l-Di-(2,6-di-iodo-4-nitrophenoxy)methane (Method F)

A solution of 2,6-di-iodo-4-nitrophenol potassium salt (50 g) in sulpholane (100 ml), dimethylformamide (10 ml) and dibromomethane (4 ml) was heated under reflux for 16 h at 130°. The solution was poured into dilute alkali, and extracted with chloroform, and the washed and dried extract was evaporated. Recrystallisation of the residue afforded the acetal (6'5 g, 7%), m.p. 197- 199°.

2,6-Di-iodo-4-nitrophenoxyaeetyl-2,6-di-iodo-4-nitrobenzoate

A mixture of the potassium salt of2,6-di-iodo-4-nitrophenol (30 g), monochloroacetic acid (14 g) and 2-ethoxyethanol (150 ml) was heated under reflux for 3 h, and added to water. The oil which separated was extracted into benzene, and the dried benzene solution was treated with thionyl chloride (25 ml), heated under reflux for 3 h and evaporated to dryness. The residue was dissolved in acetone (100 ml) and the potassium salt of 2,6-di-iodo-4-nitrophenol (30 g) was added to the solution. The mixture was heated under reflux for 3 hand added to ice, and the solid which separated was recrystallised from aqueous methanol, giving the ester, m.p. 144-148°.

4-Cyano-2-iodo-6-nitrophenyl dr/oroaeetate

This was prepared by method D. The product was crystallised from aqueous acetone and then from benzene to give the chloroacetate (44%), m.p. 149-152°. (Found: I, 34'9%. CgH4CIIN204 requires I, 34,6%.)

4-Cyano-2-iodo-6-nitrophenyl benzoate

The sodium salt of 4-cyano-2-iodo-6-nitrophenol (12'5 g) and tetramethylammonium chloride (2 g) were dissolved in benzoyl chloride (50 ml) and the mixture was heated on a steam-bath overnight, then poured into water. The oily product was extracted with ether (4 x 25 ml) and the washed and dried extracts evaporated to dryness. The residue was crystallised twice from benzene- light petroleum (b.p. 80-100°) to give the benzoate (49%), m.p. 174-175°. (Found: I, 33'3%; N, 7'0%. C14H71N204requires I, 33'6%; N, 7'4%.)

Similarly prepared (66%) was the acetate, m.p. 139- 141° (from benzene). (Found: 1,38'5%; N, 8·0%. CgH5IN204 requires I, 38·2%; N, 8,4%.)

2,6- Di-iodo-3-methyl-4-nitrophenyl propionate

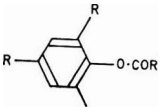
A solution of 2,6-di-iodo-3-methyl-4-nitrophenol (11'1 g) in light petroleum (b.p. 60-80°; 25 ml) and dry pyridine (10 ml) was treated slowly with propionyl chloride (3 ml) and the mixture was left at room temperature for 48 h, then poured into water and acidified with hydrochloric acid. The organic layer was separated, washed successively with water, dilute acid and water, dried and evaporated. The residue was crystallised successively from ethanol- light petroleum (b.p. 60-80°), and from acetone-water to give the propionate (6 g, 47·5%), m.p. 103- 105°. (Found: C, 26·5%; H, 1'8%; I, 55'3%. $C_{10}H_9I_2NO_4$ requires C, 26·1%; H, 2'0%; I, 55'1%.)

Preparation of other compounds related to nitroxynil

Catalytic hydrogenation of nitroxynil (lId) afforded the amine (XIV; R = R' = H), which was converted into the N,N-dimethyl derivative (XIV; R = R' = Me) by Eschweiler-Clarke methylation or by degradation of the methiodide. Acetylation gave the acetate (XV; R = Ac) which was partly hydrolysed to the acetamidophenol (XV; R = H).

TABLE III

Esters of **2,4-dihalogeno-6-nitrophenols**

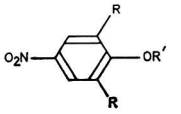


R	R'	Method[a]	Yield, %	M.p., °c	Formula	Found, %				Required, %			
						C	H	Cl		C	H	Cl	
I	CH_2Cl	Db	60	109-111	$C_8H_4ClI_2NO_4$	20·6	0·9		54·5	20·55	0·9		54·4
I	CCl_3	Db	80	109- 110	$C_8H_2Cl_3I_2NO_4$	18·0	0·7	19·8	47·4	17·9	0·4	19·8	47·3
I	$CCl_2{\cdot}CH_3$	Db	91	83-85	$C_9H_5Cl_2I_2NO_4$	20·9	1·0	\3·5	49·3	20·95	),0	13·7	49·2
Br	CH_2Cl	Dc	62	114-117	$C_8H_4Br_2ClNO_4$			9·7	42·6[d]			9·5	42'8[d]

[a] All esters were crystallised from aqueous acetone
[b] From 2,4-di-iodo-6-nitrophenol14
[c] From 2,4-dibromo-6-nitrophenol15
[d] Bromine analysis

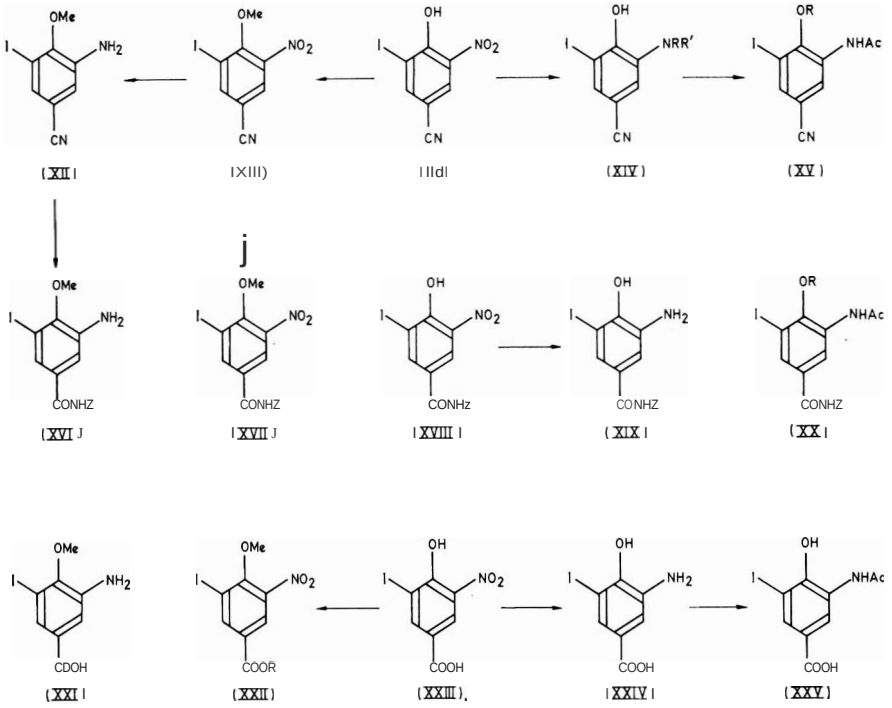
TABLE IV

Derivatives of 2,6-dihalogeno-4-nitrophenols



R	R′	Method	Yield, %	Cryst. from	M.p., °C	Formula	Found, %					Required, %				
							C	H	Cl	Br	I	C	H	Cl	Br	I
Cl	$COCH_2Cl$	D[b]	79	Aq. $COMe_2$	117–119	$C_8H_4Cl_3NO_4$			37·1					37·4		
Br	$COCH_2Cl$	D[c]	66	Aq. $COMe_2$	100–102	$C_8H_4Br_2ClNO_4$	25·8	1·0	9·8	42·4		25·7	1·1	9·5	42·8	
Br	$COCHCl_2$	D[c]	46	Aq. $COMe_2$	115–117	$C_8H_3Br_2Cl_2NO_4$	23·8	0·8	17·5	39·6		23·6	0·7	17·4	39·2	
Br	$COCCl_3$	D[c]	32	Aq. $COMe_2$	107–110	$C_8H_2Br_2Cl_3NO_4$	21·9	0·3	23·9	35·9		21·7	0·45	24·0	36·1	
I	$COCH_2Cl$	D	94	Aq. $COMe_2$	124–125	$C_8H_4ClI_2NO_4$	20·6	0·8	8·3		54·6	20·55	0·9	7·6		54·3
I	$COCHCl_2$	D	70	Aq. $COMe_2$	144–145	$C_8H_3Cl_2I_2NO_4$	19·5	0·7	14·2		50·2	19·2	0·6	14·1		50·6
I	$COCCl_3$	E	50	MeOH	141–143	$C_8H_2Cl_3I_2NO_4$					47·0					47·3
I	$COCCl_2 \cdot CH_3$	E	61	MeOH	147–149	$C_9H_5Cl_2I_2NO_4$					49·6					49·2
I	2,4-dichlorophenoxyacetyl	E	38	COMe–Pet.[a] (b.p. 40–60°)	143–146	$C_{14}H_7Cl_2I_2NO_5$					43·1					42·8
I	4-chloro-2-methylphenoxyacetyl	D	64	COMe–Pet. (b.p. 40–60°)	147–150	$C_{15}H_{10}ClI_2NO_5$					43·9					44·3
I	2,6-di-iodo-4-nitrophenoxyacetyl	E[d]	40	Aq. MeOH	144–148	$C_{14}H_6I_4N_2O_7$					61·7					61·8
I	6-chloronicotinoyl	E	73	MeOH	187–190	$C_{12}H_5ClI_2N_2O_4$[e]					47·5					47·9
I	3-(5-nitrofur-2-yl)-acryloyl	E[f]	82	Aq. $COMe_2$	218–221	$C_{13}H_6I_2N_2O_7$[g]					46·0					45·7
Br	$CH_2 \cdot OMe$	F	66	Pet.	77–79	$C_8H_7Br_2NO_4$[h]				47·5					46·9	
Br	$CH_2 \cdot OC_8H_{17}$-n	F	66	Pet.	41–42	$C_{15}H_{21}Br_2NO_4$[i]				37·3					36·4	
I	$CH_2 \cdot OMe$	F[j]	33	Aq. $COMe_2$	96–99	$C_8H_7I_2NO_4$[k]	22·5	1·6			59·6	22·1	1·6			58·4
I	$CH_2 \cdot OC_8H_{17}$-n	F	57	Pet.	66–68	$C_{15}H_{21}I_2NO_4$					47·8					47·7
I	$CH_2 \cdot O[CH_2]_2 \cdot Cl$	F	62	Pet.	77·5–78·5	$C_9H_8ClI_2NO_4$	22·6	1·7	7·0		52·8	22·4	1·7	7·3		52·7
I	2,4-dichlorophenoxymethyl	F	57	MeOH	107–109	$C_{13}H_7Cl_2I_2NO_4$			12·5		44·9			12·6		44·9
I	*p*-chlorobenzyl	F	98	EtOH	139–140	$C_{13}H_8ClI_2NO_3$[l]	30·5	1·6			49·0	30·3	1·6			49·3
Br	$\cdot CH_2 \cdot$	F[m]	9	$COMe_2$	174–176	$C_{13}H_6Br_4N_2O_6$[n]	25·9	1·1		52·7		25·8	1·0		52·8	
I	$(CH_2)_5$	F	15	Aq. $COMe_2$	174·5–175	$C_{17}H_{14}I_4N_2O_6$[o]	24·4	1·8			60·2	24·0	1·7			59·7

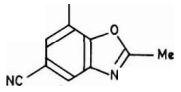
[a] Pet. = light petroleum (b.p. 60–80° except where stated). [b] From 2,6-dichloro-4-nitrophenol.[16] [c] From 2,6-dibromo-4-nitrophenol.[17] [d] From 2,6-di-iodo-4-nitrophenoxyacetic acid (see Experimental). [e] Found: N, 5·3%. Required: N, 5·3%. [f] From 3-(5-nitrofur-2-yl)acrylic acid.[18] [g] Found: N, 4·9%. Required: N, 5·0%. [h] Found: N, 4·3%. Required: N, 4·1%. [i] Found: N, 3·1%. Required: N, 3·2%. [j] First prepared by Dr. R. F. Collins. [k] Found: N, 3·1%. Required: N, 3·2%. [l] Found: N, 2·65%. Required: N, 2·7%. [m] In 2-ethoxyethanol. [n] Found: N, 4·4%. Required: N, 4·6%. [o] Found: N, 3·1%. Required: N, 3·3%.



Alkaline hydrolysis of nitroxynil yielded the acid (XXIII) which was catalytically hydrogenated to the amino acid (XXIV) and then acetylated to the amide (XXV). Hydration of the nitrile (lid) in sulphuric acid produced the nitrobenzamide (XVIII) which was likewise reduced to the aminobenzamide (XIX), acetylated to XX (R = Ac) and hydrolysed to the free phenol (XX; R = H).

Methylation of nitroxynil with diazomethane gave the methyl ether (XIII) which was successively hydrogenated to the amine (XII), and hydrolysed to the amide (XVI) (with sulphuric acid) or to the acid (XXI) (with sodium hydroxide). Partial hydrolysis of the nitrile (XIII) to the amide (XVII) was effected with manganese dioxide in methylene dichloride. For the corresponding acid (XXII; R = H), the hydroxybenzoic acid (XXIII) was methylated with diazomethane and the methyl ester (XXII; R = Me) was hydrolysed with sodium bicarbonate.

Condensation of the amine (XIV; R = R' = H) with acetic anhydride produced the benzoxazole (XXVI).



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2-Cyano-4,6-di-iodophenol (IXd)

Prepared (55%) from o-hydroxybenzonitrile by method A, this compound had m.p. **176–177°** (from aq. ethanol). (Found: N, 3·4%; I, 67'9%. $C_7H_3I_2NO$ requires N, 2'8%; 1,68'4%.)

4-Cyano-5-methyl-2-nitrophenol (VIIa)

A mixture of nitric acid (2'8 ml, sp. gr. I '42) and acetic acid (4 ml) was slowly added to a stirred suspension of 4-cyano-3-methylphenol'9 (4'2 g) in acetic acid (12 ml) maintained at 10-12°. The mixture was heated to 50-52° for I h and added to distilled water (160 ml) whereupon the product separated as yellow prisms, m.p. 138-145°. Crystallisation from aqueous methanol gave the phenol, m.p. 149·5-151°. (Found: C, 54'2%; H, 3'5%; N, 15'8%. CsHsN203 requiresC, 53'9%; H; 3'4%; N, 15'7%.) From the mother-liquors, 4-cyano-5-methyl-2,6-dinitrophenol was isolated as yellow prisms (0'1 g), m.p. 190-192° from ethyl acetate-light petroleum. (Found: N, 18'8%. CsH5N305 requires N, 18'8%.)

2-Amino-4-cyano-6-iodophenol (XIV; R = R' = H)

A mixture of 4-cyano-2-iodo-6-nitrophenol (500 g), ethanol (5 litres) and 5% platinum--charcoal (20 g) was hydrogenated at 34° and 70 Ib/in.^{2} The filtered solution was immediately added to a mixture of ice and water (20 litres), when the amine separated as pale fawn needles (401 g, 90%), m.p. 134'5-135°, unchanged by recrystallisation from aqueous ethanol (Found: 1,48'6%; N, 10'6%. C7H51N20 requires I, 48'8%; N, 10'8%.)

2-Acetamido-4-cyano-6-iodophenyl acetate (XV; R = Ac)

2-Amino-4-cyano-6-iodophenol (8 g) was dissolved in a mixture of acetic acid (5 ml) and acetic anhydride (25 ml) and the solution was heated on a steam-bath for 2 h. The product, which crystallised slowly from the reaction mixture, was collected by filtration and washed thoroughly with

water. Recrystallisation from ethanol yielded the acetate (67%), m.p. **206–207°**. (Found: I, 37·2%. ClIH.IN203 requires I, 36'9%.)

2-Acetamido-4-cyaIl0-6-iodophellol (XV; R = H)

2-Acetamido-4-cyano-6-iodophenyl acetate (4 g) was dissolved in dilute sodium hydroxide (2 N; 10 ml) and water (5 ml), the solution was heated on a steam-bath for ½ h, and filtered, and the filtrate was acidified with concentrated hydrochloric acid. The solid product was collected and crystallised twice from ethanol, affording the acetamidophenol (73%), m.p. 199-201°. (Found: I, 42·1%. CgH71N202 requires I, 42·0%.)

5-Cyano-7-iodo-2-methylbcllzoxazolc

A mixture of 2-amino-4-cyano-6-iodophenol (20 g) and acetic anhydride (100 ml) was heated under reflux for 16 h. The benzoxazole (14'6 g, 67%) separated from the cooled solution as pale yellow prisms, m.p. 234-235°, unchanged on recrystallisation from benzene. (Found: I, 44·6%; N, 9'9%. CgHsIN20 requires 1,44'7%; N, 9'9%.)

5-Cyano-2-hydroxy-3-iodo-N,*N-dimethylallilille mcthiodidc*

A mixture of 2-amino-4-cyano-6-iodophenol (41,6 g), anhydrous sodium carbonate (16,96 g), methyl iodide (40 ml) and methanol (240 ml) was heated under reflux for 10 h. The solution was cooled and the solid (9'23 g) was filtered off. Addition of ether to the filtrate gave a further quantity (15'77 g). Crystallisation of the combined products from methanol gave pale yellow prisms of the methiodide (14'7 g, 21%), m.p. 154-155°. (Found: I, 58'8%; N, 6'3%. ClOH12TzN20 requires I, 59·0%; N, 6'5%.)

5-CyaIl0-2-hydroxy-3-iodo-N,N-dimethylallililc (XIV; R = R' = H)

A mixture of 5-cyano-2-hydroxy-3-iodo-N,N-dimethylaniline methiodide (4'3 g), 2 N aqueous sodium hydroxide (25 ml) and water (200 ml) was heated at 100° for 5 min. The cooled solution was saturated with carbon dioxide and the product (1,97 g, 68'5%), m.p. 136-138° was filtered off. Recrystallisation from cyclohexane gave the amine, m.p. 139-140°. (Found: I, 44'0%; N, 9·6%. CgHgIN20 requires I, 44·1%; N, 9'7%.)

This compound was also obtained (33%) from the Eschweiler-C1arke methylation of 2-amino-4-cyano-6-iodophenol.

4-Hydroxy-3-iodo-5-IIitrobcllzoic acid (XXIIT)

A solution of 4-cyano-2-iodo-6-nitrophenol (10 g) and sodium hydroxide (40 g) in water (300 ml) was heated on a steam-bath overnight. The solution was cooled and acidified with hydrochloric acid and the product was collected. Crystallisation from acetone afforded the acid (9 g, 84%), m.p. 255-256°. (Found: 1,40'9%; N, 4'5%. C7H41NOs requires T, 41·1%; N, 4'5%.)

4-Hydroxy-2-methyl-5-IIitrobellzoic acid

This was similarly prepared (50%) from 4-cyano-5-methyl-2-nitrophenol. It was obtained as pale yellow needles (from water), m.p. 216-218° identical (mixed m.p., i.r. spectra) with an authentic specimen prepared from 4,5-dinitro-2-methylbenzoic acid by the method of Goldstein & Tardent.*zo*

4-Hydroxy-3-iodo-5-IIitrobellzamide (XVIII)

A mixture of 4-cyano-2-iodo-6-nitrophenol (10 g) and sulphuric acid (50 ml) was kept at room temperature overnight, then added to ice, when the amide separated as a yellow solid (10'25 g, 97%), m.p. 231-233° (decomp.) unchanged on crystallisation from 2-ethoxyethanol. (Found: 1,41'2%; N, 8'9%. C7HsINz04 requires 1,41'2%; N, 9'1%.)

3-AmiIl0-4-hydroxy-5-iodobellzamide (XIX)

A mixture of 4-hydroxy-3-iodo-4-nitrobenzamide (10 g), acetic acid (200 ml) and platinum oxide (I g) was shaken with hydrogen at room temperature and atmospheric pressure until the theoretical uptake had been reached (17 h). The mixture was filtered from the catalyst and the filtrate treated with hydrochloric acid (4 ml) when the amine hydrochloride separated as a pale yellow crystalline solid (6'8 g, 66%), m.p. 213-215° (decomp.). Crystallisation from methanol-ether gave the hydrochloride in pale yellow prisms, m.p. 215-215'5° (decomp.). (Found: I, 40·1%; N, 8'8%. C7H7IN20 z, HCI requires T, 40'4%; N, 8'9%.)

3-AmiIl0-4-hydroxy-5-iodobenzoic acid hydrochloride (XXIV)

This was similarly prepared (78%) from 4-hydroxy-3-iodo-5-nitrobenzoic acid. It was obtained as colourless crystals (from ethanol-ether), decomposing from 280°, after darkening from 190°. This compound has been previously prepared[21] in lower yield by reduction of the nitro-compound using stannous chloride-hydrochloric acid.

3-Acetamido-4-acetoxy-5-iodobellzamide (XX; R = Ac)

3-Amino-4-hydroxy-5-iodobenzamide hydrochloride (3'09 g) and acetic anhydride (2,04 ml) were added to a stirred and cooled mixture of dilute potassium hydroxide (10 ml, 2N) and crushed ice (10 ml). The mixture was stirred for 10 min and the solid (2,9 g, m.p. 217-220°) which separated was filtered off, washed with water and dried. Recrystallisation from ethanol gave colourless prisms of the amide, m.p. 233-234°. (Found: C, 36'7%; H, 3'2%; T, 35·3%. ClIHlITN204 requires C, 36·5%; H, 3'1%; T, 35·1%.)

3-Acetamido-4-hydroxy-5-iodobcnzamide (XX; R = H)

A mixture of 3-acetamido-4-acetoxy-5-iodobenzamide (I g), dilute sodium hydroxide ([·6 ml, 2N) and water (10 ml) was stirred and heated at 100° for 5 min. The mixture (pH 7'0) was cooled and filtered from unchanged material (0'3 g), and the filtrate was treated with excess of hydrochloric acid when the product (0'45 g) separated as colourless prisms. Crystallisation from ethanol gave the phenol, m.p. 217-219°. (Found: 1, 39'6%; N, 8·6%. CgH9INz03 requires I, 39'65%; N, 8'8%.)

3-Acetamido-4-hydroxy-5-iodobenzoic acid (XXV)

3-Amino-4-hydroxy-5-iodobenzoic acid hydrochloride (8 g) and acetic anhydride (5'6 ml) were added to a stirred and cooled mixture of dilute potassium hydroxide (28 ml, 2N) and crushed ice (28 ml). The mixture was stirred for 10 min and the solid (8 g), m.p. 242-244° (decomp.), which separated was filtered, washed with water and dried. Two recrystallisations from methanol gave colourless needles of the acetamidobenzoic acid, m.p. 236'5-237° (decomp.). (Found: 1, 39·2%; N, 4'2%. Calc. for C9HsIN04: I, 39'5%; N, 4'4%.) This compound, m.p. 234-235°, has been previously prepared[22] by reaction of 3-acetamido-5-acetoxymercuri-4-hydroxybenzoic acid with iodine-potassium iodide.

4-Cyano-2-iodo-6-nitroanisole (XIII)

A cooled ethereal solution of diazomethane (2'8 g) was slowly added to a stirred and cooled suspension of 4-cyano-2-iodo-6-nitropnenol (2'9 g) in methanol (30 ml). The mixture was kept at 20° for 16 h and evaporated to dryness *in vacuo* at 20°. The residue was triturated at 60° with a solution of N-methylglucamine (2 g) in water (50 ml) and the colourless insoluble solid (2'94 g), m.p. 100-101°, was crystallised from carbon tetrachloride to give colourless needles of the ether, m.p. 106-106'5°. (Found: I, 42'0%; N, 9'1%; OMe, 10·4%. CsHsIN203 requires I, 41'8%; N, 9'2%; OMe, 10,2%.)

Methyl 3-iodo-4-methoxy-5-nitrobenzoate (XXII; R = Me)

This was similarly prepared in quantitative yield from 3-iodo-4-hydroxy-5-nitrobenzoic acid. It was obtained as colourless needles (from cyclohexane), m.p. 134·5-135°. (Found: I, 37·7%; N, 4·1%. C9HsiNOs requires 1,37'7%; N,4·2%.)

3-lodo-4-methoxy-5-nitrobenzamide (XVII)

A mixture of manganese dioxide (15 g), 4-cyano-2-iodo-6-nitroanisole (2 g) and methylene dichloride (30 ml) was stirred for 70 h at room temperature and filtered. The filtrate was .evaporated to dryness and the residue extracted with boiling carbon tetrachloride (30 ml) when the amide was obtained as a colourless insoluble solid (0'2 g, 9%), m.p. 184-185°). Crystallisation from ethyl acetate-light petroleum gave colourless matted needles, m.p. 186-187°. (Found: I, 39·8%; N, 8'6%. CsH7IN204 requires 1,39'4%; N, 8'7%.)

3-Iodo-4-methoxy-5-nitrobenzoic acid (XXII; R = H)

A mixture of methyl-3-iodo-4-methoxy-5-nitrobenzoate (I g), sodium bicarbonate (0'25 g, anhydrous), methanol (20 ml) and water (0'2 ml) was boiled for 2·5 h and kept at room temperature for 3 days. The mixture was filtered from unchanged ester (0·11 g) and the filtrate was diluted with water (40 ml) when a further quantity (0·15 g) of ester was obtained. The filtrate was treated with an excess of hydrochloric acid and the product (0,55 g), m.p. 184-189° was crystallised from benzene, giving the acid, m.p. 186-187°. (Found: I, 39·1%; N, 4'4%. CSH61NOs requires I, 39·3%; N,4·3%.)

2-Amino-4-cyano-6-iodoanisole (XII)

A mixture of 4-cyano-2-iodo-6-nitroanisole (3,04 g), methanol (50 ml) and platinum oxide (0'1 g) was shaken with hydrogen at room temperature and atmospheric pressure until the theoretical uptake had been reached (3 h). The mixture was filtered from catalyst, the filtrate was concentrated, and the solid recrystallised from methanol, giving the amine, m.p. 154-155°. (Found: I, 46'6%; N, 10'5%. CsH7IN20 requires I, 46'3%; N, 10,2%.)

3-Amino-5-iodo-4-methoxybenzamide (XVI)

2-Amino-4-cyano-6-iodo-anisole (0'42 g) was added, with stirring and cooling, to sulphuric acid (4'2 ml). The mixture was kept at room temperature for 17 h and added to ice. The pale yellow solution was treated with an excess of anhydrous sodium carbonate and the solid (0'4 g), m.p. 166-169° was crystallised from ethyl acetate-light petroleum to give the amide, m.p. 168-169°. (Found: I, 43'7%; N, 9'3%. CsH9IN202 requires I, 43'5%; N, 9'6%.)

3-Amino-5-iodo-4-methoxybenzoic acid (XXI)

A mixture of 2-amino-4-cyano-6-iodoanisole (0'3 g) and dilute sodium hydroxide (10 ml, 2N) was stirred and heated at 100° for 18 h. The orange solution was treated with an excess of acetic acid and the product (0'23 g) was crystallised from aqueous methanol to give the acid, m.p. 205-206°. (Found: I, 43·5%; N, 4'6%. CsHsIN03 requires 1,43'3%; N, 4·8%.)

Biological Results and Discussion

Screening methods have been summarised in Part I.[1] The results and structure-activity relationships for analogues and derivatives of disophenol and nitroxynil have been discussed elsewhere.[23] Briefly, nitroxynil (lid) was found to be the only compound which exhibited high activity against both mature and immature *F. hepatica*. Its chloro- (lib) and bromo-analogues (lie), and its structural isomer (IXe) had good activity against the adult fluke, but were not effective against the young parasite.

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EFFECT OF HEAT PASTEURISATION ON SOME EGG WHITE ENZYMES

By ANN E. HENDERSON and D. S. ROBINSON

It has been shown by a potassium permanganate titration method that solutions of egg white decompose hydrogen peroxide. Using an oxygen electrode the 1st-order rate constants for the decomposition of hydrogen peroxide (during the first minute of the reaction) by different samples of newly laid and laboratory heat-treated egg white, have been calculated. An Arrhenius plot of calculated denaturation constants has shown that the activation enthalpy, free energy and entropy changes required for the heat inactivation of the 'catalase-like property' were 39'8 kcal mole-', 22·6 kcal mole-' and 51 entropy units, respectively.

The effect of heat on the lysozyme, the a-mannosidase and the N-acetyl-il-o-glucosaminidase enzymes of egg white has also been studied and it has been shown that the activity of N-acetyl-il-o-glucosaminidase enzyme is reduced by heat at 53 to 57°. The activation enthalpy, free energy and entropy changes required for the heat inactivation of N-acetyl-il-o-glucosaminidase were 62·9 kcal mole-I, 21·8 kcal mole-[1] and 124·5 entropy units, respectively.

The results are discussed with particular reference to the occurrence, disputed by some workers, of a catalase enzyme in egg white, and to a possible application of the effects of heat on the egg white enzymes as a method for measuring the effectiveness of heat pasteurisation processes.

Introduction

Corry & Barnes' and Cotterill[2] have determined the heat resistance of *Salmonella typhimllrillm* S3698/60 and *Salmonella senftenberg* 775W in egg white at various pH values, **In** egg white at pH 9'2, which had been held at 57·8° for 2½ min, Corry & Barnes[1] showed a 10^{18} reduction in the number of S, *typhimllrillm* and a 10' reduction in the number of S. *senftenberg*. Furthermore, it was also shown[1,2] that nothing is gained by lowering the pH value of egg white to 6·6-7·0 units so that higher pasteurisation temperatures can be used, as described by Cunningham & Lineweaver,[3] because the apparent advantage is reduced by the greater heat resistance of *Salmonella* at pH 7·Q As a result of the work of Corry & Barnes,[1] liquid egg white, as well as liquid whole egg (Ministry of Health[4]), is now being generally heat-pasteurised in Great Britain, although no simple chemical test is available for checking the efficiency of the heating process.

Unfortunately, the a-amylase test which is used for measuring the adequacy of the heat pasteurisation of liquid whole egg cannot be used to measure the efficiency of pasteurisation of liquid egg white because there is very little of this enzyme in egg white and the activity of a-amylase is unaffected at the lower temperatures required for pasteurisation of egg white.[5] It was therefore considered important to study the effect of heat in the temperature range **50–60°** on the activity of some of the other enzymes present in egg white.[5,7] Egg white lysozyme, the 'catalase-like' activity, a-D-mannosidase and N-acetyl-p-D-glucosaminidase have been investigated.

Experimental

Materials

All chemicals used were of Analar grade. p-Nitrophenyl-a-D-mannopyranoside was obtained from Koch-Light Laboratories. p-Nitrophenol (spectrophotometer grade) and p-nitrophenyl-2-acetamido-2-deoxy-p-D-glucopyranoside (Grade III) were obtained from the Sigma Chemical Company, Glass distilled water was used. Bacto-Iysozyme substrates, Bacto-Iysozyme and Bacto-Iysozyme buffer were purchased from Difco Laboratories, Detroit, Michigan, U.S.A,

All the shell eggs used in the investigation were bought locally from various producers and records were kept of the age of the egg white.

Bulk samples of raw and commercially pasteurised egg white were supplied by the British Egg Marketing Board and were stored at - 20° until required.

Preparation of sterile egg white

Egg white was separated from the yolk in a Perspex glove box which was sterilised with methanol before use. Before being placed in the glove box the shells of the eggs were also carefully wiped with methanol. All instruments and glass-ware were autoclaved at 120° for 15 min. Apparatus which could not be autoclaved was wiped with methanol before use.

The mixture of thick and thin egg white was placed in an M.S.E. Ato-Mix and homogenised at half-speed for not more than I min, It was then placed in sterile bottles and stored at - 20° until required. The prepared samples of egg white were tested for sterility before use.

Pasteurisation

Following the method of Shrimpton *et al.*[8] for laboratory pasteurisation of liquid whole egg, a batch method was used. The heating tube (12 ml), which was wound into a double rectangle, was made of stainless-steel tubing with an internal diameter of 2·5 mm, wall thickness of 0·5 mm and length of 240 cm. The ends were closed with a steel screw cap and rubber gasket and the samples of egg white were heated in a thermostated water bath maintained to ± O·!° of the required temperature. At the end of the selected heating time the tube was quickly cooled by being placed in a bucket of cold water. All of the pasteurised egg white was removed from the tube by blown-through compressed air and stored at - 20° until required for the enzymic assays. The tube was cleaned with a wire pull-through and cold running water. The interior of the tube was then rinsed with a mixture of alcohol and ether (I : ! by voL) and dried at 100° for 15 min.

Potassium permanganate titration method, for measurement of the destruction of hydrogen peroxide by egg white

The assay procedure used was similar to that of Maehly & Chance,[9] based on the methods of Bonnischen *et al.*[10]

Homogenised egg white (5 ml) was pipetted into a conical flask containing 0·05 M potassium phosphate buffer (5 ml) at pH 7·0 and the contents were stirred slowly at room temperature (20°) for a few minutes using a magnetic stirrer. To give a final concentration of 2·5 mg/ml, 2·3 vol. hydrogen peroxide (5 ml) was quickly added. Stirring was continued at room temperature throughout the experiment. Aliquot parts (I ml) of the reaction mixtures were transferred about 4 and 40 min at room temperature to 10 ml volumetric flasks containing 15% trichloroacetic acid (3 ml) and diluted to 10 ml with distilled water. Long reaction times were selected for convenience as the rate of destruction of hydrogen peroxide was relatively slow under the experimental conditions used. The flasks were shaken gently and the precipitated proteins removed by centrifugation for 10 min in an M.S.E. bench centrifuge at 6000 rev/min. The supernatant was transferred to 25 ml volumetric flasks, 2% sulphuric acid (2 ml) was added and the whole diluted to 25 ml with distilled water. The diluted superantants were titrated with a solution of potassium permanganate, standardised against arsenious oxide using ferroin (I, IO-phenanthroline ferrous complex solution) as an indicator[11] and the amount of hydrogen peroxide destroyed by the egg white samples was calculated.

Oxygen electrode method for measurement of oxygen produced during the destruction of hydrogen peroxide by egg white

The method used was similar to that described by R0rth & Jensen[12] for the determination of catalatic activity using a Clark[13],[14] oxygen electrode (Yellow Springs Instrument Co. Ohio, U.S.A.). The circuit diagram for polarising the electrode and coupling to a 10 mY Rikadenki recorder (B24) is shown in Fig. I. The pOlemial difference across the resistances RI-R6, which was proportional to the current passing at the electrode, was measured continuously at the recorder. The electrode was operated at a polarising voltage of -0,8 Y, as recommended by the manufacturers.

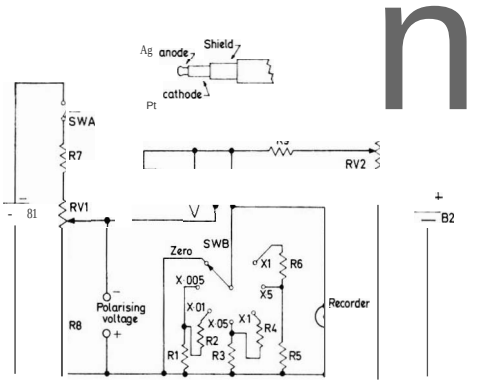

FIG. I. *Diagram of the electrical circuit used for measurillg the currelll passillg at the oxygell electrode*

Component **values:**
R1, R2, 220 ohm metal film ±1%; R3, R4, 2·2 K ohm metal film ±1%; R5, R6, 22 K ohm metal film ±1%; R7, 270 ohm carbon film ±5%; R8, 680 ohm carbon film ±5%; R9, 1 M ohm metal Oxide ± 2%; RVI, 250 ohm W.W. I watt ± 10%; RV2, 20 K ohm 5 turn Helical. CLR2405 Colvern; Bl, 82. RM.12R Mallory cell I·35 volts; SWA, 2 pole C.O. toggle; SWB, I pole 7 position wafer

Oxygen concentration was recorded in millivolts. Before each experiment the electrode was calibrated with air-saturated distilled water at **37°** which contained 0·217 **μmole** of 02/ml at 760 mm atmospheric pressure.[15] Corrections for barometric pressure were applied.

Egg white was diluted with 0·1 M potassium phosphate buffer at pH 7·0 so that stock solutions contained 5% by vol. of egg white. Aliquot parts (6 ml) of these solutions were placed in a water-jacketed glass reactions vessel held at 37° and stirred magnetically for several minutes to ensure complete equilibriation with the atmosphere. The electrode was carefully placed in the vessel so that air bubbles were excluded and, using the 'back off' resistance (RY2, Fig. I) the position of the recorder pen, which measured the oxygen content of the test solution, was adjusted to zero. The chart motor and magnetic stirrer were then started at speeds of 12 cm/min and 580 rev/min, respectively, and 25 vol. hydrogen peroxide solution (25 **μl**) was added through the 2 mm bore groove in the side wall of the electrode holder with a micro-syringe. The traces obtained showed that the rate of oxygen production was generally of the order of 0'1 I,mole of oxygen/ml/min and that up to 0·6 "mole of oxygen/ml could be measured accurately.

N-acetyl-li-o-glucosaminidase assay

The procedure was that described by Levy & Conchie[16] and later used by Lush & Conchie.[7] The test solutions contained citrate buffer (10 ml) at pH 4'0, distilled water (0'5 ml), diluted egg white (0'5 ml) and a 10 mM solution of p-nitrophenyl-2-acetamide-2-deoxy-li-o-glucopyranoside (I.5 ml). The diluted egg white was prepared immediately before the test by adding egg white (0'50 ml) to distilled water (4'5 ml). The test solutions were held at 38° for up to I h. The reaction was terminated by the addition of a 0·**4** M glycine-NaOH buffer (4 ml) at pH 10·5 and the p-nitrophenol liberated by the egg white was measured at 430 nm by reference to standard solutions of p-nitrophenol in the glycine-NaOH buffer solution.

a-Mannosidase assay

Test solutions[7,16] contained I M acetate buffer solution at pH 5·0 (0'5 ml), distilled water (1'5 ml), diluted egg white (0'5 ml) as used for the N-acetyl-li-o-glucosaminidase assay and 16 mM p-nitrophenyl-a-o-mannopyranoside (1'5 ml). Incubation at 38° and measurement of the liberated p-nitrophenol were carried out as described above for the assay of N-acetyl-li-o-glucosaminidase.

Lysozyme assay

Difco lysozyme substrate (approximately 50 mg), which is a dry-killed culture of *Micrococcus lysodeikticus*, was dissolved in 100 ml of Difco lysozyme buffer solution at pH 6·2 (9'6 g of a combination of NaH2P04 and K2HP04 in 1000 ml of distilled water). To determine the lysozyme activity of raw and heat-pasteurised samples of egg white 5 ml of diluted egg white (I : 4000 by voL) were added to the freshly prepared solution of substrates (5 ml) and held at room temperature (20°) for 20 min. The optical densities of the solutions were measured at 540 nm against distilled water.

Results

Titrations, with standardised potassium permanganate, of the supernatants obtained from reaction mixtures, were first carried out with different initial amounts of hydrogen per-

oxide. These showed that the proportion of hydrogen peroxide destroyed by egg white was directly proportional to the initial concentration of hydrogen peroxide, up to 3 mg{ml (Fig. 2). At higher initial concentrations of hydrogen peroxide the proportion destroyed was found to decrease, probably owing to inactivation of the catalatic property present in egg white by excess hydrogen peroxide.[17] Consequently, comparisons between different samples of egg white were all carried out at peroxide concentrations of 2·5 mg{ml in order to minimise the deleterious effect of higher concentrations.

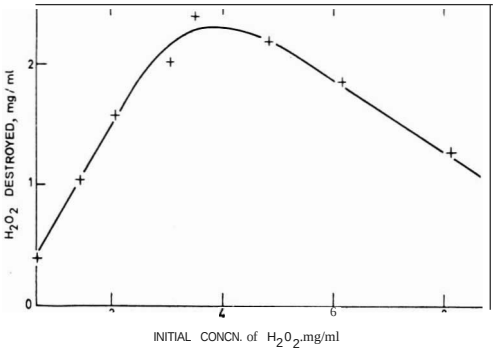

FIG. 2. *Effect oJ concell/ration oJ hydrogen peroxide all catalatic activity as determined by titration*

The results, given in Tables I and II, show that sterile samples of egg white destroyed approximately 95% of added hydrogen peroxide, even after storage of the egg white at 25° for as long as 3 weeks, and that the ability to destroy hydrogen peroxide was impaired when the egg white had been previously heated at a temperature of 57·8°. Experiments with samples of commercial unpasteurised egg white obtained from a number of different packing stations showed that heat at 57.8° in the laboratory pasteurising tube caused a similar but greater effect on the catalatic activity of these samples (Table III). However, the amount of hydrogen peroxide destroyed by these commercial samples of egg white was variable. All the samples were received frozen and were held at - 20° until heat treatment. Furthermore, there was very little difference between the ability of thick and thin egg white fractions obtained from newly laid eggs to destroy hydrogen peroxide (Table IV). The cause of the variation between samples was not, therefore, apparent and it can only be assumed that the different samples of egg white contained different amounts of the 'catalase-like' property. To test whether the commercial pasteurisation technique carried out

TABLE I

Effect of storage on the catalatic activity of egg white

Storage		% H202 destroyed	
Temperature, °c	Time	After 5 min	After 40 min
	Raw	44·9	94·7
	Raw	46·2	95·1
+ 25	3 weeks	75·8	95·4
+ 15	3 weeks	78·1	95·7
10	3 weeks	82·4	95·6
+ 5	3 weeks	82·2	94·8
- 20	3 weeks	75·2	97·3

TABLE II

Effect of laboratory heat treatment on the catalatic activity of sterile samples of egg white from newly.laid eggs

Heat treatment		% H202 destroyed	
Temperature, °c	Time, min	After 5 min	After 40 min
50	2·5	61	92
50	4	65	92
50	7	42	92
55	2·5	31	92
55	4	26	92
55	7	15	87
57·8	2·5	33	71
57·8	4	9	52
57·8	7	4	17
control unheated	0	72	94
58	2·5	47	90
58	4	38	72
58	7	26	44
58·5	2·5	59	66
58·5	4	31	51
58·5	7	23	28

TABLE III

Effect of laboratory heat treatment at 57·Soc for 2·5 min on the catalatic activity of commercial samples of egg white

Sample No.	% H202 destroyed			
	Untreated egg white		Pasteurised egg white	
	After 5 min	After 40 min	After 5 min	After 40 min
P.S.360	75	96	24	52
P.S.211	44	91	19	34
P.S.440	44	89	31	44
P.S.246	34	76	22	30
P.S.954	63	95	25	59
P.S.837	63	95	23	42
P.S.976	55	92	30	38
P.S. 822	64	81	20	48
P.S.401	59	95	29	45
P.S. 468	69	95	41	54
P.S.107	55	95	13	29
P.S.990	68	92	29	41
P.S.145	53	96	17	21
P.S.494	88	92	51	90
P.S. 076	54	89	22	40

TABLE IV

Catalatic activity of thin and thick egg white fractions

Egg white fraction	% H202 destroyed	
	After 5 min	After 40 min
Thick	Test I 40 2 33	Test I 91 2 89
Thin	Test I 28 2 25	Test I 82 2 83

at 57·8' for 2'5 min also reduced catalatic activity, ten samples of commercial heat-treated egg white were examined using the permanganate titration method. The results given in Table V show that the catalatic activity of these commercially pasteurised samples of egg white was low.

The addition of small quantities of hydrogen peroxide to solutions of egg white in which the Clark oxygen electrode was immersed gave a measurable increase in electrode current, which was almost linear for the first few seconds. Addition of 0·1 M sodium dithionite to the test solutions caused a rapid decrease in the measurable current and the 'catalase-like' reaction was appreciably inhibited by 0'19 M potassium cyanide. The reaction was not affected by prior dialysis of egg white samples before dilution for the polarographic test. Other tests showed that the amount of oxygen produced during the first minute of the reaction was proportional to the amounts of egg white and of hydrogen peroxide in the reaction mixture, which is compatible with the findings of other workers who have studied the complex kinetics of catalatic reactions. Tests with different samples of egg white showed that the catalatic activities as measured at the oxygen electrode were variable (Table VI) and therefore confirmed the results obtained with the potassium permanganate titration method.

Because of the high sensitivity and specificity of the oxygen electrode and because it permits an accurate measure of oxygen evolution during the first few minutes, and even seconds, of the reaction after the addition of hydrogen peroxide,[12] (Fig. 3), the 1st-order rate constants for the breakdown of hydrogen peroxide to oxygen were calculated using the equation:

$$k1 = \frac{1}{t} \times 2{\cdot}30 \times 10\text{glO}\,\frac{Xo}{X,} \qquad (I)$$

where Xo = initial concentration of hydrogen peroxide
$X,$ = residual concentrations of hydrogen peroxide after time I
I = reaction time (30 sec)

The 1st-order reaction rate constants calculated for the destruction of hydrogen peroxide by egg white samples which had been heat-treated at 53-6')' for 2·5 min in the laboratory pasteurisation tube (Table VII) were used to calculate the 1st-order reaction rate constants (K_p) for the destruction of the catalatic activity by heat using the equation:

$$K_p = \frac{2.5}{1} \times \frac{60 \times 2'30310g.o}{} \frac{k_o}{k_1} \qquad (2)$$

where k_o = rate constant for the control unheated egg white
k_1 = rate constant for the heat-treated egg white

TABLE V

Catalatic activity of commercially pasteurised (2'5 min at 57·S'c) egg white

Sample No.	% H202 destroyed	
	After 5 min	After 40 min
1	15	21
2	12	22
3	13	19
4	14	31
5	24	38
6	25	40
7	33	38
8	35	43
9	18	22
10	37	51

TABLE VI

Production of oxygen from hydrogen peroxide by egg white

Sample	02 produced, I'mole fml fmin
A	0·1656
B	0·1440
C	0·0396
D	0'0378
E	0·0864
F	0·0486
G	0·1728

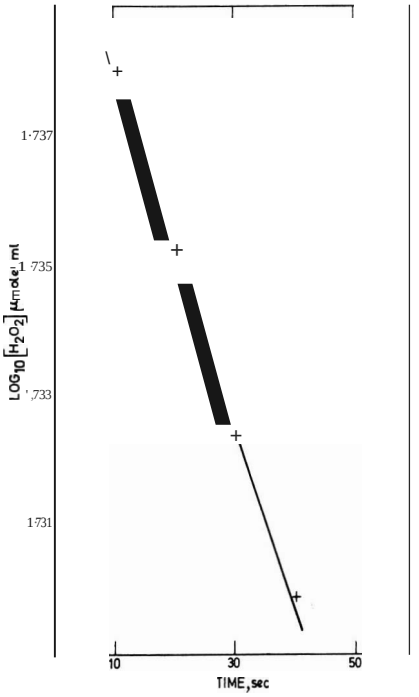

FIG. 3. *Relation between concell/ration oj Irydrogen peroxide and reaction time at tire oxygen electrode*

TABLE VII

Effect of heat treatment for 2½ min on *k,* for the catalytic activity of egg white

Temperature, °c	*k"* 1st-order reaction rate constant, sec-I
53	2·51 x 10-4
54	2·12 x 10-4
55	2·31 x 10-4
56	I·90 x 10-4
57	1·60 x 10-4
58	1·29 x 10-4
59	9·12 x lO-s
60	9·12 x lO-s
Unheated conlrol	5·78 x 10-4

The activation enthalpy (ΔH) was calculated to be 39·8 kcal mole-I from the slope of an Arrhenius plot (Fig. 4) using the equation:

$$\Delta H = \Delta E + RT \quad (3)$$

where T = 331° K (58°)

Using the Eyring theory for absolute reaction rate processes[18,19] where:

$$K_p = \frac{k'T}{h} e^{-F/RT} \quad (4)$$

and k' is the Boltzmann constant (1.38 x 10-16 erg/°) and h is the Planck constant (6'27 x 10-27 erg sec), the free energy change was calculated to be 22·6 kcal mole-I. The activation entropy change (ΔS) derived from the equation:

$$T\Delta S = \Delta H - \Delta F \quad (5)$$

was calculated to be 51 cal/° (entropy units).

The use of the selected substrates p-nitrophenyl-N-acetyl-/i-o-glucosaminide and p-nitrophenyl-a-o-mannopyranoside has verified the findings of Lush & Conchie,[7] that egg white contains both N-acetyl-/i-o-glucosaminidase and a-mannosidase enzymes. N-acetyl-/i-o-glucosaminidase has been determined for six separate samples of fresh egg white and it is shown in Table VIII that there can be at least a 6-fold variation in enzymic activity between samples. The results, given in Table IX, show that only egg white N-acetyl-/i-o-glucosaminidase is affected by heat within the temperature range 53-60°. The reaction rate constants for the hydrolysis of p-nitrophenyl-/i-o-glucosaminide (Table X) were calculated for samples of egg white which had been heat-treated in the laboratory pasteurisation tube for 2·5 min at 53-60°, using Equation (I), and the rate constants for the destruction by heat of the enzyme N-acetyl-/i-o-glucosaminidase at each temperature were calculated using Equation (2). From Fig. 5 and Equations (3), (4) and (5), as described for the inactivation of the catalatic activity of egg white, the activation parameters ΔH, ΔF and ΔS for heat inactivation of N-acetyl-/i-o-glucosaminidase were calculated to be 62'9 kcal mole-I, 21·8 kcal mole-I and 124·5 E.U.

Lysozyme was not affected by heat-treatment up to 58° for 2½ min. However, the enzymic activity of lysozyme decreased in samples of egg white which had been heated at 59° and 60° to 50% and 33%, respectively, of the original enzymic activity measured for unheated control samples of egg white.

Discussion

Although Pennington & Robertson[20] showed that egg white possessed catalatic activity, Baker & Manwell'l and then Corbin & Brush[22] and Lush'3 have claimed that catalase is not present in egg white. As the results of the present work show clearly that hydrogen peroxide is destroyed by egg white, that oxygen is produced during this reaction, and that the reaction is inhibited by potassium cyanide and not reduced by prior dialysis of egg white, it is by no means certain that the catalatic activity is due to the presence of a catalase enzyme. Indeed, these findings, which show that the catalatic activity is equivalent to only about 7 Sigma units of bovine liver catalase/ml egg white, tend to support the view'l that a true catalase enzyme is not present in egg white. However, the destructive effect of heat on the catalatic activity of egg white

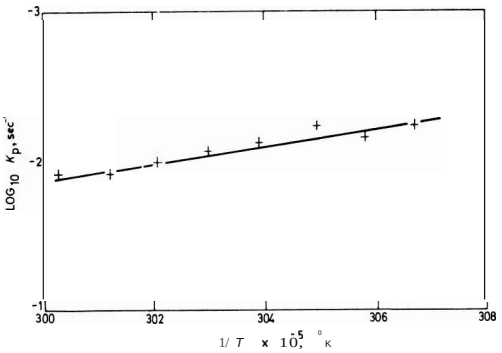

FIG. 4. *Arrhenius plot for hear inactivation of catalatic activity*

TABLE VIII

N-acetyl-/l-o-glucosaminidase activity of six egg white samples

Sample	μg p-nitrophenolliberated/ml of egg white	
	After 30 min incubation at 38°c	After 60 min incubation at 38°c
M_1	954	2044
01	238	613
Cl	136	340
EI	408	954
K_1	626	1267
Jl	613	954

TABLE IX

Effect of heat treatment for 2t min on N-acetyl-/l-D-glucosaminidase and a-mannosidase in egg white

Temperature, °c	μg p-nitrophenolliberated by I ml egg white after I h at 38°c	
	a-mannosidase	N-acetyl-/l-D-glucosaminidase
53	272	2344
54	299	1772
55	245	627
56	272	491
57	272	300
58	272	245
59	327	177
60	408	232

TABLE X

Effect of heat treatment for 2t min on kl for N-acetyl-/l-D-glucosaminidase of egg white

Temperature, °c	*kl*, 1st-order reaction rate constant, sec-1
53	1·21 x 10-3
54	6·61 x 10-'
55	5·51 x 10-'
56	2·89 x 10-'
57	1·25 x 10-'
58	1·IOxIO-'
59	1·25 x 10-'
60	1'41 x 10-'
Unheated control	3·47 x 10-3

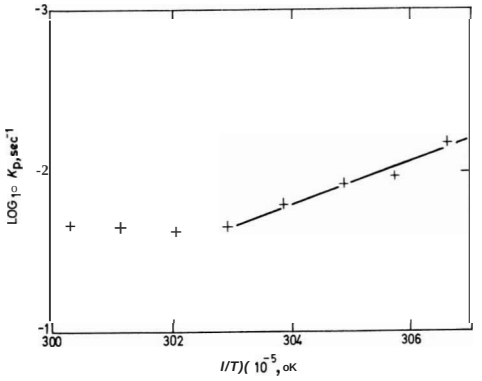

FIG. 5. *Arrhellius plot for* ***heat*** *illactivatioll of N-aeetyl-ß-o-* ***glucosamillidase***

and the high values calculated for the enthalpy change, the entropy change and the free energy change do suggest that a natural undenaturated protein is required for the catalatic activity of egg white. Moreover, the free energy change of 22·6 kcal{mole occurring during the heat inactivation of the catalatic property is identical to that found for the denaturation of many other proteins.·[4] Therefore, the claim by Baker & Manwell·[1] and later by Corbin & Brush·· and **Baker,**[25] that catalatic activity is associated with the main protein fractions of egg white found on starch-gel electrophoretograms, warrants a more detailed investigation, although it seems possible that their observations, with the potassium iodide-hydrogen peroxide staining technique, could have been due to a reaction between the iodine liberated and some of the amino acids present in the protein fractions, rather than with the starch gel.

Unfortunately, the differences in catalatic activity found between different samples of egg white were of the same magnitude as the observed decreases in catalatic activity caused by heat treatment and this limits therefore the general application of a test based on catalatic activity for monitoring the pasteurisation processes now used in Great Britain. The results of this work also show that N-acetyl-ß-D-glucosaminidase is affected by heat, at such temperatures as are used commercially. However, again the differences in activity between samples of sterile egg white are of the same magnitude as the observed decreases in activity caused by heat pasteurisation. Nevertheless, it is possible, in the absence of more suitable tests, that measurements of both the catalatic and N-acetyl-ß-D-glucosaminidase activities may prove useful for testing the efficiency of heat pasteurisation of bulk egg white. Furthermore, if measurements both before and after pasteurisation are made on a given sample of egg white, then the decrease in the activity of N-acetyl-ß-D-glucosaminidase and in the catalatic activity afford a measure of the efficacy of heat pasteurisation.

As the enzymic activity of lysozyme in egg white was only affected at the higher temperatures, an observation which has now also been reported by Cunningham & Lineweaver!6 the use of the lysozyme assay as a test for the satisfactory heat pasteurisation of egg white is not possible, unless higher temperatures are used than are required to destroy sufficient numbers of the most commonly isolated *Salmollella* from human sources.[2] Furthermore, as the egg white samples which had been heated at these higher temperatures contained a precipitate, and as Kline *et 01.*[27] have shown that egg white coagulates at 138·**4–140**°F (59-60°c), then it seems likely that such a pasteurisation technique would be inadvisable. A useful test for the excessive heat treatment of natural egg white would be the application of these findings on the effect of heat on egg white lysozyme.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

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I.-AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Pedogenesis during the Quaternary [period]. M. L. Jackson and F. D. Hole *(Soil Sci.,* 1969, 107, 395-397).-A short outline of typical changes occurring during this period [the glacial Pleistocene and the Recent (since the appearance of man) periods], due to weathering of original rocks and rock debris, and the effects of these changes on some characteristics of the soils formed.
A. G. POLLARD.

Principles for dating pedogenic events in the Quaternary. R. V. Ruhe *(Soil Sci.,* 1969, 107, 398-402).- Dating methods are explained and illustrated by reference to several soil formations. (29 ref.) A. G. POLLARD.

Some aspects of soil evolution in north-east Scotland. E. A. Fitzpatrick *(Soil Sci.,* 1969, 107, 403-408).-The development of these soils is traced from the Tertiary period until the present day. (16 reL) A. G. POLLARD.

Gilgai [soils] in the Quaternary. E. G. Hallsworth and G. G. Beckmann *(Soil Sci.,* 1969, 107, 409-420).-The nature, formation and classification of gilgai (contorted surface) soils are discussed, together with factors which influence the final form of these soils. (44 ref.) A. G. POLLARD.

Eolian sediment influence on pedogenesis duriog the Quaternary. J. K. Syers, M. L. Jackson, V. E. Berkheiser, *el 01. (Soil Sci., 1969,* 107, 421-427).-Accretions of some Eolian material, e.g., loess and aerosolic dust, into soils are demonstrated by observations of the oxygen isotopic composition of soil quartz. Data for a number of soils and wind-blown dusts are presented and discussed. (25 ref.)
A. G. POLLARD.

Pedotranslocation: Eluviation-illuvialion in soils during the Quarternary. J. A. McKeague and R. J. St. Arnaud *(Soil Sci.,* 1969, 107, 428-434).-Translocation of sol. matter and of particles in suspension is discussed in relation to the micromorphology of soils, to the mechanisms of clay mobilisation and to the weathering products (notably Fe and AI) in spodosols. (47 ref.)
A. G. POLLARD.

Arrangement of constituents in Quaternary soils. R. Brewer and J. R. Sleeman *(Soil Sci.,* 1969, 107, 435-441).-Soil materials can be classified broadly as relatively inert 'skeleton grains' and 'plasma' (which includes particles which may be relatively easily rearranged by pedological processes). Evidence is noted of soil formation from transported parent materials; such transport produces a parent material containing an intimate mixture of constituents. Profile constituents are discussed, especially those readily recognised in thin sections under a polarising microscope. (50 reL)
A. G. POLLARD.

Pedoeementalion; induration by silica, carbonates and sesquioxides in the Quaternary. K. W. Flach, W. D. Nettleton, L. H. Gile and J. G. Cady *(Soil Sci.,* 1969, 107, 442-453).-The formation of cemented layers in soil is largely attributable to the presence of opal-like Si compounds, or to CaC03 or sesquioxides, with or without org. matter. Possible mechanisms of formation of such layers are discussed. (32 ref.) A. G. POLLARD.

Pedosesquioxides; composition and colloidal interactions in soil genesis during the Quaternary. J. M. de Villiers *(Soil Sci., 1969,* 107, 454-461).-A discrete amorphous form of Al_2O_3, boehmitic in character, present in certain soils is examined. Its formation is possibly related to the tendency of various soil constituents to inhibit the crystallisation of sesquioxides. (32 reL)
A. G. POLLARD.

Mineral interactions and transformatiOlls in relation to pedogenesis during the Quaternary. C. Pedro, M. Jamagne and J. C. Begon *(Soil Sci.,* 1969, 107, 462-469).-Various processes of mineralogical weathering concerned in soil formation during the Quaternary period are discussed. Transformations occurring in the feldspar and phyllosilicate sequences during weathering of micaceous phyllites are considered. (36 ref.) A. G. POLLARD.

Pedohumus; accumulation and diagenesis duriag the Quaternary. F. J. Stevenson *(Soil Sci.,* 1969, 107, 470-479).-The somewhat limited information concerning humus in buried soils and associated deposits of the Pleistocene period is reviewed. (25 ref.)
A. G. POLLARD.

Significance of magnesium and iron in montmorillonite formation from basic igneous rocks. G. S. R. Krishna Murti and K. V. S. Satyanarayana *(Soil Sci.,* 1969, 107, 381-384).-The chemical and mineralogical composition and the cation-eXChange capacity *(CEC)* of clays separated from soils of the Malwa plateau, India, were subjected to multiple correlation analysis. Results show that octahedral Fe is far more effective than octahedral Mg in the formation of montmorillonite *(M)* and in assessing *CEC*. The *CEC* and the *M* content (%) were more closely correlated with the Mg/Fe and Fe/A] ratios than with the Mg/AI ratio of the clay. (11 reL) A. G. POLLARD.

Iron-rich montmorillonite formation in soils derived from serpentine. W. E. Wildman, M. L. Jackson and L. D. Whittig *(Proc. Soil Sci. Soc. Am.,* 1968, 32, 787-794).-Soils of three series derived from serpentine contained Fe-rich montmorillonite *(M)* as the predominant B-horizon clay mineral. Fe comprised I·8 to 2·7 of the four octahedral cations per unit cell of the *M,* the balance being AI and Mg. Weathering of the high magnesian serpentine to Fe-rich clay resulted in a 5- to 10-fold increase in Fe and AI, and a decrease of similar magnitude in Mg.
A. H. CoRNFIELD.

MetaI-organic chemistry of the geochemical cycle. J. D. Saxby *(Rev. pure appl. Chem.,* 1969, 19, 131-150).-The review covers associations of metals with carbonaceous material, metal-org. compounds in living organisms, metal-org. compounds in sediments (porphyrins and salts of long-chain org. acids) and potential org. metal binders in sediments (amino acids, N and S heterocyclic compounds, O-containing compounds and org. complex formation from minerals). There is evidence that during weathering, sedimentation and diagenesis, eo-ordination and organometallic compounds are sometimes present, originating from biological precursors without major structural changes or formed during sedimentation from org. mol. and metal ions derived from various sources. (227 ref.) J. M. JACOBS.

Particle size variation and weathering in [Canadian] Orthic Podzol pr06les. K. W. G. Valentine and J. F. G. Millette *(J. Soil Sci.,* 1969, 20, 11-22).- The extent of breakdown of gravel, sand and silt, and the accumulation of clay were related to the porosity of the parent material and the type of rocks in the gravel. There was no clay accumulation in the B horizon when the results were expressed on the %wt. per unit vol. basis. The sp. gr. of the gravel was less than that of any sand fraction, and the sp. gr. of the latter increased with decreasing particle size. The sp. gr. of the gravel, but not of the sand, increased with profile depth. The proportions of gravel, sand, silt and clay were used to calculate an index of weathering intensity. (18 reL) A. H. CoRNFIELD.

Genesis, chemical properties and mineralogy of Caribbean grumusols. N. Ahmad and R. L. Jones *(Soil Sci.,* 1969, 107, 166-174).-Profile characteristics of these soils are described. In general they are dark clay soils formed on calcareous material under various conditions of rainfall (100-190 cm/annum) or on compacted volcanic ash with rainfall < 125 cm/annum. Numerous chemical and mineralogical data are recorded. (18 ref.)
A. G. POLLARD.

Mineralogy of some arid and semi-arid land soils of Iraq. A. H. AI-Rawi, M. L. Jackson and F. D. Hole *(Soil Sci.,* 1969, 107, 480–486).—**Mineralogical** analyses of a Brown soil developed from fine alluvial matter on Pleistocene terraces (northern Iraq), and of three other alluvial soils formed on deep, stratified, fine-textured Holocene alluvium in north and central Iraq, are recorded. A comparison of the sand, silt and clay fractions of these soils is made. (24 reL) A. G. POLLARD.

Morphology and genesis of greyish c1aypan soils in Oklahoma. I. Morphology, chemical and physical measurements. II. Mineralogy and genesis. J. R. Culver and F. Gray *(Proc. Soil Sci. Soc. Am.,* 1968,32,845-851; 851-857).–Characteristics of the soils are presented and their genesis is discussed. (35 **ref.)**
A. H. CoRNFIELD.

Properties and mineralogy of soils derived from argillised tuff in the Mount Carmel region, Israel. A. Singer *(Proc. Soil Sci. Soc. Am.,* 1968, 32, 839-844).-One profile described developed ill *silll* on partly argillised tuff, the other on alluvium derived from the alteration products of tuff. The alteration product « 2 **μm)** of the tuff rocks, as well as the clay from the soil, consisted chiefly of Fe-rich saponite. A large part of the clay was in the sand- and silt-sized aggregates, which are difficult to disperse. Both profiles contained some lime and exhibited weak textural horizon differentiation. (21 ref.) A. H. CoRNFIELD.

Nature and genesis of aridisols in Kenya. J. Makin, J. Schilstra and A. A. Theisen *(J. Soil Sci.,* 1969, 20, I11-125).- Some soils of the semi-arid lowlands are described and the 7th Approximation soil classification is adapted to include them. A. H. CORNFIELD.

Soil structure in Vertisols of the Blue Nile clay plains, Sudan. J. H. de Vos t.N.C. and K. J. Virgo *(J. Soil Sci.,* 1969,20,189-206). -Morphological characteristics of the soils are described and compared with those of similar soils in other parts of the world. Soil cracking, surface mulching, structure and micromorphology are discussed. (42 ref.) A. H. CoRNFIELD.

Phosphorus transformations in a chronosequence of soils developed on wind-blown sand in New Zealand. I. Total and organic phosphorus. J. K. Syers and T. W. Walker *(J. Soil Sci.,* 1969, 20, 57–64).—**In** a chronosequence of weakly weathered soils, loss of total P was linearly related to time between 500 and 10,000 years. Total loss of P was 1,910 kg per hectare-metre during 10,000 years. In the original sand, org. P was very low; it increased rapidly during the first 300 years of soil development and much more slowly thereafter. Net accumulation of org. P after 10,000 years was 1,050 kg per ha-m. (28 ref.) A. H. CoRNFIELD.

Phosphorus fractions of Some Venezuelan soils as related to their stage of weathering. F. C. Westin and J. G. de Brito *(Soil Sci.,* 1969, 107, 194-202).- The 23 soils examined included representatives of eight orders of the U.S. Dept. Agric. Comprehensive System *(eS)* drawn from areas of varied rainfall from 'wet' to 'arid'. Relationships between (i) cation-exchange capacity x 100/ %c1ay, *(il)* classification in *eS,* ***(iii)*** amount and form of soil P, and *(iv)* pH are examined. With advancing stages of weathering, the eS classification moves from mollisols, inceptisols and entisols through alfisols to ultisols and oxisols. This grading is associated with falling pH and total, active, and org. P, with reduced % active P in total P, and with a shift of active P from Ca-P to Fe-P, but appreciable change in Al-P or reductant P does not occur. (15 **ref.)** A. G. POLLARD.

Fractionation of phosphate in a maturity sequence of New Zealand basaltic soil profiles. II. J. D. H. Williams and T. W. Walker *(Soil* Sci., 1969, 107, 213-219: cL *Idem, ibid.,* 1969, 107, 22).- Estimates of mean values for whole profiles of Ca-bound phosphates, I.e., P in apatite, org. P, occluded and non-occluded forms of secondary inorg. P occurring during pedogenesis, are illustrated by a 'maturity sequence' of the basaltic soils. Difficulties in defining and measuring these categories are discussed. Mineralogical differences between and within profiles may contribute to the similarity of behaviour of org. P and NH,F-sol. P within profiles; a state of equilibrium between org. P and non-occluded secondary inorg. P is suggested. The hypothesis that apatite-P is transformed into occluded P via these forms may also account for changes in P distribution between the fractions with the advancing profile maturity. (II ref.) A. G. POLLARD.

Characterisation of tillage pans in selected Coastal Plain soils. A. Kashirad *(Diss. Abslr., B,* 1967, 28, 1314-1315).-Tillage pans from several localities are compared with adjacent cultivated and with virgin soils; sampling sites were selected by vertical penetrometer readings. Tillage pans showed higher soil strength and bulk density and lower pore space than did samples at corresponding depths in the cultivated or virgin soils. Cementing agents in the compacted soil were examined by sequential extraction with peroxide (I), **citrate–dithionite** reagent (TI) and hot 0·5 N-NaOH (Tl1). Significant linear regressions were found between soil org. matter and the AI, Fe and Si extracted with I. II removed more Fe and Si, and 10 more AI and Si from the pans than from virgin soil at corresponding depths. The cementing agents were probably Fe or A1 silicates or hydroxides. The pH, org. matter and acetate-extractable Ca, Mg, K and P were higher in the pans than in the virgin soil. A. G. POLLARD.

Soil texture patterns in the alluvium of the lower Indus plains. D. A. Holmes and S. Western *(J. Soil Sci.,* 1969, 20, 23-37).- Textural mapping of the immature alluvial soils is described.
A. H. CORNFIELD.

Channelling of alluvial depression soils in Iraq and Sudan. L. P. White and R. Law (J. *Soil Sci.,* 1969,20, **84–90).—Characteristics** of the channel patterns formed in the floors of alluvial clay depressions are described and their possible modes of formation are discussed. (11 **ref.)** A. H. CORNfiELD.

Radio-carhon dating of soils. III. Soils with a B, horizon and fossil chernozems. H. W. Scharpenseel and F. Pietig(Z. *PflEmiilzr. Bodellk.,* 1969, 122,145-152).- M. LONG.

Basis for the classification of soil. C. N. Macvicar *(J. Soil Sci.,* 1969,20, 141-152). –The scope of pedology and definition of 'soil' is discussed. Certain principles are suggested for devising a classification applicable over large regions. A. H. CORNFIELD.

Natural system of soil classification. J. W. Muir *(J. Soil Sci.,* 1969,20, I53-166). –Definitions of natural classification are given which emphasise the importance of theory and concept in natural systems. The proposed system is based on two principles of soil formation: *(a)* significance of the three-dimensional nature of soil bodies, *(b)* the principle of developmental sequences which is concerned with stages of soil development. The many developmental sequences are correlated by means of a small number of constituents which are lost and gained during soil formation, and are used to rank the properties on which the proposed system is based. Soil horizons are defined according to the ranked properties and the classes of lhe system are, in turn, defined in terms of the horizons, so that the central statement of the system is the Principle of Developmental Sequences. A. H. CORNFIELD.

Physical and chemical properties of soils in the Beqa'a Plain, Lebanon. A. H. Sayegh and A. J. Salib *(J. Soil Sci.,* 1969, 20, 167-175). –Characteristics of regional red soils, white rendzina, light chestnut, dark chestnut, grey, black and recent alluvial soils are presented and compared statistically. (12 **ref.)**
A. H. CORNFIELD.

Constancy of potassium micaceous minerals in a given weathered system. S. Paxinos and C. Alexiades *(Aris/oleleioll Pallepislemioll Tilessaloll. Geopoll. Dasolog. Schole, Epislem. Epel.,* 1967, 1-30). – A general discussion of the literalure. The clay fractions were dispersed, fractionated, prepared for elemental and X-ray diffraction analysis and the cation-exchange capacity was determined. Carbonates were removed by washing and org. matter by treatment with H,O,; free iron oxide was removed by Na,S,03. In highly micaceous clays, weathering in the initial stages resulted only in the production of vermiculite and montmorillonite. (21 **ref.)** (In Greek with English summary.) S. C. HAWORTH.

Composition and properties of the < 2 **μm** [clay] fraction of soils derived from basic igneous rocks. U. Schwertmann and E.-A. Niederbudde *(Z. PflEmiihr. Bodellk.,* 1969, 122, 193-206).-The **6–2, 2–0·2** and < 0·2 **μm** particle-size fractions in most of these German soils contained montmorillonite *(M)* as the predominant cryst. layer silicate. Others derived from diabase and phonolite contained, e.g., vermiculite, while loess contamination led to the occurrence of typical loess mineral associations. The *M* was similar to Wyoming bentonite, and differed from mica-derived *M* in sedimentary soils; it contained considerable amounts of X-ray amorphous material however, which may be an intermediate in *M* formation in these soils. (20 **ref.)** M. LONG.

Effect of eoneentration and movement of solutions on the swelling, dispersion, and movement of clay in saline and alkali soils. D. L. Rowell, D. Payne and N. Ahmad *(J. Soil Sci.,* 1969, 20, **176–188).—** Changes in permeability *(P)* of a soil containing montmorillonite, and the swelling of oriented aggregates of extracted clay, were measured for a range of exchangeable Na (%) (Na_{ex}) and electrolyte concn. *P* began to decrease at lhe same concn. as the

clay began to swell; changes in *P* were directly controlled by the swelling until clay dispersion began. The concn. at whIch clay dispersed depended on the mechanical stress applied; at low stresses the proportion of clay which swelled and dispersed depended directly on Na.x. Large stresses dispersed most of the clay even at low Na_{ex} levels. (14 ref.) A. H CoRNFIELD.

Pbenomenological soil-polymer parallels. R. J. Krizek *(Am. Scien/.,* 1968,56, 279-287).-Clay soils and polymers are discussed and their behavioural patterns are compared, with special reference to the temp. of the polymer and the moisture content of the soil. Polymer mol. wt. and soil particle size are compared and the response characteristics are reviewed, attention being called to the similarities in time-dependent response of polymer and clays. The differences are also examined but it is suggested that the application of the parallels can greatly accelerate progress in soil mechanics. C. V.

Settling of soil as an aspect of structural stability. K. H. Hartge *(2. PflErniihr. Bodenk.,* 1969, 122, **250–259).—A** hand-operated apparatus for determining the settling behaviour of **mechanically-**disturbed soils is described. Lime greatly reduced the settling angle of a loess soil, whilst Na alginate did not. In this **respect** the method differs from the standard wet slevmg techmque and It IS concluded that the settling characteristics of a soil and its water stability are different aspects of soil structure. (16 ref.) M. loNG.

InDuence of texture on the moisture characteristics of soil. V. Relationships between particle size composition and moisture cootents at the upper and lower limits of available water. P. J. Salter and J. B. Williams (J. *Soil Sci.,* 1969,20, 126-13I). –The effects of **sand,** silt, clay and org. matter contents in 26 soils on the moisture **contents** *(MC)* at the upper and lower limits of **available** water were determined. Regression equations are presented whIch enable *MC* to be estimated on a wt. or vol. basis from mechanical analysis data. Accuracy of predictions ranged from ± 8 to 22 % of the measured values. Estimated mean values of *MC* at the upper and lower limits of available water for 22 textural classes are presented. A. H. CoRNFIELD.

Effect of initial clod size on characteristics of splasb and wasb erosion. W. C. Moldenhauer and Jajah Koswara *(Proc. Soil Sci. Soc. Am.,* 1968, 32, 875-879).-Wash erosion (sheet Dow) from clods of a silty clay loam subjected to artificial rainfall increased with clod size from O'5-2'0 rom to 8-30 mm with clods from continuous maize. Wash loss from clods from bromegrass meadow was not related to clod size. Splash loss (detachment of particles from clods) was highest from the 8-30 mm-size clods from continuous maize, but lowest from the corresponding clods from bromegrass. Material lost by splash was larger than that removed by wash. (11 reL) A. H. CoRNFIELD.

Evaporation from drying surfaces by tbe combination metbod. M. Fuchs, C. B. Tanner, G. W. Thurtell and T. A. Black *(Agron. J.,* 1969, 61, 22-26).-The combination formula, which explicitly **includes the** surface temp., is used to predict the hourly evaporation from a drying bare soil. The formula is valid regardless of the water vapour saturation deficit at the surface, but the measurement of the surface temp. limits its application to surfaces having a well-defined boundary with the atm. Agreement with hourly evaporation measured by Iysimetry, was only fair **because** of a thermal lag in the energy balance of the Iysimeter, but was very good for average daily values where the effect of thermal lag was negligible. (22 reL) A. H. CoRNFIELD.

Drying patterns in soil following anbydrous ammonia injection. J. F. Parr and F. E. Khasawneh *(Pl. Soil,* 1968 29, **460–470).—** Drying patterns in soil, caused by a more rapid rate of watcr evaporation from the retention zone after injection of NH_3, are described. Development of moisture gradients inclined towards the centre of the retention zone from the periphery over a period of several months, even when pots were subjected to alternate wetting and drying. Explanations of NHa-induced drying patterns and their possible agronomic significance are discussed. (12 reL) A. H. **CORNFIELD.**

Effect of bysteresis of **pore-water** on bydraulic conductivity. A. Poulovassilis *(J. Soil Sci.,* 1969,20, 52-56).-For two sands, the hydraulic conductivity during drainage was greater than that during corresponding wetting. The max. hysteresIs effects occurred at intermediate moisture contents. (16 reL) A. H. CoRNFtELD.

Determination of bydraulic conductivity in unsaturated soils witb a double membrane pressure apparatus. K. L. Henseler and M. Renger (2. *PflErniihr. Bodenk.,* 1969, **122,** 220–228).—**The** apparatus, which has porous ceramIc plates, and ItS use are descnbed. M. loNG.

Resistance to tbe movement of water tbrougb soil. W. Tepe and A. Darlowsky (2. *PflEmiihr. Bodenk.,* 1969, 122, 206-219).-An electrode system is described, consisting of triangular Zn and Cu electrodes separated by absorbent paper held in a specially designed clamp. At const. voltage and temp. the current passing through the system is proportional to the. water. **absorbed** by tbe paper. Using this apparatus, water mobIlity, **viscosity** (η) .and apparent density *(d)* were investtgated for two SOIls; increasmg SOll water increased water mobility and decreased η and *d.* Increasmg the electrolyte content and temp. separately had the same effect. M. loNG.

Saturation-unsaturation transition in infiltration to a non-uniform soil profile. D. Zashavsky *(Soil Sci.,* 1969, 107, **160–165).—** Distinction is drawn between saturated and unsaturated Dow of water in a soil profile, and effects of a 'more-permeable' or 'less permeable' layer at particular **depths** below or. at the **surface** are examined. A column of sand 10 whIch a senes of tensIOmeters at various depths were installed, contained, as a surface (control) layer, much finer sand than was in the **body** of the column. Rates of infiltration were measured with varymg depths of ground water. Ground-water depth did not affect the infiltration rate unless it reached an interface between layers in the profile. Infiltration rates from ponded water were proportional to the head of water except when the head became very small. The bearing of results obtained on problems of leakage of water from reservOirs and on irrigation practices is considered. A. G. POLLARD.

Selective adsorption of water from benzene by soil minerals. I. Degree of surface covering. O. Schincariol and M. Tschapek. U. Determination of surface bydrophilic [character]. M. Tschapek and O. Schincariol (2. *PflEmiihr. Bodenk.,* 1969, 122, 129-137; 137-144).-1. The selective adsorptIOn of water, by **soils** and SOll minerals, from C.H. was studied at various reduced concn. *(ClCo,* where *C* and Co are the actual and max. content of water in the benzene, respectively). Values of C/C_0 of up to 0'.7 resulted in incomplete mol. layers. Differences **in** the formation of water layers from gas and from benzene may arise from differences in the degree of adsorption of air and benzene mol. It was ImpOSSIble to calculate surface area by the adsorption of water from benzene. (24 reL) (In English.)

II. The hydrophilic characteristics for SOll and clay mmerals of known surface area are presented. These are based on Ihe adsorption of water from benzene. (11 reL) M. loNG.

Construction of calibration curves for determining water content from radiation counts. D. A. Holland *(J. Soil Sci.,* 1969, 20, 132-140).-The principles involved in the calibration of a radiation counter are considered. calibration of a **β-gauge** and of a neutron probe for determining moisture contents of leaves and offield soils, respectively, is described. (22 ref.) A. H. CORNFIELD.

Water table, soil moisture and oxygen diffusion relationsbips on two drained **wetland** forest sites. C. E. Young, jun. *(Soil Sci.,* 1969, 107, **220–222).—Data** are presented for the soil moisture content 6-18 in below the surface and the 0 2-dIffuslOn rate *(ODR)* at 12 **in** below the surface in relation to the depth of the water table *(WT)* in two soil types. The significance. of these observations is **discussed** and the possible use of *WT* as a SImple basIS for calculating *ODR* is indicated. A. G. POLLARD.

Capillary conductivity and soil water diffusivity values from vertical soil columns. W. J. Flocker, M. Yamaguchi and D. R. **Nielsen** *(Agron. J.,* 1968,60, 605-61O).-Changes in the distribution of **soil** water, capillary conductivity and **diffusivity** are presented, durnng water evaporation from lOQ-cm vertIcaI columns of a Yolo loam over 200 days allO', 20' and 30'. A. H. CoRNFIELD.

Sprinkler and surface irrigation of crops in an arid environment. F. E. Robinson, O. D. McCoy, G. F. Worker, Jun. and W. F. **Lehman** (*Agron. J.,* 1968, 60, 696-7oo).-Sprinkler irrigation removed more sol. salts from the upper soil layer than did Dood irrigation of a clay or furrow irrigation of a sandy clay loam. Emergence and early growth of 10 crop species under. **sprinkler** was equal to or better than **that** under **flood** or furrow lITIgation. Water use efficiency was hIgher under sprinkler than under the other systems of irrigation. A. H. CoRNFIELD.

Soil-water Dux below a ryegrass root zone. M. E. LaRue, D. R. Nielsen and R. M. Hagan *(Agron. J.,* 1968,60,625-629). The net water Dux below the root zone of a ryegrass sward was measured in the field for plots receiving equal total quantities of irrigation

water but applied in unequal amounts at different frequencies. Hydraulic conductivity values, calculated from field-measured soil-water potential gradients and laboratory-measured soil-water characteristics were used to compute the soil-water flux. The amount of water moving into and out of the root zone was influenced by the irrigation frequency. The nature of the hydraulics of a natural field soil, its variability in space, and its implications for the interpretation of soil-water depletion are discussed.
A. H. CoRNFIELD.

Effect of carbon dioxide on the chemical equilibrium of soil solution and ground water. K. L. Dyer *(Diss. Absfr., B,* 1967, 28, 160I).-Relationships between dissolved CO" HCO.-, CO_3^{2-}, H^+, solid phase CaCO., exchangeable H^+ and ionic strength were examined by digital computer. The routine was combined with an existing program, successfully used to relate the equilibrium of sol. CI-, SO_4^{2-}, NO.-, sol. and exchangeable Ca^{++}, Mg^{++}, Na^+, and solid phase $CaSO_4.2H_2O$. This model made possible the calculation of equilibrium concn. resulting if one or more of these values were arbitrarily changed. Soils of a wide range of types were used and the chemical predictions obtained were approx. as accurate as were the chemical methods used to determine the various constituents. The system of analysis, with minor modifications, can probably serve to predict changes in ground-water quality as water percolates through strata of known chemical characteristics. A. G. POLLARD.

Measurement of soil pH. R. E. White *(J. Ausf. Insf. agric. Sci.,* 1969,35, 3-14). –A review. (64 ref.) E. G. BRICKELL.

Equilibria of potassium-aluminium exchange in clay minerals and acid soils. B. S. Coulter *(J. Soil Sci.,* 1969, 20, 72-83).- The K-Al equilibria in montmorillonite, vermiculite, illite and two soils were studied, using an isotopic dilution technique. (22 ref.)
A. H. CoRNFIELD.

Effect of prolonged cropping on the exchange surfaces of the clays of Broadbalk field. P. H. T. Beckett and M. H. M. Nafady (1. *Soil Sci.,* 1969, 20, 1-1O).-There were substantial differences in Ihe K-(Ca + Mg) exchange isotherms between K-enriched and K-depleted soils of the long-term Broadbalk trials. The changes probably result from removal of inter-lamellar K and the consequent increase in surface area available for cation exchange. (27 ref.) A. H. CoRNFIELD.

Contribution of adsorbed strontium to its **self-diffusion** in a moisture-saturated soil. C. J. B. Mott and P. H. Nye *(Soil Sci.,* 1968, 105, 18-23).-The self-diffusion coeff. was measured by use of a ^{89}Sr-labelled solution of $SrCl_2$. Initially the soil sample in a Perspex cell was treated with 10-' M-SrO, to saturate all the pores, air being evacuated slowly from above the soil level. The process was repeated until the surplus diffusate showed constant composition in successive fractions. Labelled $SrCl_2$ solution was then added and the soil was stirred, constant temp. being maintained. Samples of the solution were tested over a period of a week for ^{89}Sr, the decrease in concn. of which served as a measure of the rate of diffusion of Sr into the soil pores. Calculation of relationships between Sr on the solid particles of soil, that in the solution and their relative effects on the diffusion rate are shown. A. G. POLLARD.

Relative adsorption and desorption of strontium and calcium to and from soils and soil clays; column saturation-displacement and acid displacement. E. O. McLean, C. Lakshmanan and F. P. Miller *(Soil Sci.,* 1969, 107, 206-212).-Samples of the Ap, B'I and CI horizons of a silt-loam were leached with aq. $CaCl_2$–$SrCl_2$. Other samples of the Ap horizon were leached with aq. $AlCl_3$ or HCI, dried and column-leached. Aliquots of the acid-leached soils were limed to various extents, dried and also column-leached. Clay fractions from various horizons were saturated with Ca and Sr and equilibrated with half-symmetry HCI ('symmetry' = cation-exchange capacity in mequiv.). Column-leaching showed marked preferential adsorption of Ca in the Ap horizon, small preferential adsorption of Sr in the B2l sample and equal adosrption of Ca and Sr in the CI sample. Acid leaching eliminated preferential adsorption of Ca in the Ap horizon; liming and incubation of the soil re-established the preferential effect. (16 reL)
A. G. POLLARD.

Movement of algal- and fungal-bound radiostrontium as chelate complexes in a calcareous soil. W. H. Fuller and M. F. L'Annunziata *(Soil Sci.,* 1969, 107, 223-230).-In a sandy loam SOIl (pH 7·8-8·0) there was evidence of the migration of 8'Sr-Iabelled complexes, formed by extracellular secretions of algae (e.g., *Anacysfis* Menegh) and fungi. Leaching with 0·067 M-DTPA (diethylenetriaminepenta-acetic acid) moved 0'2-1'6% of the algal- and fungal-bound "Sr to depths > 20 em in the soil. In control (water-heated) columns the 8'Sr remained within the upper 2·5 em where it had been applied. A. G. POLLARD.

Rôle of organic matter in soil manganese equilibrium. D. J. Cotter and U. N. Mishra *(Pl. Soil,* 1968, 29,439-448). –Addition of ground cottonseed hulls to an alluvial loam (pH 8) before incubation resulted in rapid and marked increase in exchangeable Mn. Some increase occurred even during incubation of air-dry soil, but the increase was considerably greater with incubation at field capacity moisture content, and at 38° rather than at 21°. This increase was derived almost entirely from the soil and not from the added org. material. (18 reL) A. H. CORNHELD.

Forms of manganese in soils as influenced by organic matter and iron oxide. S. G. Misra and P. C. Mishra *(Pl. Soil,* 1969, 30, 62-70).-The retention of Mn^{++} in exchangeable and easily-reducible forms by black, red and alkali soils decreased with extent of removal of org. matter. Increased removal of iron oxides decreased the easily-reducible form of Mn. (13 reL)
A. H. CoRNFIELD.

Successive extraction of soluble organic substances disseminated in rocks. V. Th. Cerchez, F. Vasilescu and I. Ardeu (*Chim, analyf.,* 1969, 51, 219-226).-Procedures are described for successive extraction (20°, 2-4 days) by various solvents, alone or admixed, and for identification of, e.g., saturated and aromatic hydrocarbons, asphaltenes, resins, in each extract by i.r. and u.v. spectrophotometry. Extractions are made on **50–200** g dry powdered rock. Results for various sedimentary rocks (clays, shales, marls, sandstones, limestones, etc.) are in good agreement with those obtained by SiO,-gel column chromatography of one total extract. The method also permits extraction and identification of traces of S.
W. J. BAKER.

Method of extraction and anal)'sis of higher fatty acids and triglycerides in soils. T. S. C. Wang, Yu-Cheng Liang and Wei-Chiang Shen *(Soil Sci.,* 1969, 107, 181-187).-Soils are treated with a I : I mixture of 1'15% HF and 1·25% HCI, and extracted in succession with CHCb- MeOH and methanolic NaOH (pH II '0). The lipids are resolved by t.l.c. Higher fatly acids are further purified by chromatographing on SiO, gel containing 10% Na_2CO_3. Triglycerides are saponified, acidified, and the acids are methylated and determined by g.c. The importance of the acid pretreatment of soils is stressed. (14 rer.) A. G. POLLARD.

Retention of low levels of copper by humic acid. R. I. Davies, M. V. Cheshire and I. J. Graham-Bryce *(J. Soil Sci.,* 1969, 20, 65-71). –Most of the Cu^{++} added to sedge fen peat humic acid (I) was easily removed by washing with HCI, but the strength of binding increased with decreasing Cu content. The capacity of I to retain Cu against extraction by N-HCI was reduced to about half by pretreating I to block CO,H or OH groups separately or together. (15 reL) A. H. CoRNFIELD.

Influence of root CfN ratio on nitrogen availability in soils. R. A. Wood *(Proc. S. Afr. Sug. Technol. Ass., 421ld Anll. COllgr., 1968,* 157-161).-Three soils were mixed with O'5% or with 1% of sugar-cane root material, having CjN ratios of 50 and 100, moistened to 30% capacity with water or a tagged solution of $(NH_4)_2SO_4$ and then incubated at 30° for 112 days. Controls were prepared with no added rool material. With or without N-fertiliser, the time required to reach max. immobilisation of N increased as the CjN ratio and/or the level of root material increased. It is concluded that the CjN ratios within the rhizosphere may strongly influence the N nutrition of crops. Differences in the available N after incubation were due largely to the extent to which mineral N was immobilised by micro-organisms associated with the rhizosphcre. (II ref.) P. S. ARUP.

Microbial populations in stubble-mulched soil. F. A. Norstadt and T. M. McCalla *(Soil Sci.,* 1969, 107, 188-193).-Comparisons of conventional tillage operations with stubble-mulching were made in 3 localities with 2 crop rotations (maize-oats-wheat and wheat-clean fallow replicated 3 times). Sub-tillage, as compared with ploughing, increased the total counts of bacteria actinomycetes and total fungi. *Penicillium urficae,* Banier numbers were higher in sub-tilled than in ploughed soil, and in sub-tilled soils without removal of previous crop residues than when the residues were removed. Sub-tillage did not increase counts of *Trichoderma* spp. as compared with ploughing or sub-tillage after removal of crop residues. Growth of associated organisms appeared to convert wheat straw into a suitable substrate for *P. urficae,* B., numbers of which were greatest in spring and autumn and lowest in summer in sub-tilled soil. Counts of *Trichoderma* spp. showed the reverse

seasonal trend. Reduced crop yields following sub-tillage may be partly attributable to the production of patulin by *P. urticae*, B. (13 rer.) A. G. POLLARD.

Effect of soil amendments on chemical and microbiological properties of an alkali soil. S. A. Z. Mahmoud, S. M. Taha, A. El Damaty and F. Anter *(Pl. Soil,* 1969, 30, 1-14).—Decrease in exchangeable Na and incrcase in exchangeable Ca + Mg during 60 days following treatment of alkali soil and leaching, occurred more quickly when S was added as CaSO. than as elementary S. The rate of change in these constituents was proportional to the amount of S added (sufficient to release Jrom 25% to 100% of the exchangeable Na). The treatments increased counts of total microbial flora, Azotobacter, nitrifiers, and aerobic cellulose-decomposers, and decreased those of aerobic spore.formers, streptomyces, and clostridia. Clover yields also increased with rate of application of S. (33 rer.) A. H. CORNFIELD.

Micro-Qrganic colonisation of forest soil after burning. M. Jalaluddin *(Pl. Soil,* 1969,30, 150-152).-The pattern of microbial succession occurring in an acid sandy soil after bonfires was studied. The fire sites remained largely free of many of the organisms that normally inhabit the soils and were gradually colonised, particularly at the margins, by species characteristic of burnt sites. Micro-organisms isolated from the centre of the burns within a few days of burning probably arose from spores carried by wind and rain. A. H. CoRNFIELD.

Enzymic activity of a soil profile in the Sudan Gezira. M. M. Musa and N. O. Mukhtar *(Pl. Soil,* 1969,30, 153-156).- Urease and dehydrogenase activity in a semi-arid soil under natural vegetation were better indicators of biological activity than was catalase activity. Biological activity was marked in the top 2 in soil layer, but decreased gradually to low levels in deeper horizons. (14 rer.) A. H. CORNFIELD.

Accumulation of nitrate in fresh soils after gamma irradiation. P. A. Cawse and T. White *(J. agric. Sci., Comb.,* 1969,72,331-333). - Increases in N03-·N were found in 8 of 10 soils after irradiation (0,25-2'5 Mrad) with a max. at 0·75 Mrad. Arable or grassland sites gave the max. response to irradiation and there was a highly significant correlation between nitrification in the normal soil and oxidation rate after irradiation. It is suggested that radiation-released NH3 is less limiting to N03--N formation than is the original population or activity of nitrifying organisms which are favoured by alkaline conditions. (12 ref.) M. LoNG.

Electrolytic respirometer for measuring oxygen uptake in soils. J. W. Birch and M. Melville *(J. Soil Sci.,* 1969,20, 101-110).- An instrument providing cumulative records of 02 uptake during incubation of soil is described. (10 ref.) A. H. CORNFIELD.

Visual record of the decomposition of 14C-labelled fragments of grasses and rye added to soil. E. Grossbard *(J. Soil Sci., 1969,* 20, 38-51).—The rate of decomposition of 14C-labelled material placed on or buried in the soil was studied by autoradiography. As decomposition proceeded, 14C disappeared from the site of application and dispersed several cm. Ryegrass decomposed faster than cocksfoot and ground material decomposed differently from plant fragments. Labelled micro·organisms, particularly fungi, appeared in the vicinity of the plant residues as the latter decomposed. These materials, as well as faecal pellets of soil mesofauna which accumulate 14C, were highly resistant to further decomposition. (32 ref.) A. H. CoRNFIELD.

Sulphate reduction in waterlogged soils. C. Bloomfield *(J. Soil Sci.,* 1969 20, 207-221).- Flooded soils were incubated with plant material and either Na2S0. or S. Less H2S was evolved from a periodically waterlogged soil than from a well-drained soil of similar HCI- and dithionite-sol. Fe content. Addition of a slight excess of sol. Fe relative to the added SO_4^{2-} prevented loss of H2S. Native org. matter from a well-drained soil did not promote loss of H2S, but the nature of added plant material affected the FeS: H2S ratio. When either Na2S0. or S was added, more H2S was evolved when lucerne than when rice blade tissue was added before incubation. The pH was little affected by S, but increased when Na2S0. was added; more free H2S was evolved under the latter conditions, suggesting that it is the initial mobilisation of Fe that governs the distribution of reduced S, rather than the pH-solubility relations of FeS. (35 rer.) A. H. CORNFIELD.

Effect of sodium chloride and sulphate on sulphur oxidation in soil. P. Keller *(Pl. Soil,* 1969 30, 15-22).-The rate of oxidation of S to SO_4^{2-} during incubation of two calcareous soils decreased with increasing level of added NaC!. This rate was reduced slightly by 0'25% NaC! and was completely inhibited by 10% NaCI. Max. recoveryofS as SO_4^{2-} after 70 days of incubation was ~54%. Addition of SO_4^{2-} (3000 ppm S as Na2S0.) decreased the rate of oxidation only slightly. (II ref.) A. H. CORNFIELD.

Effect of some factors on transformation of elemental sulphur in soils. S. L. Chopra and J. S. Kanwar *(J. Indian Soc. Soil Sci.,* 1968, 16, 83-88).-Reactions between S and various soil components are influenced by concn., time, temp. and the cations involved. All reactions studied were pseudo-unimol., and were accelerated by rise in temp. Single-cation soils (Na, K, Ca, Mg) prepared from a sandy loam were mixed with water (⅓ field capacity) and incubated at 25° or 35° with S for 1-7 weeks. Rates of reaction were compared with those of the whole soils. The sp. reaction rates were influenced by the cations in the order: Na > H > K > Ca > Mg. The amount of S transformed was greater in a soil of pH 7·8 than in one of pH 5·35.
A. G. POLLARD.

Soil testing and plant analysis. Symposium sponsored by Soil Science Soc. of America, Crop Science Soc. of America and American Soc. of Agronomy. 1967, 114 pp. (Madison, Wis.: Soil Science Society of America, Inc.). S. C. H.

Chlorophyll compounds and nitrogen availability in West Indian soils. I. S. Cornforth *(Pl. Soil,* 1969,30, 113-116).-The amounts of chlorophyll·type compounds *(CC)* extracted by 90% aq. acetone from 70 West Indian soils were inversely correlated with pH. CC were significantly correlated with N availability to maize in pot tests and with mineralisable N for soils only in the pH range 5·0-5·9. CC may persist in acid soils. A. H. CoRNFIELD.

Volatilisation of ammonia from urea (and urine) in soils under pasture. J. R. Rajaratnam *(J. Aust. Inst. agric. Sci.,* 1969, 35, 57).-An apparatus ('volatilisation chamber') for collecting volatilised NH3 is described. On the soils studied (gley, podzolic and black earth), volatilisation, especially during summer or under conditions of high stocking rates, is a serious factor in the pastoral ecosystem and is likely to limit the levels of available N in soil.
E. G. BRICKELL.

Effect of nitrogen fertilisation on release of soil nitrogen. Z. Aleksic, H. Broeshart and V. Middelboe *(Pl. Soil,* 1968,29, 474-478).-Pot tests with millet in soils from 9 countries, treated with I5N-labelled (NH.)2S0. (50-200 kg N per hal showed that the % N in the plant derived from the applied N increased with level of application. The availability of native soil N, calculated from the ratio of I5N enrichments of fertiliser and plant samples, was not affected by rate of N fertilisation in any of the soils.
A. H. CoRNFIELD.

Manganese status of black soils of Indore District. O. P. Sharma and D. A. Shinde *(J. Indian Soc. Soil Sci.,* 1968, 16, 65–69).— Numerous surface soils are examined for various forms of Mn and associated soil properties. In general, the available (water-sol. + exchangeable) Mn contents were low whereas easily reducible, active and total Mn were high. Significant positive correlations are shown between available Mn (I) and org. C and org. C and clay contents, between I and Mn extracted by wheat seedlings, but not between I and CaC03 content. (16 rer.)
A. G. POLLARD.

Manganese–phosphate reactions in aqueous systems and effects of applications of monocalcium phosphate on availability of manganese in an alkaline fen soil. S. G. Heintze *(Pl. Soil,* 1968, 29, 407-423).- Electrometric titrations and chemical analyses of MnSO.-H3PO. aq. systems showed that the Mn phosphate compounds formed were usually completely pptd. at pH 6'5-9'0; phosphate dressings may benefit soils containing toxic levels of Mn. Application of $Ca(H_2PO_4)_2$ (≡ 750-1500 lb superphosphate per acre) to an alkaline Mn-deficient soil decreased grain yields. When neutral and alkaline Mn·deficient fen soils were incubated with Ca(H2PO.)2, with and without added Mn, the phosphate treatments had little effect on soil pH or on exchangeable and easily reducible Mn. (23 rer.) A. H. CoRNFIELD.

Influence of boron, nitrogen, and potassium on yield, nutrient uptake and abnormalities of cotton. W. N. Miley, G. W. Hardy, M. B. Sturgis and J. E. Sedberry, jun. *(Agron. J.,* 1969,61,9-13).- The effects of applying N (54-80 kg), K (45 kg), and B (1.7 kg per hal to a B·deficient sandy loam (pH 7·3), on the performance of colton was studied. B or N increased yields only when the other was applied, whilst K had no effect. Plant B% was increased by B, decreased by N, and unaffected by K. At the early and medium boll stages, B decreased leaf petiole N%. At the early square stage, B decreased petiole N% only when N was applied. At 2 of 3 maturity stages, B decreased whilst N increased the occurrence of

ringed petioles (symptom of B deficiency). At the early square stage, K increased ringed petioles where no B was applied. (29 ref.)
A. H. CORNFIELD.

Ionic balance and com [maize] growth in a Port Byron soil. R. W. Blanchar and L. R. Hossner *(Agron. J.,* 1968, 60, 602–605).—The org. anion content of maize plants varied with source of N (NH_4^+ or NO_3^-) and level of applied $CaCl_2$, but was not related to maize growth. Plant wt. was inversely related to pU (negative log: of the ionic strength of the SOll solutIOn). Plant Ca% was lmearly related to pCa, plant Mg% was related to pMg-o·5 pU and plant K% to pK-pU in the soil solution, respectively. Maize P% was higher where NH_4^+ than where NO_3^--N was applied; NH_4^+ stimulated uptake of Sand P. Maize Cl% increased with level of applied $CaCl_2$. (14 rer.) A. H. CORNFIELD.

Soil improvement trials using scarification and fertilisation in stagnant white spruce plantations, Quebec, Canada. J. D. Gagnon *(Pl. Soil,* 1969,30, 23-33).-Scarification (incorporation of ground vegetation into the soil) of a deteriorated sandy soil resulted in marked increase in growth of white spruce *(Picea glauco)* after 3 years. Additions of buckwheat residues, NPK fertilisers and composts in addition to scarification did not improve growth further. In the root zone of treated soils over 5 years, K increased, P and pH remained almost constant, whilst total Nand org. matter decreased. (16 ref.) A. H. CoRNFIELD.

Effect of conifers on available soil nitrogen. E. L. Stone and R. F. Fisher *(PI. Soil,* 1969, 30, 134-138).- The N% in herbaceous vegetation growing under larch and pine (10–14-year old) was significantly higher than that of the same species growing in the open within a few metres of each conifer plantation. Average NH_4^+-N was higher in the canopy position than in the open for the combined locations at each sampling date, and for each location over all dates. Average NO_3^--N was also higher (canopy position) at each location for all dates combined but over all locations only in May and June. Increased mineralisation rather than increased fixation of N is the more probable explanation.
A. H. CoRNFIELD.

Fixation and plant-recovery of ^{137}Cs. E. J. Evans and A. J. Dekker *(Soil Sci.,* 1969, 107, 175-180). –Relationships are examined between the fixation of ^{137}CS and the moisture content and previous cropping history of the soil. Two acid soils, *(a)* a fine sandy loam and *(b)* a silt loam, were used; half of each soil was cropped heavily with oats and then treated with $Ca(NO_3)_2$ and $Ca(H_2PO_4)_2$ with added ^{137}CS and cropped with lucerne. Fixed ^{137}Cs (not extracted by neutral N-NH_4OAc) increased in moist soils during incubation at 75' F to extents greater in samples precropped with oats than when not precropped. Both soils fixed more ^{137}CS when cropped with lucerne than when uncropped before incubation, but on further cropping with lucerne the difference between cropped and uncropped soils in this respect disappeared. In both soils, alternate wetting and drying caused greater fixation of ^{137}CS than did moist or dry incubation at 0', 36' or 75' F. NH_4Cl and KCl leached more ^{137}CS from soils than did $MgCl_2$, $CaCl_2$ or $AlCl_3$ but appreciable amounts of ^{137}CS were taken up by oats from that remaining after leaching with NH_4Cl or KCI. Thus, ^{137}CS added to, and fixed by soil seems to be available to plants and NH_4^+ causes a greater intake of ^{137}CS by plants than other cations.
A. G. POLLARD.

Correlation between plant uptake and different methods of extraction of soil cobalt. S. K. Rana and G. J. Ouellette *(J. Indian Soc. Soil Sci.,* 1968, 16, 89-91).- Co was extracted from soil samples from 9 major soil series and compared with the amounts removed by oats from the soils. Close correlation was shown between the Co extracted from the soils by 2·5% AcOH (pH 2·5) and that taken up by the plants. Neither N-NH_4OAc nor 0·01 N-HCI extracted amounts of Co showing any consistent relationship with the plant uptake. A. G. POLLARD.

Fonns of potassium in soils of Rajasthan. S. Dhawan, B. L. Pareek and C. M. Mathur *(J. Indian Soc. Soil Sci.,* 1968, 16, 55-60). -The total K content of these soils is considerable but its availability to plants is inadequate, particularly in soils on which intensive cropping is practised. Determinations of water-sol., exchangeable and non-exchangeable K were made. Fairly wide variations in pH, texture, org. C, $CaCO_3$ and cation-exchange capacity were found to exist among these soils. Differences in K status between the soils are related to soil-forming processes and clay content. (17 ref.) A. G. POLLARD.

Relationship between exchangeable and boiling nitric acid. extractable potassium in seventy-five West Virginia soil series. E. M. Jencks *(Agron. J.,* 1968, 60, 636-639).-The contents of exchangeable K *(K_{ex})* and boiling HN03-extractable K *(Kacld)* in 75 soils were not related to either topographic position or soil series. Amounts of both forms were higher in limestone than in non-limestone soils. For the soils as a whole, both K_{ex} and *Kacld* were closely related to cation-exchange capacity and pH, but there were considerable variations among topographic positions and parent material classes. K-fixing capacity was more closely correlated with *Kacid* than with K_{ex} for the soils as a whole. There was generally good positive correlation between K_{ex} and *Kacid* in the aggregate and among topographic groups and parent material classes. In bottomland non-limestone soils there was a high negative relation between the two forms of K, due in part to the high org. matter content of these soils. (24 ref.)
A. H. CORNFIELD.

Available potassium in soils of Madhya Pradesh. O. P. Verma and G. P. Verma *(J. Indian Soc. Soil Sci.,* 1968,16, 61-64).-The available (water-sol. + exchangeable) K in soils from 24 districts is determined. Values obtained are correlated with other soil characteristics, notably pH and contents of $CaCO_3$ and org. C.
A. G. POLLARD.

Limitations in isotopic measurements of labile phosphate in soils. F. Amer, S. Mahdi and A. Alradi *(J. Soil Sci.,* 1969, 20, 91-100).- Addition of reactive Fe_2O_3 to calcareous soils gave erroneous results for labile P, especially when carrier-free 32p was used. Methods involving the use of 0·2 ppm P carrier solution or the addition of an unlabelled carrier to a labelled soil may give satisfactory labile P measurements in soils of low and medium phosphate-fixing capacity. All methods tested (carrier-free, carrier, and inverse dilution) gave unsatisfactory results for high phosphate-fixing soils. (18 ref.) A. H. CORNFIELD.

Photoelectric colorimetric method for detennining nitrate-nitrogen in soils. G. C. Shukla and M. Singh *(J. Indian Soc. Soil Sci., 1968,* 16, 77-81).- The method is based on the formation of a violet-coloured compound of NO_3^- with Crystal Violet at pH 6'0, and its extraction by chlorobenzene. Such extracts containing NOs-N up to 1·5 ppm conform with Beer's Law. A. G. POLLARD.

[A] Extraction of organically bound iron from sandy soils. H. Wiechmann and H. Grimme. [B] Method for extraction of organically bound iron from soils. H. Grimme and H. Wiechmann *(Z. PflErniihr. Bodenk.,* 1969, 122, 260–267; 268-279).-[A] Six sandy soils were extracted repeatedly with 0·01–0·1 N-NaOH, with or without 0·01 M Na-EDTA. Increasing NaOH concn. extracted more org. C from the Ahe than from the BI or B, horizons; addition of Na-EDTA also increased extraction of org. C and organically bound Fe in certain cases. Distribution of org. C and organically bound Fe in the different horizons is discussed.
[0] NaOH and NaOH + EDTA were satisfactory extractants. With increasing NaOH concn. increasing amounts of org. matter were extracted but Fe in the extracts increased only up to a certain NaOH concn., which was different for each horizon. It was found that ~ 50% of oxalate-sol. Fe could be extracted by NaOH from Ah horizons and ~ 10% from B horizons. (to reL) M. LONG.

Soil acidity and liming. Ed. R. W. Pearson and F. Adams. 1967,274 pp. (Madison, Wis.: Amer. Soc. Agronomy, No. 12).- Basic chemistry of soil acidity. N. T. Coleman and G. W. Thomas (128 ref.). Physiological effects of soil acidity. W. A. Jackson (420 reL). Liming materials and practices. S. A. Barber (t 11 ref.). Crop response to lime in the southern United States aod Puerto Rico. F. Adams and R. W. Pearson (107 ref.). Crop response to lime in the mid-western states. C. M. Woodruff (48 reL). Crop response to lime in the north-eastern states. M. E. Weeks and D. J. Lathwell (43 reL). Crop response to lime in the western states. T. L. Jackson, E. G. Knox, A. R. Halvorson and A. S. Baker (17 ref.).
S.C.H.

Evaluation of sodium as a nutrient for three horticultural crops. J. K. Ferguson *(Chilean Nitrate agric. Servo Inf,* 1969, No. 108, 4 pp.).-Practical trials with $NaNO_3$ on red beet, cabbage and carrot are reported. Increased yield, rate of maturity, quality and financial return are claimed. E. G. BRICKELL.

InOuence of subterranean clover pastures on soil fertility. m. Effect of applied phosphorus and sulphur. E. R. Watson *(Aust. J. agric. Res.,* 1969, 20, 447-456).-Annual soil and pasture measurements were made using superphosphate over 11 years. The relationship between age of pasture and soil N was linear at the lowest level of fertiliser but curvilinear at all other levels. There was a curvilinear relationship between total superphosphate and soil N. Pot trials at the end of the period showed that the different levels of

superphosphate had induced large differences in the P status of the soil but had had much smaller effects on that of the S. (18 ref.)
E. G. BRICKELL.

Potassium movement in fallowed soils. F. C. Boswell and O. E. Anderson *(Agron. J.,* 1968, 60, 688-691).-The movement of K (317 and 1900 kg per hal after surface application of KN03 in Nov. was studied over 76 weeks in fallowed sandy loam and sandy clay loam soils. 14 weeks after treatment virtually all the applied K was found in the top 30 cm of both soils except for the high K rate on the loamy sand. The high rate K tended to move faster and to greater depths compared with the moderate rate. Irrespective of application rate the highest concn. of K was found in the 15-30 cm depth of both soils 76 weeks after application. The data indicate that for these two soils K will not leach below the root zone during the growing season of most field crops. (14 ref.)
A. H. CORNFIELD.

Recovery of potassium fertiliser from mixed crystals of earnallite and kainite produced by solar evaporation of inland (Kuch) bitterns. M. V. Chandorikar, D. J. Mehta and D. H. Oza *(Chem. Age India,* 1969, 20, 1I6-118).- Mixtures containing KCI 22, MgSO, 19, $MgCl_2$ 17, and NaCI 12%, suspended in liquors containing 13-23% of MgCl, were, floated with 2% of octadecyl aminoacetate and 10% of oleic acid in white spirit. The concentrate was decomposed with water to yield a fertiliser containing 47-49% of K,O (wet). Overall K rccovery was 79%. K. GRAUPNER.

Nitrogen fertilisers as a petrochemical operation. Y. N. Kanaan *(WId Cllem., Beirut,* 1969, 1,63-60, 58-55).- The manufacture of NH3 by high pressure synthesis is described, the mcrits and limitations of large vs. small NH3 plants are discussed, togethcr with the use of NH3 for making fertilisers such as NH_4 and NH,Ca nitrates, $(NH_4)_2SO_4$ and urea. Emphasis is placed on co-operation between the petrochemical and fertiliser industries. (In English.)
W. J. BAKER.

Deterioration of jute in contact with various fertilisers. S. Varma and V. P. Singh *(Technology, Q. Bul/. Ferlil. Corp. India,* 1968, 5, 235-236).-Contact between a hygroscopic fertiliser and the jute bag, evcn when polythene-lincd, causes loss in mechanical strength. The extcnt of swelling and disengagement of individual fibres when they are dipped into saturated solutions of various fertilisers was determined. Urea solution caused swelling and complete disengagement, whereas $(NH_4)_2SO_4$ caused no swclling. Between these extremes, swelling and disengagement decreased in the order NH,N03 > NH,Cl > diammonium phosphate > monoammonium phosphate> KCI. (II ref.) M. GREENAWAY.

Organic chelating compounds. Chemolimpex Magyar Vegyiaru Kulkereskedelmi Vallalat (Inventors: J. Szava, M. Sarosi and R. Totos) (B.P. 1,159,462, 16.6.67).-The compounds, which form chelates with, e.g., Fe, Co, Ni and Mn, suitable for trealing iron chlorosis in plants, have general formula $(Ar\text{-}CH_2\text{-})_2A$, where Ar is a 2-hydroxyphenyl radical with a halogen or acid (carboxylic or sulphonic) group in the5-position and A is -NH- or -NH'$[CH_2]_2NH$-. Prep. is from NH3 or $NH_2(CH_2)_2NH_2$ by reaction with 2 moles of HCHO and treatment of the product with a 4·substituted phenol. E.g., dimethylamino-bis·2-hydroxyphenyl-5-sodium sulphonate is prepared by reacting 40% HCHO solution with aq. NH3 at 25-30° and then adding Na p-phenolsulphonate solution neutralised with NaOH and refluxing for 8 h. S. S. CHISSICK.

Plant Physiology, Nutrition and Biochemistry

Effects of superficial wax on leaf wettability. P. J. Holloway *(Ann. appl. BioI.,* 1969,63, 145-153).-Contact angles *(a)* of water were mcasurcd on a variety of leaf surfaces *(LS)* before and after removal of wax (with $CHCl_3$), and on smooth films of the isolated superficial waxes. Differences in wellability of *LS* were not wholly accounted for by the differing chemical and hydrophobic properties of thcir waxes. Waxes isolated from leaves having $\alpha < 90°$ were usually more hydrophobic than thc *LS* itself. On most leaves having $\alpha > 90°$, wax was the dominant factor governing water repellency; the isolated wax contributed at least 60% to the α measured on the *LS.* Additional factors, such as roughness, responsible for the occurrence of $\alpha > 110°$ on ccrtain *LS,* resided in the wax layer. The hydrophobic properties of some leaves were unaffected by washing with $CHCl_3$. (32 ref.)
A. H. CoRNFIELD.

Regular yearly variations in effect of irrigation in southern dry regions. G. Rappe *(Pl. Soil,* 1968, 29, 362-368).-Wheat and oats were sown at 2-weekly intervals through the year and grown for 28-35 days in photothermostats under constant conditions of light, temp., water supply and atm. C02 concn. Dry mailer yields of tops and roots were consistently approx. twice as high from winter and early spring sowings than from summer and late autumn sowings. Variations in yield could not be explained on the basis of soil analysis. If similar results are applicable in other regions it is suggested that where irrigation water is in short supply it should be applied in the period of the year where growth potential is at a max. (16 ref.) A. H. CORNFIELD.

Critical plant nutrient composition values useful in interpreting plant analysis data. S. W. Melsted, H. L. Mollo and T. R. Peck *(Agron. J.,* 1969, 61, 17-20).-Critical composition values (levels of N, P, K, Ca, Mg, Mn, Fe, B, Cu, Zn, and Mo) for maize, soyabean, wheat and lucerne used for diagnostic interpretation of the nutrient status of the plants are given. Maize and soyabean leaf nutrient compositions are tabulated for various locations and times of sampling. A. H. CoRNFIELD.

Trace metal deficiencies in orange trees. I. Relationships between deficiency symptoms and trace metal content of orange tree leaves. E. Primo, J. M. Carrasco and P. Cunat *(Revta Agroqulm. Teenol. Aliment.,* 1969, 9, 240--253).-Results of foliar analysis for B, Fe, Mn and Zn are reported for a large number of orange trees from normal orchards and orchards showing deficiency symptoms. Symptoms of deficiencies of Fe, Mn and Zn were as reported in the literature; symptoms of B deficiency were the development of gummy granules in the fruit albedo and exceptionally coarse venation of the leaves. Symptoms were particularly noticeable during maturation of the fruit, when the colour change from green to orange was delayed in areas of peel above the gummy granules. Normal levels found were: B, 35–68; Mn, 25-58; Fe, 110–150; and Zn, 25-60 ppm. Clear deficiency symptoms were present at leaf contents less than 30, 18, 60 and 19 ppm, respectively; at intermediate levels occurrence of symptoms varied with variety and growth conditions. (30 ref.) E. C. APLING.

Cause of differential susceptibility to zinc deficiency in two varieties of navy beans, *Phaseolus .ulgaris* L. J. E. Ambler and J. C Brown *(Agroll. J.,* 1969, 61, 41-43).-Studies in split medium, soil, and nutrient solutions showed that the Sanilac variety (susceptible to Zn deficiency) absorbed more Fe and P than the Saginaw variety (less susceptible) where Zn supply was low and Fe and P relatively high in the growth medium. A. H. CoRNFIELD.

Response of plant species to concentrations of zinc in solution. II. Rates of zinc absorption and their relation to growth. M. D. Carroll and J. F. Loneragan(Aust. J. *agric. Res.,* 1969,20,457-463). -Rates of Zn absorption by whole plants of eight species grown for 46 days in a flowing culture system were determined over a range of constantly maintained Zn concn. Absorption increased almost linearly from 2 to 400 ng of Zn/g of fresh roots/day as concn. increased from 0·0\ to 6·25 μM. At particular Zn concn., rates of absorption were about one-tenth of those reported for excised roots and up to 50 times greater than calculated rates of absorption from standard culture soln. E. G. BRICKELL.

Interactions of zinc and phosphorus with soil temperatures in rice. K. C. Sharma, B. A. Krantz, A. L. Brown and J. Quick *(Agron. J.,* 1968,60, 652–655).—In glasshouse tests, growth of waterlogged rice in a clay loam increased with soil temp. (15-30°). At 15°, application of 5-25 ppm Zn (ZnSO,) on the soil basis increased Zn% in the roots, but not in the tops; the response to Zn decreased with increasing soil temp. At 15°, Zn% in tops of plants grown with added Zn was lower than that in plants grown without added Zn at 22° and 30°. Application of 25-400 ppm P, as Ca(H2PO,)2, reduced Zn% in tops but not in roots. Plant P% was not affected by Zn applications. (12 ref.) A. H. CoRNFIELD.

Effects of nitrogen, phosphorus, and potassium and their interactions on yield and kernel weight of barley in hydroponic culture. L. B. MacLeod *(Agron. J.,* 1969, 61, 26-29).—Barley plants were grown in sand culture receiving 10·200 ppm K and N, and 25-100 ppm P, in factorial combination. There were significant interactions of NK and NP on tillering, yield and kernel wt. of grain. Adequate P and K were required to obtain max. response to increasing levels of N; K was more important than P in this respect. Max. grain yields were obtained when the plant tissue, sampled at heading, contained more than 4% N, O·7% P, and 4% K (dry basis). These critical nutrient levels were higher than for barley grown in soil. A. H. CoRNFIELD.

Effect of aluminium on lucerne seedlings. L. Dessureaux *(Pl. Soil,* 1969, 30, 93-98).-Addition of 20–100 ppm Al^{3+} to the nutrient

solution had no toxic effects on the germination metabolism of two lucerne genotypes or of their F. hybrids. However, seedling growth (measured by appearance of the unifoliate leaf) was retarded by 20 ppm Al and almost completely suppressed at higher concn.; this was accompanied by lack of root elongation. There were some strain differences in tolerance to Al.

A. H. CoRNFIELD.

Alkali metal interactions in the nutrition of sugar-beets. A. Mahmoud EI-Sheikh *(Diss. Abstr., B,* 1967, 28, 1306).- lnteractions of K and other alkali metals are examined in relation to absorption of Fe, mineral composition and possible substitution of other alkali metals for K in the nutrition of beet. Analysis of culture solutions at intervals during plant growth yielded data comparable with those obtained by excised root studies and provided additional evidence of the existence of carrier sites in ion absorption. Rb and K appear to be absorbed by two carrier sites, one indifferent to Na and the other having a higher selectivity for K and being the only site for Na absorption. The 'cation balance' hypothesis is put forward to explain the results observed for K, Rb and Na. Li and Cs were both toxic to sugar-beet.

A. G. POLLARD.

Effects of temperature on growth and nutrient uptake in subterranean clover during recovery from phosphorus stress. I. Growth changes. II. Phosphorus uptake and distribution. D. Bouma and E. J. Dowling *(Aust. J. bioi. Sci.,* 1969, 22, 505-514; 515-521).- I. Effects of temp. on the growth of *Trifolium subterraneum* L., cv. Mt. Barker, at different P levels, and on growth changes during the early stages of recovery from P deficiency were investigated. Seedlings germinated under 25/20° day/night temp. conditions were grown on nutrient containing 170 and 850 μg of P, respectively, and grown under 15/10, 21/16 or 27/22° temp. regimes. After 18 days pre-treatment, seedlings were transferred to full nutrient concn. (5 ppm of P) and leaf areas and dry wt./plant were measured over 7 days. Both parameters at the end of pre-treatment increased with temp., but reduced temp. response at low P levels suggested a higher P requirement at higher temp. Deficient plants had lower relative growth rates, the effect being most marked at high temp. Relative rates of leaf expansion were not affected. Differences in yield between P levels and growth response after removal of stress would thus be expected to decrease with temp. (13 reL)

II. In seedlings grown under the above conditions, the P uptake in the 7 days following transfer to non-deficient medium and its distribution to shoots was greater at high temp., suggesting a greater demand under these conditions. Uptake of P by plants recovering from P stress was little affected by temp. and was always greater than that by non-deficient plants. At higher temp., the proportion distributed to the leaves was greatest, owing to the greater severity of the stress under these conditions. J. B. WOOF.

Effects of changes in boron nutrition on growth and development of subterranean clover. D. Bouma *(Aust. J. bioi. Sci.,* 1969, 22, 523-533).-seedlings of *Trifolium subterraneulll* L., cv. Mt. Barker, were placed in pre-treatment nutrient soln. containing different amounts of B in order to obtain groups of plants of different B status whose subsequent growth could be followed. In the early stages, total dry wt. increase was not affected by B deficiency but leaf matter increased at the expense of rootlets. Distribution of dry matter to new leaf tissue was progressively restricted at later stages of deficiency. Plants deficient in B showed a greater wt. increase in existing leaves when transferred to soln. without B than to soln. with B, but the growth rate of leaves formed after transfer was less. After removal of B stress, assimilates were preferentially distributed to Ihe roots. It is suggested thai wt. distribution reflects changes in demand for assimilates resulting from the effect of B on meristematic activity. (20 reL)

J. B. WOOF.

Calcium requirement of plants. J. F. Loneragan and K. Snowball *(Aust. J. agric. Res.,* 1969,20,465-478).- The quant. relationships between growth and Ca concn. in grasses, cereals, legumes and herbs were re-examined for Ca concn. of O'3-1000 μM in flowing culture soln. of pH 5·7. The results indicated that variation in the conditions of ea supply, under which Ca deficiency develops, partly accounts for the wide range of critical values used by various workers to diagnose deficiency in plants. (27 ref.)

E. G. BRICKELL.

Rate of calcium absorption by plant roots and its relation to growth. J. F. Loneragan and K. Snowball *(Aust. J. agric. Res.,* 1969, 20, 479-490).--Grasses, cereals, legumes and herbs grown in flowing culturesoln. of pH 5·7 with Caconcn. of O'3-1000 μM were studied. Increasing concn. from 0·8 to 10 μM increased the avo rates of Ca absorption (similarly for increasing concn. to 100 or 1000 μM) but decreased effectiveness. Conditions of ea supply, under which deficiency develops in soln. and in soils, must be taken into account when assessing the relative susceptibility of plant species to Ca deficiency. (17 ref.)

E. G. BRICKELL.

Interaction between calcium level and nitrogen source on growth and ^{45}Ca distribution in subterranean clover. C. R. Millikan, B. C. Hanger and E. N. Bjarnason *(Aust. J. bioi. Sci.,* 1969, 22, 535–544). -seedlings of *Trifolium subterraneulll* L., cv. Dwalganup, were supplied with basic nutrient containing K^+, Mg^{2+}, PO_4^{3-} and Fe Na EDTA, together with NH_4^+, NO_3^- or urea as N sources at both high (8'0) and low (0'1 mequiv./l) of Ca^{2+}. With NH_4^+ and NO_3^- together, the yield was independent of Ca level, but when they were added separately, growth was best under normal Ca nutrition. With urea as N source, growth was best at low Ca levels. The main factor determining distribution of ^{45}Ca was the concn. of ea in the substrate. At low levels, the % retained in the roots was the greatest and this was most noticeable in plants fed with NH_4^+ and least with $NH_4^+ + NO_3^-$. The concn. of ^{45}Ca in plant tops was equal in the presence of urea or $NH_4^+ + NO_3^-$, and greater than with NO_3^- or NH_4^+ alone. Lamina : petiole, leaf edge : leaf centre and peliole distal : petiole proximal ratios of ^{45}Ca concn. showed differences with the different N sources when the Ca concn. was low but nOI when it was normal. (27 ref.)

J. B. WOOF.

Soluble and total silicon in sugar-cane. R. L. Fox, J. A. Silva, D. L. Plucknett and D. Y. Teranishi *(Pl. Soil,* 1969,30,81-92).- Total Si and sol. Si (that extractable with 2% CCl_3CO_2H) were higher in leaf sheaths than in leaf blades. Total Si was much higher in leaf sheaths and blades than in internodal tissue. Sol. Si was highest in least mature, whilst total Si was highest in recently mature, tissue. There was little change in total Si with time after the leaf had matured. Total Si in leaf blades was more responsive to basic slag applications than was total Si of leaf sheaths, but the reverse was true for sol. Si. The mature stalk tended to be the most responsive tissue. Both sol. and total Si in the plant were affected by the Si present in the soil solution and irrigation water. (19 ref.)

A. H. CORNFIELD.

Silica in the oat plant. IV. Silica content of plant parts in relation to stage of growth, supply of silica, and transpiration. K. A. Handreck and L. H. P. Jones *(PI. Soil,* 1968, 29, 449-459).- Although the SiO_2 % of the tops of oat plants increased with level of SiO, in the soil solution of three soils, the pattern of its distribution in the various plant parts was similar on all soils. The SiO_2 % in the dry matter was highest in the palea, lemma glumes, awn and leaves. The pattern of Si02 distribution in the tops suggests that monosilicic acid and waler move concomitantly in the transpiration stream and that SiO, deposition is proportional to water losses in the various parts. (16 ref.) A. H. CoRNFIELD.

Uptake of silica by *Trifolium incarnalum* in relation to concentration in the external solution and to transpiration. L. H. P. Jones and K. A. Handreck *(PI. Sail,* 1969,30, 71-80).- Uptake of Si by crimson clover increased with monosiJicic acid [Si(OH),] level in the culture solution (0'4-60 ppm SiO_2) or in the soil solution (7-67 ppm SiO_2). Amounts of SiO, in the tops were always less than those which were theoretically carried to the roots in the mass flow of water. The SiO, content of the roots was higher than in the corresponding tops and seemed to be largely associated with the epidermis; also the Si(OH), concn. in the xylem sap is lower than that in the external solution. It is suggested that a proportion of the Si(OH), from the transpiration stream is excluded by the plant, probably by a barrier in the rool. SiO, distribution among the parts of the plant top was unaffected by the level of Si(OH), in the external solution or by the quantity which entered the tops. SiO, deposition is proportional to water losses in the various parts. (See preceding abstr.) (13 ref.)

A. H. CoRNFIELD.

Effect of low temperatures on fatty acid biosynthesis in plants. P. Harris and A. T. James *(Biochem. J.,* 1969, 112, 325-330).- Rate of formation of unsaturated fatty acids *(UFA)* increases with decrease of temp. in bulb (narcissus) tissue, but not in plant (castor oil and spinach) leaf tissue or in intact green algal *(Chlorella vulgaris)* cells. In bulb tissue, [O,] is rate-limiting for synthesis of *UFA* at $\leq$ 10°, but increases with temp. at high $[O_2]$. Photosynthetic tissues do not respond to lower temp. or to increased [O,J in presence of light, probably because of photosynthetic production of excess of 0 2. [HCO,-] only slightly affects formation of *UFA.* The increased content of *UFA* in plants at lower temp. is attributed to increased [02] in solution. (17 ref.)

J. N. ASHLEY.

Temporal changes in carboxylate content of ryegrass with stepwise chaoge in nutrition. W. Dijkshoorn, D. J. Lathwell and C. T. de Wit *(PI. Soil,* 1968, 29. 369-390).- Changing the type of nutrient supply during growth in nutrient cultures showed that translocatIOn of KN03 to the shoot and subsequent N03- metabolism was the only process capable of supplying the shoot with sufficient carboxylates and of removing the excess from the foliage to the root system so as to maintain normal carboxylate content. Absorbed HC03- was a good source of carboxylates in the roots, but the rate of translocation to the plant tops was slow. Constancy of carboxylate concn. in the dry matter was related to the **early** establishment of the proportion of carboxylates to dry matenal in the new growth, making it independent of subsequent changes m water content of the tissues. (24 ref.) A. H. CoRNFIELD.

Accumulation and translocation of sorbitol in apple phloem. R. L. Bieleski *(Aust. J. bioi. Sci.,* 1969,22, 611-620).- I·year old shoots of *Malus sylvestris* (L.) Mill. cv. Granny Smith were used on the tree for translocation experiments or harvested and stripped to provide phloem tissues for accumulation studies. Using "C02, it was shown that sorbitol (1) rather than sucrose is the main carbohydrate involved in phloem transporl. In freshly excised or aged (24 h) phloem, sucrose, glucose and I were actively accumulated. the rates being highest at high concn. and with aged prepn. When sucrose or glucose was accumulated, the former was the main product and no 1 was detected. When [was accumulated, it was the main product, though in aged phloem, sucrose formed as well. 1 was readily accumulated from conc. soln. but only slowly metabolised. Thus 1 probably preferentially accumulates in the phloem where it is metabolically inert and remains unchanged until it reaches the destination tissue where, as in aged phloem, it is readily utilised. (12 ref.) J. B. WOOF.

Nutrient status effects on loss of amides and amino acids from pine roots. G. D. Bowen *(PI. Soil,* 1969, 30, 139-142).- Loss of amides and amino acids from *Pillus radiata* roots was 2·5 times greater from roots grown in P·deficient nutrient than from those grown in complete nutrient and 10 times greater than from plants grown in N·deficient nutrient. The major factor in increased loss from P·deficient plants was an increase in free amides and amino acids in these plants. (13 ref.) A. H. CORNFIELD.

Effect of nitrogen and sulphur on nitrogen fractions of barley at various early stages of growth and on yield and amino acid composition of grain. W. Eppendorfer *(PI. Soil,* 1968,.29, 424-438).- Pot tests with barley analysed 23-55 days after sowmg showed that the contents of total and N03-·N, free amino acids and amides varied with time, level of applied Nand S, and level of light intensity preceding sampling. At 55 days of age, high N without S caused a Hold increase in asparagine content and lesser increases in glycine and serine. S deficiency, as judged by content of S·amino acids and amides, increased with time. There was a positive interaction between effects of Nand S on the yield and cystine and methionine contents of grain. Absence of applicd S reduced the cystine content of grain much more than that of methionine. particularly at the high level of applied N. (24 ref.) A. H. CORNFIELD.

Mucilaginous layer of citrus roots as part of the rhizosphere. E. Brams *(PI. Soil,* 1969, 30, 105-108).- The prescnce of a mucilaginous layer on the young feeder roots of citrus was confirmed. Leaching the roots with N·NH,CI removcd the layer and the m,cro·organisms associated with il. The extract contained 17-21 % of the specific root Ca'+ and K+ and **3–1·4** %of the Cu'+ and **Al^{3+},** respectively. The mucilaginous layer is **probably** part of the rhizosphere and may act as a reservOIr of plant nutrients.
A. H. CORNFIELD.

Enzymic breakdown of pectin and acid·inhibition of the infection of *Medicago sati,a* roots by *Rhizobium meli/oti.* D. N. Munns *(PI. Soil,* 1969,30, 116-120).- Change in pH of the nutrient over the range **4·0–6·0** had little effect on pectinase production, but both nodulation of roots by *R. meliloti* and the rate of breakdown of pectin by pectinase, which were low at pH 4-5, increased with pH. Since the production of pectinase precedes infection, it is possible that nodulation may be limited by the effect of low pH on pectinase activity. A. H. CORNFIELD.

Seedling growth of **summer-dormant** and **non-dormant** ryegrasses in relation to temperature. J. H. Silsbury *(Aust. J. agric. Res., 1969,20. 417-423).–Lolium rigidum* Gaud. and a summer·dormant and a non·dormant form of *L. perellle* L. were studied. DIfferences in total dry matter production were related to initial differences in seedling dry wt. and the general responses to temp. were similar for each ryegrass. Total dry matter production was greatest at 20° and lowest at 10°. A temp. of 30° did not induce dormancy in the summer dormant ryegrass but did depress growth. (10 ref.) E. G. BRICKELL.

Effect of soaking and sowing of lentil seeds *(Lens culinaris* Moo.) with and without seedcoats on seedling growth. T. Gaspar, A. Xhaufflaire and J. Lacoppe *(Experielllia,* 1969, 25, 654).- Diffusates were prepared by soaking seeds with *(WC)* and without *(WOC)* coats, in distilled water and then germinating them in the dark. Seedling growth was better on distilled water *(DW)* than on soaking water *(SW)* and results were better in the *WOC* series. The results showed that there is an inhibiting substance in the seed coat. *WOC·soaked* embryos showed better root growth on *DW* than on *SW,* showing that root **growth** inhibitors occurred also in the cotyledons. The protective effect of seed coats against osmotic processes must be considered and best growth is obtained when seeds are soaked with the coats and sown with them off. (12 ref.)
C. Y.

Reversion of senescence: effects of 2,4-dichlorophenoxyacetic acid and indoleacetic acid on respiration. ethylene production and ripening of banana fruit slices. M. Yendrell *(Aust. J. bioi. Sci.,* 1969, 22. 601-610).-Slices (6 mm) of bananas (Dwarf Cavandish) were treated with the growth regulators by vac. infiltration and respiration and ethylene production was followed. At 10^{-5} to 10^{-3} M (higher levels caused injury), 2,4-D and IAA delayed ripening and delayed but increased the peaks in respiration and ethylene production induced by cutting. 2,4-D prevented or delayed ripening which could be induced by ethylene treatment (10 ppm) for 24 h but did not prevent a c1imacteric-like rise in respiration. IAA had a similar but less pronounced effect. These effects are the reverse of those produced by spraying or dipping treatments. It is suggested that these compounds cause reversion of the tissue to a more juvenile state in which it is less sensitive to ethylene treatment. (25 ref.) J. B. WOOF.

Effect of CCC [2-chloroethyltrimethylammonium chloride) and glycine betaine on growth and growth·substance content of primary leaves of dwarf French bean. *Phaseotus .ulgaris* L. A. W. Wheeler *(Ann. appl. Bioi.,* 1968,63, 127-133).–Application of CCC to the nutrient medium (moist sand) decreased growth of primary leaves and stem internodes above the bean hypocotyl, probably due to decreased auxin production from tryptophan and phenolic esters in the primary leaves. Growth of leaves was less affected than that of stems, and the gibberellin conlent of the primary leaves, associated with their expansion, was unaffected by CCC. The treatment delayed death of the primary leaves, the breakdown of cWorophyll, and the increase in auxin associated with death of bean leaves. CCC had less effect on the growth of leaf discs, probably because they are not sites of growth-substance production. Glycine betaine did not affect growth of bean plants, their metabolism of growth substances or the longevity of their primary leaves, but inhibited growth of leaf discs. (14 ref.) A. H. CORNFIELD.

Effect of the growth regulant CCC on the growth of cereals. I. Experiments 1963-1964. P. F. J. van Burg and G. H. Arnold *(Neth. Nitrogen Tech. Bull.,* 1969, No.7, 32 pp.).–Response was shown to depend on species, variety, level of N supplied, rate of CCC and date of application. Spring wheat and winter wheat showed the greatest response. Early application of CCC shortened the lowermost internodes, and late application the topmost ones, and late application therefore caused the greatest reduction in culm length. (22 ref.) (In English.) E. G. BRICKELL.

Detection of iron ethylenediamine di(o-bydroxyphenylacetate) (FeEDDHA) in plant tissue. R. A. Jeffreys and A. Wallace *(Agroll. J.,* 1968, 60. 613-616).-A method for the extraction and determination of intact FeEDDHA in plant tissue is described. Absorption of FeEDDHA by tomato and tobacco plants from solution culture increased with level of chelate in the culture (10^{-5} to 10^{-3} M). There were higher concn. of the chelate in the upper and middle than in the lower leaves of tobacco. In tomato the concn. in the plant ranged from 0·057 to 0·393 **µmoles** and in tobacco leaves from 0·06 to 0·26 **µmoles** per g dry wt. of material. (10 ref.) A. H. CoRNFIELD.

Ashing methods for plant tissues for mineral nutrition. Y. Aiba. *(Bull. expo Forests, Tokyo Vlliv. Agric.,* 1968, **No** 7, **45–46).—** Many ashing methods were studIed but they were tIme consumIng. The procedure here described uses **1·0–1**.5 g of tissues, 10 ml of HCIO, and 2 011 of **HNO_3;** time 90 min. (From English summary.)
C. Y.

Qualitative and quantitative analysis of natural carotenoids. J. L. Fiasson, N. Arpin, P. Lebreton and M. P. Bouchez **(*Chim. analyt.,*** 1969, **Sl,** 227-236).-The analytical schemes described provide for

(i) extraction of sample with COMe, (optionally containing 10–20% MeOH) and back-extraction into light petroleum, (ii) determination of total carotenoids (I) by absorption spectrophotometry of the extract, (iii) qual. determination of mixed 1 in the extract by circular paper chromatography, *(iv)* determination of relative concn. of constituents by column chromatography of the extract on Al_2O_3, *(v)* identification of separated 1 by spectrophotometry and chemical means. Error for quant. methods is within ± 4% for non-chlorophyllic samples and ± 8% for green material; the sensitivity for qual. methods is ~ I ppm. W. J. BAKER.

Plant injury by air pollutants: influence of humidity on stomatal apertures and plant response to ozone. H. W. Otto and R. H. Daines *(Science, N. Y.,* 1969, 163, 1209-1210).-03 injury to Bel W3 tobacco and pinto bean plants increases with increasing humidity: the degree of plant injury sustained correlates with the porometer measurements indicating that size of stomatal aperture increases with increasing humidity (Lh.). R.h. may therefore influence plant response to all pollutants and may account in part for the greater sensitivity of plants to an 03-type injury found in the eastern U.S. as compared with that occurring in the S.W. region. C. V.

Crops and Cropping

High speed, precision, centrifugal seed planting. A. U. Khan *(Diss. Abstr., B,* 1968,29, 582).- The main principles of this new method were control of seed-cell exposure time, increase in the force needed to move the seeds into the cells by use of a centrifugal force and the delivery and placement of seeds by high speed ejection of seeds to permit embedding in the furrow. The machine was practical at speeds of 1100 cells/min and above, and seed damage was comparable to that with conventional planters.
F. C. SIJTON.

Grain production and water use of wheat as affected by plant density, defoliation and water status. N. C. Turner (J. *Aust. Inst. agric.* Sci., 1969, 35, 56).-Field and glasshouse experiments showed that in a community of plants the lower leaves contribute < 15% to the grain yield after anthesis, though they act as a source of N for the developing grain if other sources are low. Water stress reduced ear emergence by 55% and the fertility of those ears which emerged by > 30%. Removal of the lower leaves after anthesis increased the efficiency of water use by up to 25% in plants subjected to low water levels but did not increase the economic yield.
E. G. BRICKELL.

Growth and yield of three varieties of wheat, with particular reference to the influence of unproductive tillers. P. M. Bremner *(J. agrie. Sci., Camb.,* 1969, 72, 281-287).- None of the varieties (Jufy I, Yeoman and Cappelle) responded to the higher N application [4 cwt/acre 12 : 12 : 18 NPK plus 2 cwt $(NH_4)_2SO_4$ in early spring). Once unproductive tillers had died, productive shoots grew rapidly and caught up with productive tillers which had not been affected by intertiller competition. M. LONG.

Effects of time and rate of nitrogen application on tillering, 'sharp eyespot' *(Rhizaetania solani)* and yield in winter **wheat.** P. M. Bremner *(J. agrie. Sci., Comb.,* 1969, 72, 273-280).-Various rates of N were applied in early and late spring to two plant-densities of winter wheat. Grain yield was slightly greater with the late-applied N, with more but smaller ears from the early application. Main shoots produced at least 70% of the grain on the high-density plots (303 plants/m') but < 50% on the low-density plots (151 plants/m'). Tillers appearing after early March contributed little on the high-density but did so substantially on the low-density plots. Tillers produced after early April died without heading. The incidence of ***Rhizoctani solani*** was higher where N was applied early and increased with increasing N applications up to 120 units N. It was slightly higher at the higher plant density. (16 ref.)
M. LONG.

Effect of early-summer seeding of winter wheat on yield, soil moisture and soil nitrate. W. G. Dewey and R. F. Nielson *(Agron. J.,* 1969, 61, 51-55). –Under dryland conditions, sowing winter wheat in June or July exhausted most of the available moisture and soil NO_3^- by autumn and produced much lower yields than did sowings made from Aug. to Oct. Although irrigation and application of N maintained soil moisture and NO_3^- near optimum with June and July sowings, yields, particularly from June sowings, were still lower than from later sowings. Winter survival from June sowings was also lower, under both dryland and irrigated conditions. (13 ref.) A. H. CORNFIELD.

Winter wheat yields and response to nitrogen as affected by soil and climatic factors. H. V. Eck and B. B. Tucker *(Agron. J., 1968,* 60, 663-666).-Soil moisture, pptn., temp., and soil org. matter content were related to winter wheat yields and yield response to N fertiliser. Although significant simple and multiple correlations were found, relationships were not sufficiently close to give satisfactory equations for predicting wheat yields or response to N application. (13 ref.) A. H. CORNFIELD.

Nitrogen content of [wheat and sorghum] grain as influenced by water supply. J. F. Stone and B. B. Tucker *(Agron. J.,* 1969, 61, 76-78). –Studies at 3 locations over 8 seasons showed a significant decrease in the N% of grain of sorghum and wheat with increasing level of water supplied (10–90 em) just prior to and through vegetative growth of the crops. A. H. CORNFIELD.

Changes in microfloral composition of moist sorghum stored under hermetic conditions. M. Gonen and M. Calderon *(Trop. Sci.,* 1968, 10, 107-114). –The grain was stored in air-tight bins over 10 months; one group of bins was opened and resealed several times, the other remained sealed throughout the experiment. *Penicillium* and *Aspergillus,* initially present, disappeared and gave way to yeast-like fungi *(Hansenu/a anornala* and *Trichosporon* sp. and/or *Endomyeopsis* sp.) in both groups of bins, although the total population counts in the sealed bins were slightly lower after storage than at the beginning. The [O,J in the sealed bins varied from 0·0 to 0'4%. P. P. R.

Timing and correlation of major developmental events in maize, *Zea mays* L. E. G. Siemer, E. R. Leng and O. T. Bonnett *(Agron. J.,* 1969,61, 14-17).- The timing and sequence of these events were studied in dent inbred lines and hybrids, using two dates of planting and two growing seasons. (I3 ref.) A. H. CORNFIELD.

Effect of plant population on production and distribution of dry matter in maize. J. C. S. Allison *(Ann. appl. Bioi.,* 1969, 63, 135-144).- On a clay loam in Rhodesia, grain yield was increased by 50% and total dry wt. by 30% when population *(P)* was increased from 23,000 to 48,000 plants per ha. There were no further increases with population up to 74,000 per ha. Between 6 and 10 weeks after sowing, crop growth *(C)* increased by 40% with increase in leaf-area index *(LAI)* from 2 to 5·5, despite concomitant increase in moisture stress. Immediately after flowering (10–11 weeks after sowing) C increased up to the greatest *LAI* attained, 6·7. 18 weeks after sowing (*LAI* 3-5'5), C was max. when *LAI* was 5. The proportion of grain in the dry matter accumulated after flowering increased from 73%, when *P* was 23,000, to 82% when *P* was 74,000. Stem wt. increased until 3-4 weeks after flowering, then remained constant when *P* was 23,000, but decreased in denser *P.* The fraction of the ear consisting of grain increased with *P.* (12 ref.) A. H. CORNFIELD.

Effect of plant type and nitrogen level on the growth characteristics and grain yield of indica rice in the tropics. S. K. De Datta, A. C. Tauro and S. N. Balaoing *(Agron. J.,* 1968,60, 643-647).- During a dry, sunny season, tall indica rice varieties showed best grain yields with 30–60 kg N per ha. All the tall varieties lodged at some stage of growth. The dwarf varieties showed increasing grain yields (up to 9477 kg/hal with 90–120 kg N per ha and did not lodge. In a wet, cloudy season, when there was reduced response to N applications by either tall or dwarf varieties, the latter produced higher grain yields. (12 ref.)
A H. CORNFIELD.

Influence of placement on uptake of [labelled) nitrogen by rice. F. E. Broadbent and D. S. Mikkelsen *(Agron. J.,* 1968, 60, 674-677). - Tiller number and bearing tillers were increased by application of N (30–60 ppm soil basis) to waterlogged rice, but source [^{15}N-labelled $(NH_4)_2SO_4$ or urea] and method of placement (broadcast, banded, topdressed, or split) had no effect. Grain yield was increased similarly by both sources of N with any particularly method of placement. Split application (half banded at planting time and the rest topdressed 47 days later) gave the highest, and broadcasting the lowest, yields. $(NH_4)_2SO_4$ was more effective in increasing grain N% than was urea, and this resulted in less loss of N from the soil-plant system. (13 ref.) A. H. CORNFIELD.

Absorption of manganese by rice under flooded and unflooded conditions. C. S. Weeraratna *(PI. Soil,* 1969,30, 121-125).-Rice grown under flooded conditions in a lowland soil, used for growing rice for many years, absorbed more Mn and gave higher dry matter yields than when grown under unflooded conditions. The reverse was true when rice was grown on an upland soil. The poor performance of the upland soil under flooded conditions may have been due to the very high sol. Fe content causing interference with the absorption of Mn. (14 ref.) A. H. CORNFIELD.

Growth and nutrient uptake of rice at different growth stages and nitrogen levels. J. L. Sims and G. A. Place *(Agron. J.,* 1968, 60, 692-696).-About 50% of the total N and K and 66% of the total P absorbed by waterlogged rice plants were taken up after dates previously established as optimum for mid-season top-dressed N application (50–79 days). About 66% of the dry matter production also occurred after these dates. The results partially explain why top-dressed N at mid-season (jointing stage) is more efficient in increasing grain yields than earlier applications. On average, 232 kg N, 48 kg P and 171 kg K per ha were absorbed at dates of max. nutrient accumulation (near soft dough stage); 25 kg additional grain were produced per kg of N applied. Losses of N and K occurred from all varieties between the tillering and jointing stages. Total N and K content of the plants was much lower at maturity than at the soft dough stage. (14 ref.)

A. H. CORNFIELD.

Recommended varieties of potatoes. Anon. *(Fmrs' Leaf. nafn. Inst. agrie. Bot.,* 1969, No. 3, II pp.).- 21 potato varieties recommended for general or special use are grouped in three tables according to their most useful time of marketing (First Early, Second Early and Maincrop) and their performances in various trials are listed on a 0–9 scale. Characters listed include yield, properties of tubers, cooking quality and field characters (resistance to rot, blights, elc.). The properties of each variety are summarised. Outclassed varieties and varieties still under trial are listed.

P. C. W.

Yield and quality of red beet seed as affected by desiccant sprays and harvest date. R. B. Austin and P. C. Longden *(Weed Res.,* 1.968, 8, 336-345).-Diquat and Na monochloroacetate were used to desiccate seed crops; the seeds became brown and dry ~ 7 days after spraying, and were judged to be suitable for combine harvesting. Seed yields were not much affected, but some adverse effect on germination and emergence was noted. The technique may be useful when harvesting conditions are poor. (II ref.) P. P. R.

Effects of magnesium fertilisers on yield and chemical composition of sugar-beet. A. P. Draycott and M. J. Durrant *(J. agrie. Sci., Camb.,* 1969,72, 319-324).–Trials were conducted on fields with previous histories of Mg deficiency symptoms showing on beet. Kieserite and dolomite were tested at zero, 2·5 and 5 cwt/acre with nil or 3 cwt/acre crude NaCI and with 0·8 or 1·2 cwt/acre N as Nitrochalk. Kainit (7 cwt) and KCI (2 cwt/acre) were also applied on extra plots. Kieserite and dolomite increased sugar yields, the latter, at 5 cwt/acre, being the most effective. NaCl and N were profitable and neither interacted with Mg on average. The Mg in kainit was beneficial, but insufficient. All the Mg fertilisers increased the Mg content of the plants and reduced the incidence of Mg deficiency symptoms. The Mg content of the plants was related to the yield response of the fertiliser and to the percentage of plants with deficiency symptoms. M. LONG.

Recommended varieties of grasses. Anon. *(Fmrs' Leaf. natn. Inst. agrie. Bot.,* 1969, No. 16,23 pp.).-The main characteristics of varieties of perennial, Italian and hybrid ryegrass, timothy, cocksfoot and meadow fescue recommended for general or special use are listed. Most of the species are subdivided into maturity groups (e.g., early, medium, late) and recommendations are made in relation to other varieties in the same group. New varieties under trial are listed. P. C. W.

Use of halophytes for forage production on saline wastelands. C. V. Malcolm *(J. Aust. Inst. agrie. Sci.,* 1969, 35, 38-49).- A review. (68 ref.) E. G. BRICKELL.

Sudan grass and sorghum-Sudan grass hybrids for forage. Anon. *(Fmrs' Bu/l., U.S. Dep. Agrie.,* 1969, No. 2241,12 pp.).- Varieties, cultural practices, harvesting and pest control are described.

E. G. BRICKELL.

Some effects of fertilisers and cutting frequencies on Giant Rhodes and veld grasses. J. N. Boultwood *(Rhod. agrie. J.,* 1969, 66, 61-64).-Little difference is reported in yields of grasses given the same amounts of fertilisers and with the same frequencies of cutting. Yields were proportional to the amount of fertiliser applied. When the grass was allowed to grow unchecked for a complete season, the effects of frequent cutting for four seasons largely disappeared and the original species composition was restored.

E. G. BRICKELL.

Effect of applying nitrogen in various ways on the herbage yield of Giant Rhodes grass *(Chloris gayana* Kunth). M. G. W. Rodel *(Rhod. agrie. J.,* 1969,66, 43-45).-Greatest yields of herbage were recorded when N was applied in four equal amounts at four-weekly intervals during summer. Kraal manure also increased yields, indicating that on heavily grazed pastures, manure is likely to affect herbage production even where very large amounts of N have been applied in chemical form. E. G. BRICKELL.

Effect of nitrogen and phosphorus placements and rates on turfgrass establishment. J. W. King and C. R. Skogley *(Agron. J.,* 1969, 61, 4-6).–A silt loam (pH 6·5) of low fertility was given adequate K and treated with superphosphate (490–3920 g P) and NH4N03 (375-1470 g N per 100 m^2) *(a)* as surface application, *(b)* mixed with the top 10 cm or *(e)* 75% mixed into the top 10 cm and 25% in the top I cm of soil before sowing a bluegrass-red fescue mixture. Both spring and autumn sowings showed that the effects of treatments lasted for only a few months. The highest N rate gave the best turfgrass quality and top growth and did not cause seedling injury. Treatments *(a)* and *(e)* gave equally satisfactory results. Treatment did not affect root wt. Maintenance fertiliser improved grass quality and top growth. (11 ref.)

A. H. CORNFIELD.

Effect of fertiliser treatments on production of culms of American beachgrass, *Ammophila bre.iligulata.* M. T. Augustine and W. Curtis Sharp *(Agron. J.,* 1969, 61, 43-45).- Culm production on a coastal dune over 3 years was increased to the greatest extent by an initial application of a Mg/NH, phosphate (I) or by annual applications of 10–10–10 mixed NPK fertiliser. After the 4th year, culm production dropped sharply where I had been used.

A. H. CORNFIELD.

Management of land diverted from crop production. I. Perennial forages. R. G. Robinson *(Agron. J.,* 1968, 60, 619-622).-Removing clover and clover-grass forages over 5 years compared with nonremoval (mowing or chopping and leaving the forage) reduced stands of timothy, but not of other forages, slightly increased weed control, and reduced soil fertility, as shown by subsequent yields of maize grain. Effects of mowing or chopping were similar with regard to weed control and subsequent maize yields, except that chopping sweet-clover resulted in higher maize yields than did mowing. Cutting 1-3 times each year, compared with no cutting, favoured growth of lucerne, red clover and foxtail and injured bromegrass, timothy and quackgrass. Uncut bromegrass-lucerne gave the densest ground cover and best weed control. Without bromegrass or timothy present, cutting was essential for weed control. and two cuttings were best. For prep. of land for maize the following year, ploughing in July and spraying with weedicides in Sept. gave the best weed control. A. H. CORNFIELD.

Moisture equilibrium values for grass and legume seeds. J. F. Harrington *(Agron. J.,* 1968, 60, 594-597).-Although different seeds had different moisture contents at a particular R.H., all the seeds showed essentially parallel changes in moisture content with changes in R.H. A method is described for forecasting the equil. moisture % which each kind of seed will reach in a mixture of seeds in a sealed container. A. H. CORNFIELD.

Moisture use by forage crops. O. P. Cohen and E. Strickling *(Agron. J.•* 1968, 60, 587-591).-Lucerne, tall fescue and Bermuda grass used approx. the same amount of water during the growing season. Soil moisture loss continued at a uniform rate until ~ 63·5 mm moisture was used; thereafter the rate decreased. Growth of plants was reduced even though available water was present at 15·2 cm depth and below. Application of N (450 kg per hal to Bermuda grass increased dry matter yields more than did application of 112 kg per ha, with no significant difference in water consumption. After rain which did not wet the whole profile, moisture was used from the whole root zone and not only from the depth wetted by the rain. (17 ref.) A. H. CORNFIELD.

Annuallespedezas. Culture and use. P. R. Henson and W. A. Cope *(Fmrs' Bull., U.S. Dep. Agrie.,* 1969, No. 2113, 16 pp.).–Striate and Korean varieties are described followed by soil and fertiliser requirements, seeding, hay pasture and harvesting practices. Insect pests, weed and common diseases are discussed.

E. G. BRICKELL.

Response of subterranean clover *(Trifolium subterraneum* L.) to foliar applications of phosphorus. D. Bouma *(Aust. J. agrie. Res.,* 1969, 20, 435-445).-Dry wt. of tops and roots were significantly increased by most spray treatments, foliar applications of 50 mM-H3PO, giving the greatest response, but the relatively slow rate of P uptake and poor growth responses compared with root applications of P indicated that foliar spray applications are not suitable for hastening recovery from P deficiency.

E. G. BRICKELL.

Shoot numbers, shoot size and yield of regrowth in three lucerne cultivars. G. J. Leach *(Aust. J. agrie. Res.,* 1969,20,425-434).-

Shading plants before cutting produced a large and uniform decrease in the wt. of tops, stubble and roots. The no. of shoots remaining after cutting, the no. produced during regrowth, and the size to which they grew all varied between cultivars and between cutting treatments. Shoot size depended mainly on the length of its growing period, but on severely cut plants the few shoots remaining grew larger than those of the same age on leniently cut plants. E. G. BRICKELL.

Effect of time of molybdenum application on soyabean yield and on N, oil and Mo contents. F. C. Boswell and O. E. Anderson *(Agron. J.,* 1969,61, 58-6O).-Application of Mo to the seed or as a foliar spray to soyabeans growing on moderately acid soils sometimes increased seed yields, especially on a shaley soil. The treatments increased N %and Mo %of leaves, and protein %of the beans. For the sites as a whole there was a significant negative correlation between protein %and oil %in the beans. (12 ref.) A. H. CoRNFIELD.

Methods for evaluating protein and oil in soyabeans and mass selection by seed size and specific gravity in soyabean populations. W. R. Fehr *(Diss. Abstr., B,* 1967,28, 1306-1307).- The relative efficiency of seed density (SD), sp. gr., n.m.r., Kjeldahl value *(Kj),* and solvent extraction *(SE)* for determining protein and oil in the seeds was examined. Relative error among 80 duplicates placed the methods in the (descending) order, sp. gr., *SE,* n.m.r., *Kj, SD.* For selecting high-protein samples for genotypes, *Kj* was superior for direct and n.m.r. for indirect measurement; n.m.r. was superior to *SE* as a method of oil analysis. Seed size *(SS)* was correlated positively with sp. gr. and protein and negatively with oil content. In the selection of genotypes, large SS and high sp. gr. tended to produce high-protein-low-oil beans, whereas small SS and low sp. gr. led to high-oil- low-protein types. A. G. POLLARD.

Factors affecting uptake, yield response, and carryover of molybdenum in soyabean seed. W. H. Gurley and J. Giddens *(Agron. J.,* 1969, 61, 7-9).-Yields of soyabeans increased with level of Mo in the seed sown. Seed with high Mo content was obtained when soil was limed to pH 6·5 and foliar Mo sprays applied. High-Mo seeds supplied the Mo needs of the crop, but there was little carryover of Mo into the next generation of seed. Mo accumulated in the cotyledons of seed. Seed from the lower part of the plant had a higher Mo content than that from the higher part. A. H. CORNFIELD.

Nutrient distribution during development of three market **types** of peanuts [groundnuts]. I. Phosphorus, potassium, calcium, and magnesium contents. D. L. Hallock, D. C. Martens and M. W. Alexander. **II.** Boron, copper, manganese and zinc contents. D. **e.** Martens, D. L. Hallock and M. W. Alexander *(Agron. J.,* 1969,61, 8I-85; 85-88).-The major and micro nutrient levels in various plant parts of three groundnut cultivars were determined at different stages of growth. A. H. CORNFIELD.

Separation of aflatoxin-infected groundnut kernels. I. Pattinson, P. Crowther and H. El. Noubey *(Trop. Sci.,* 1968, 10, 212-221).- Results of a field trial show that by use of air separation and colour sorting equipment (two commercial machines are illustrated and flow diagrams given) it is possible to effect almost complete removal of infected from non-infected material. (12 ref.) P. P. R.

Effect of irrigation on recovery of applied nitrogen by cotton. B. D. Doss and C. E. Scarsbrook *(Agron. J.,* 1969, 61, 37-40).-On a fine sandy loam, irrigation (applied when 50 % of the available water had been used in the top 60 em of soil) decreased cotton plant·N%, but increased total N uptake due to increased dry matter *(DM)* production. Over 5 years data for both irrigated and non-irrigated cotton closely fitted the equation log $Y = 2{\cdot}333 + 0{\cdot}727 \log X$ (Y = *DM* yield and X = total N uptake). (10 ref.) A. H. CoRNFIELD.

Effect of urea on germination and yield of jute *(Corcho,us capsularis).* D. H. Khan and B. C. Mandai *(PI. Soil,* 1968, 29, 471-473).-Whenjute seed was sown immediately after application of urea, germination decreased rapidly with level of application of urea-N (**60–360** lb per acre). When sowing was delayed after application of urea this inhibitory effect disappeared after ~ 7 days. Green-wt. yields and fibre yields increased with level of urea up to 150 lb N per acre, but decreased with higher rates. A. H. CoRNFIELD.

Effect and mode of action of latex and silicone coatings on shoot growth and water use by citrus. C. V. Malcolm and L. H. Stolzy *(Agron. J.,* 1968, 60, 598-6(1).-Spraying sweet orange seedlings with a latex emulsion decreased water usage over 20 days both in the glasshouse and out-of-doors; a silicone spray had no effect. There was no significant **effect** on growth in anyone environment due to treatment. Scorch was less on latex-coated plants but shoot distortion was greater than for silicone-coated or uncoated plants. (10 ref.) A. H. CoRNFIELD.

Development of banana plant roots in various soils. Relationship with fertility. J. Godefroy *(Fruits d'omre mer,* 1969, 24, 101-104).- Differences in effecls on root development have been observed between typical soils of the Cameroons, Madagascar, and the Ivory Coast, but the differences are considered of minor importance in comparison with such overriding factors as depth of tillage and adequacy of drainage, manuring, and nematode control. (II ref.) P. S. ARUP.

Influence of date of planting and of weight of rejects on growth of pineapples in the Cameroons. J.-P. Gaillard *(Fruits d'oUlre mer,* 1969, 24, 75-87).- Various growth measurements concerning chiefly leaf and fruit production, and examination of the quality of rejects, for plantings made at 4 or 6 different seasons over 2 years, were recorded. A system of treatment with NK (N : K = I : I·5), ranging from 2·5 g of N per plant (Nov. planting) to 4 g (Mayor Aug. planting) as well as growth regulator treatment, was planned, enabling the production of exportable fresh pineapples of good quality during the whole year. P. S. **ARUP.**

Relationship between tissue zinc levels and maturity period of field beans *[Phaseolus vulgaris].* L. C. Boawn, P. E. Rasmussen and J. W. Brown *(Agron. J.,* 1969,61, 49-51).- The number of days to maturity of field beans decreased with increasing level of Zn in the mature leaf tissue or total tops up to ~ 20 ppm (dry basis). When tissue Zn was < 15 ppm, up to 30 extra days were required to reach maturily. A. H. CORNFIELD.

Growing pumpkins and squashes. Anon. *(Fmrs' Bnl/., U.S. Dep. Agric.,* 1969, No. 2086, 21 pp.).- Discusses varieties, culture, storage, diseases, and other aspects. P. P. R.

Importance of soil air for tea root growth. R. L. de Silva and L. A. Seevaratnam *(Tea Q.,* 1968, 39, **42–49).—In** poor, clayey soils, plant growth was much improved by increasing the size of the planting holes. Precautions against wilting in poor soils during drought, by thickening the layer of topsoil, and where possible, improving the subsoil by forking, etc., are discussed. P. P. R.

Behaviour of root system of sugar-cane at and aftcr harvest. J. Glover *(Proc. S. Afr. Sng. Technol. Ass., 42nd Ann. Congr., 1968,* 133-135).-Exisling roots cease to grow 3 days after harvest, but some of them retain some activity over several weeks, being able to translocate moisture and 32p from deeper levels and thus assisting the development of incipient shoots in the absence of surface moisture. New roots and shoots are quickly developed in the presence of moisture. The old roots probably help the stool to survive a dry spell. P. S. ARUP.

Magnesium: its **rôle** in rubber cultivation. Anon. *(Pirs' Bull. Rubb. Res. Inst. Malaya,* 1969, No. 102, 99-102).- The effects of Mg deficiency on *Hevea brasiliensis* trees were studied, and the development of chlorosis is discussed. Mg contents of Malayan soils are considered, and correction of Mg deficiency by fertiliser application is described. E. G. BRICKELL.

Pest Control

Pesticide chemicals official compendium, Association American Pesticide Control Officials Inc. 1966, 2 vol. (A-L, M-Z), 1297 pp. (Topeka, Kansas: Kansas State Board of Agriculture). S. C. H.

Acute toxicity data for pesticides (1968). K. H. Jones, D. M. Sanderson and D. N. Noakes *(Wid Rev. Pest COll/rol,* 1968, 7, 135-143).-Eight pages of oral and dermal LD_{50} values (mainly for the rat) of pesticides, arranged in *(a)* order of decreasing toxicity and *(b)* alphabetically. P. P. R.

Chemical characteristics of petroleum products used in crop protection applications. D. W. White *(Wid Rev. Pest COlllrol,* 1968,7, 144-154). –The basic operations carried out in the manufacture of petroleum products for use with pesticides, and the analysis of these products are reviewed. P. P. R.

Mixtures of specific pesticides as opposed to broad spectrum soil fumigants for multiple pest control. B. B. Brodie, J. M. Good, **e.** A. Jaworski and N. C. Glaze *(PI. Dis. Reptr,* 1968, 52,193-197). -Specific mixtures (insecticides, nematocides, herbicides and fungicides) were compared with broad spectrum soil fumigants on a sandy loam growing tomatoes. A. H. CoRNFIELD.

Chemical constitution and activity of bipyridylium herbicides. V. Diquaternary salts of trans-l,2-di(4-pyridyl)ethylene. J. E. Dickeson and L. A. Summers *(J. chem. Soc.,* C, 1969, 1643-1645).-The di-iodide and di-methosulphate of *trans-I* ,2-di(4-pyridyl)ethylene (IVA and IVB, respectively) were stable in aq. solution below pH II·5. Polarographic examination of IVA and IVB at O·()()I M and 0'()()15 Mgave rcsults consistent with the reduction of IV di-cation in two successive one-electron steps. The methosulphate IVB was slightly active as a hcrbicide at 8 lb/acre on sugar beet but inactive against five other plant species. This result is consistent with the theory that for high herbicidal activity of pyridylium salts, the compounds must be capable of being reduced at appropriate potentials to radical cations which are stable in aq. solution and are quant. reoxidiscd by air to the diquaternary salts.

J. I. M. JONES.

AdsorPtion and desorption of simazine by some Rothamsted soils. J. D. H. Williams *(Weed Res.,* 1968, 8, 327-335).-Adsorption of simazine (I) from, and desorption into, 0·01 **M-CaCl$_2$** was investigated on 23 soil samples. Org. C content was the only factor affecting the ability of the soils to adsorb I. (14 ref.) P. P. R.

Phenylurea herbicide behaviour in soil as related to hydrogen ion activity. K. E. Savage *(Diss. Abstr., B,* 1967, 28, l309).- The toxicity, persistence and reaction with soil of some phenylurea (I) herbicides were examined with particular reference to the influence of Ca'+ and H+. Titration of these herbicides with dil. acid revealed diffcrcnces due to the polarity of the herbicide mol.; buffer capacities increased with electronegative substitition in the urea chain. Studies of lon, e.g., kaolin, charcoal, suggests that adsorption occurs largely through non-coulombic forces and is influenced by H+ activity in the equilibrium solution and in close proximity to the adsorptive surface. The ability of I to form coordination complexes with Ca is discussed. A. G. POLLARD.

Alrazine and the nitrate content of corn [maize]. J. D. Doll and W. F. Meggitt *(Agrol/. J.,* 1968, 60, 655-657).-Pre-emergence treatment of maize with atrazine resulted in increased concn. of NO,' in the plant tissue but this occurred only in the early stages of growth and where maize was planted early (May 10). At the silage stage, atrazine treatment had no effect on tissue NO,' concn. (II ref.) A. H. CoRNFIELD.

Inlluence of crop sequence, nitrogen fertiliser, and herbicides on weed seed populations in sugar-beet fields. A. D. Dotzenko, M. Ozkan and K. R. Storer *(Agrol/. J.,* 1969,61, 34-37).- The crop sequence where sugar-beet followed beans had lower weed seed populations than where sugar-beet followed barley and maize. Density of weed seeds increased with level of N applied to sugar-beet. Six chemical weed control treatments reduced weed populations to a greater extent than did cultivation treatments. (14 ref.)

A. H. CORNFtELD.

Selectivity of Phenmedipham as a post-emergence herbicide in sugar-beet. F. Arndt and C. Kotter *(Weed Res.,* 1968, 8, 259-271). Sugar-beet are remarkably tolerant to Phenmedipham [3-methoxy-carbonylaminophenyl·N.(3'.methylphenyl) carbamate] (I), which can thus be used for selective weed control in this crop. A test with 29 varieties of sugar-beet and one variety of fodder-beet showed no interaction between phytotoxicity and variety. The mode of action of I and the nature of its selectivity are discussed. ([n German.) P. P. R.

Use of diuron for **weed** control in mature low-grown tea. D. T. Wettasinghe *(Tea Q.,* 1968, 39, 119-120).-The use of a diuron formulation, after a preliminary application of Gramoxone (paraquat formulation), to control dense stands of weeds (especially *Paspalum cOl/jugafllm* and *Spermacoce lati/olia)* is recommended.

P. P. R.

Effect of July applications of dalapon on growth and botanical composition of an *Agrostis/Lolium* pasture. G. P. Allen *(Weed Res.,* 1968, 8, 309-320).-For selective control of weed grasses *Agrostis stolol/ifera, Poa trivialis* and *Holcus lal/atus* in the pasture, the most promising treatment was with 3·3 lb/acre of dalapon-Na, applied on July 4th. P. P. R.

Quantitative analysis of phenolic saponification products of 1,2,4-trichlorobenzene after previous separation by gas or thin-layer chromatography. D. Ehrhardt, H. FUrst and K. Michael *(Chem. Tech., Berl.,* 1969,21,416-417).- 2,4-, 2,5- and 3,4-dichlorophenol, phenol and *o-* and p-chlorophenol in the intermediate products in the prep. of selective weedkillers were separated by g.c. in the form of their Me,Si ethers ina 3-m column of 15% polytetrafluoroethylene QFI on Chromosorb W. Separation time was 30 min, the carrier gas was Ar and a flame-ionisation detector was used. T.l.c. was carried out with the azo dyes formed with diazotised p-nitroaniline on 250-l'm-thick impregnated Kieselgel G. Brief treatment of the chromatogram with NH3 improved the detection limit of the phenols from 0·2 to 0·05 **μg.** In both methods, calibration with mixtures of known composition was necessary. G.c. was faster and more accurate with phenol contents < 5% but above this t.l.c. was preferred. M. GREENAWAY.

Sulphonohydrazides and related compounds. XI. Some substituted aryl ether sulphonohydrazides. R. J. W. Cremlyn and R. Hornby *(J. chem. Soc.,* C, 1969, J341-J345). –A series of benzenesulphonohydrazides were prepared as potential pesticides, and were converted into a number of deriv., e.g., hydrazones and azides. In *in vitro* fungicidal tests against *Botrylis allii,* several deriv. of 3-acetamido-4-methoxybenzenesulphonohydrazide inhibited the fungal spores at 25 ppm and caused stunting at J3()() ppm. (II reL) J. I. M. JONES.

Relative inlluence of chemical and physical properties on the fungitoxicity of tetrachloroisophthalonitrile and some of its analogues. N. J. Turner and R. D. Battershell *(Colllr. Boyce Thompsol/ II/st. PI. Res.,* 1969, 24, J39-147).-Fungicidal activity was correlated with chemical reactivity, oil/water partition coeff. and vapour pressure. The most effective member was tetrachloroisophthalonitrile, a broad spectrum foliage fungicide. The mode of action is thought to involve reaction with thiol groups. (13 ref.)

E. G. BRICKELL.

Secondary mechanisms of antifungal action of substituted 8-quinolinols. II. Substituted quinolines. H. Gershon, R. Parmegiani, M. W. McNeil and Y. J. Hinds *(Cow. Boyce Thompsol/ II/st. PI. Res.,* 1969,24, 149-150).- Theantifungal activities of 15 substituted quinolines containing only H in the 8-position were studied. The results indicated that substituted 8-quinolinols possess a secondary mechanism of antifungal action in addition to chelation. This mechanism appears not to be concerned with the 1- and 8- position relationship of the mol. as found in 8-quinolinol.

E. G. BRICKELL.

Fusarium wilt of coffee in India. T. S. Govindarajan and S. Subramanian *(II/dial/ Coff.,* 1968, 32, 27o-271).-Predisposing factors for infection by *F. javallicum* Koorders are low soil pH, high temp. and moisture stress, exhaustion due to heavy crop, inadequate shade, and wounds. Lime applications controlled spread of the disease. (18 ref.) P. P. R.

Bacterial blight of peas. J. M. Young, D. W. Dye and R. C. Close *(II/! Ser. Dep. sciel/t. il/d. Res. N.Z.,* 1969, No. 70, 14 pp.).-Symptoms of, and control measures for, blight caused by *Pseudomol/as pisi* are described. Decontamination of machinery, hygiene in pea seed crops, and inspection procedures arc discussed.

E. G. BRICKELL.

Inlluence of nitrogen fertilisation on the attack of peas by **powdery** mildew *(Erysiphe polygoni).* T. Wijngaarden and J. Ellen *(PI. Soil,* 1969,30, 143-144).-Application of NH,NO, (120 mequiv. per 5-l pot) had no effect on growth but reduced powdery mildew infection to very low levels compared with the control pots.

A. H. CORNFIELD.

Effect of mineral nutrition on *Fusarium* brown foot-rot of wheat. P. E. Onuorah *(PI. Soil,* 1969,30, 99-104).-When inoculated with spore suspensions of *F. culmorum* at various stages of growth, the extent of infection in young plants was not consistently related to N, P or K application. In older plants there was a trend for P and K to decrease and for N to increase infection. The effects of applying N, P and K together in varying proportions on young and adult plants are discussed. (14 ref.) A. H. CoRNFIELD.

Effects of nitrogen and glucose on saprophytic survival of *Ophiobolus graminis* in buried straw. P. R. Scott *(Alll/. appl. Bioi., 1969,* 63, 27-36).-When applied to the soil or to the straw before burial of colonised straw in soil, N [as **Ca(NO$_3$)$_2$**] prolonged the saprophytic survival of *Ophiobolus gramil/is,* but glucose shortened it. Whilst N prolonged survival in straw that remained undecomposed, under certain circumstances it reduced net survival by accelerating straw decomp., so that viability was limited by substrate exhaustion. **Very** high N03· concn. also had an inhibitory effect on *O. gramil/is.* (14 ref.) A. H. CORNFtELD.

Tetramethylthiuram disulphide: Microbial degradation. K. Maeda and K. Tonomura *(Rep. Fermelll. Res. II/SI.,* 1968, No. 33, 1-8).-This compound has been used as an accelerator for vuicanisation and as a seed fungicide. It is sparingly sol. in water. Organisms similar to *Pseudomol/as repfilivora* or *P. boreopolis* were isolated from the soil and were capable of using the C, Nand S atoms to support their growth, dithiocarbamate, Me,NH, HCHO, S and

methionine being detected amongst the degradation products. (10 ref.) (From English summary.) C. V.

Fungicidal effectiveness of chemicals on *Phymatotrichum omni-.orum* [cotton root rot) in laboratory soil cultures. B. Sleeth *(Pl. Dis. Reptr,* 1968, 52, 232-234).- Soil drenches of DMIT (tetra-hydro-2H-3,5-dimethyl-I,3,5-thiadiazine-2-thione) and SMDC (Na methyldithiocarbamate) were the most effective of ten chemicals tested in penetrating the soil and killing sclerotia.

A. H. CORNFIELD.

Effects of soil fumigation on disease incidence, growth and yield of spring wheat. D. L. Ebbels *(Ann. appl. Bioi.,* 1969,63,81-93).- Plots were injected with D-D (dichloropropene-<lichloropropane, 200-800 lb per acre) or with 85% dazomet dust (100–400 lb) broadcast and incorporated by rotovation. Dazomet increased yield and decreased take-all disease *(Ophiobolus graminis)* of spring wheat in the first crop after treatment, but increased the disease in the second crop. Although D-D increased take-all slightly, it increased yield in the first year, but in the second year it decreased yield and caused severe ear deformity. Fumigation treatments had little effect on eyespot, sharp eyespot, root browning *(Fusariulll* spp.), or browning root rot *(Pythiulll* spp.), but decreased nematode damage where nematodes were numerous. (29 rer.)

A. H. CORNFIELD.

Relationships of peanut [groundnut] seed treatment fungicides to seed mycoflora and germination and seedling emergence. D. K. Bell *(Pl. Dis. Reptr,* 1968, 52, 240-243).-When 13 fungicide powders were tested alone or as blends of two materials, as dressings on seed, the blends were generally superior to single fungicides in reducing seed-borne fungi, improving laboratory germination, and enhancing emergence in the field. A. H. CORNFIELD.

Effect of ozone on *Penicillium* mould decay and sporulation. P. R. Harding, jun. *(PI. Dis. Reptr,* 1968,52, 245-247).-Excellent control of sporulation and some control of decay were obtained on oranges and lemons in open boxes stored in rooms containing I ppm 03 in the atm. Sporulation and decay of fruit stored in vented or unvented cartons could not be controlled effectively by 03.

A. H. CORNFIELD.

Control of European canker of apple by eradicative and protective fungicides. E. E. Wilson *(PI. Dis. Reptr,* 1968, 52, 227-231).- Application of C.CI50Na (3 lb per 100 gal) after leaf fall to apple trees affected by canker suppressed initiation of perithecia by *Nectria galligena* 40–80% and conidial development 80–100%. Pre-ieaffall application of C.CbONa or Bordeaux mixture (10–10–1(0) gave better control than did application after leaf fall. (14 ref.) A. H. CoRNFIELD.

Comparison of zineb and maneb with a nickel-maneb mixture for control of oat crown rust. L. J. Michel and M. D. Simons *(Pl. Dis. Reptr,* 1968,52, 205-208).-At 3 lb in 80 gal per acre, nickel-maneb was superior to zineb and maneb in controlling *Puccinia coronata,* particularly when applied in late June. A. H. CORNFIELD.

Antibiotic treatments to prevent stem rusts on jack pine seedlings in nursery seedbeds. G. W. Anderson, R. L. Anderson, D. W. French and P. R. Flink *(PI. Dis. Reptr,* 1968,52, 538-541).-Treatment of I-year-old seedlings had no effect on the extent of eastern gall rust infection. Treatment with 200 ppm phytoactin, 5-20 ppm cycloheximide, 25-200 ppm semicarbazone or methyl hydrazone, or NiSO, (0'5-1'5 lb per acre) as foliar sprays and drenches delayed development of symptoms of sweetfern rust.

A. H. CoRNFIELD.

Ripe fruit rots in tomato and tbeir control by oils. K. S. Aulakh and R. K. Grover *(PI. Dis. Reptr,* 1968,52, 555-559).-Satisfactory protection against rot due to *Alternaria tenuis* was obtained by spraying with emulsions of coconut, cottonseed, paraffin, and Mobil oils in I% soap solution. Rot due to *Cladosporium fulyulll* was controlled by cottonseed and Mobil oils, that due to *Fusarium roseum* by castor, coconut, cottonseed and paraffin oils, and that due to *Sclerotinia sclerotiorum* by coconut and Mobil oils. Treated fruit remained healthy for 5-10 days longer than did untreated fruit. (10 ref.) A. H. CORNFIELD.

Chemical control of feeder root necrosis of pecans caused by *Pythium* spp. and nematodes. W. M. Powell, F. F. Hendrix, jun. and D. H. Marx *(PI. Dis. Reptr,* 1968,52, 577-578).–The no. of *Pythium* spp. were reduced by soil treatment with Dexon [Na p-(dimethylamino)benzenediazosulphonate, 84 lb) and captan (12 lb per acre). Treatment with DBCP (I,2-dibromo-3-chloropropane, 6 gal per acre) reduced the population of stunt and stubby-root nematodes but not of root-knot and lesion nematodes.

A. H. CoRNFIELD.

Apple scab. V. Effect of late-season application of fungicides on prevention of perithecial development by *Venturia inaequalis.* S. R. Connor and J. W. Heuberger *(PI. Dis. Reptr,* 1968,52,654-658).- A single late-season spray application of 50% I-(butylcarbamoyl)-2-benzimidazole carbamic acid methyl ester (IIb) with surfactant (60 ml); per 100 gal, and 2-3 applications of 0·1% PhHgCI gave complete control. (15 ref.) A. H. CoRNFIELD.

Control of bean rust *(Uromyces phaseoli)* by the use of fungicides. J. M. del Rivero, J. L. Gasc6, J. J. Tuset and F. J. Roig *(Revta Agroquim. Tecnal. Alimelll.,* 1969,9, 258-260).-Field trials of the efficiency of the systemic fungicide oxycarboxine (formulated commercially as a wettable powder containing 75% a.i. under the name of Plantvax) in disease control on rust-sensitive varieties in two growth areas are reported. In one area, infection was low and results were not statistically significant, but in the other, spraying at 0'5% on first appearance of symptoms gave almost complete control. Spraying at O'3% and application to the soil at planting gave less effective control. E. C. APLING.

Control of *Armillaria mellea* with systemic chemicals. P. C. Cheo *(PI. Dis. Reptr,* 1968,52, 639-64I).-Culture tests showed that cycloheximide was lethal to *A. mellea* at 20 ppm and 2,4-dichlorophenoxyacetonitrile at 50 ppm. Acrizane chloride was fungistatic at 10 ppm. A. H. CORNFIELD.

Control of rot in pimento plant taproot. J. M. del Rivero and F. J. Roig *(Reyta Agl'oquim. Tecnol. Alimellt.,* 1969,9,254-257).- The efficiencies in control of pimento plant disease, caused by *PhytophrllOra capsic!,* of several pesticides and pesticidal combinations were compared with application (7-1 of solution per 100 plants) to the base of plants just before irrigation. Excellent results were obtained with 0'15% Cupfram Z and 0'30% Dexon (losses 0'9% and 2'4%, respectively, compared with 64'6% in controls). Useful but less remarkable control was also obtained with 0'10% KMnO" 0'15% Dexon + 0'15% Antracol, 0'20% Pentacilline and 0'15% Dexon. E. C. APLING.

Blight of pears, apples and quinces. Anon. *(Leaft. U.S. Dep Agric.,* 1969, No. 187,6 pp.).- Control by pruning. spraying and dusting is described. E. G. BRtCKELL.

Control of root rot in garden peas with a soil fungicide. F. R. Harper *(PI. Dis. Reptr,* 1968, 52, 565-568).-In-furrow application of 0-1991 [50% 1-(butylcarbamoyl)-2-benzimidazole carbamic acid methyl ester (I), 6·2 lb/acre) to captan-treated peas reduced root rot and increased yields. The treatment reduced damage from *Fusarium* and *Rhizoctol/ia,* but not from *Pythium* spp. When I was mixed with seed before sowing, the germinating seed was not protected from attack by seedling pathogens. (13 ref.)

A. H. CORNFIELD.

Oecurrence of *Cylindrocladium* blights on nursery crops and control with Fungicide 1991 [I-(butylcarbamoyl)-2-benzimidazole carbamic acid methyl ester] on azalea. R. K. Horst and H. A. J. Hoitink *(PI. Dis. Reptr,* 1968,52, 615-617).-Fungicide 1991 gave control of C. *scoparium* on azaleas. A. H. CORNFIELD.

Comparison of Fungicide 1991, thiabendazole and sodium a-phenylphenate for control of *Penicillium* moulds of post harvest citrus fruits. P. R. Harding, jun. *(PI. Dis. Reptr,* 1968, 52, 623-625).-Additions of 500 ppm Fungicide 1991, (I), 500 ppm thiabendazole, (II), and 2% Na o-phenylphenate telrahydrate, (III), to the wax emulsion applied to citrus fruits were equally effective in controlling decay during storage of tangors. I and II were better than III in controlling decay in oranges, mandarins, tangerines and tangelos. (12 ref.) A. H. CORNFIELD.

Systemic aetivity of Fungicide 1991, a derivative of benzimidazole, against grass diseases. J. R. Hardison *(PI. Dis. Reptr,* 1968, 52, 205).- The new fungicide has promising systemic activity against *Gloeotinia temulellIa* and *Clayiceps purpurea.* A. H. CoRNFIELD.

Control of stripe smut *(Ustilago striiformis)* in Kentucky bluegrass turf with a systemic fungicide. P. M. Halisky, C. R. Funk and P. L. Babinski *(Pl. Dis. Reptr,* 1968, 52, 635–637).—Of 8 chemicals applied as drenches to heavily smutted turf, only Fungicide 1991 (6 oz per 1000 ft') effectively controlled the disease.

A. H. CoRNFIELD.

Systemic control of powdery mildew on curcubits with Fungicide 1991 applied on soil drenches and seed treatments. W. T. Schroeder and R. Provvidenti *(PI. Dis. Reptr,* 1968, 52, 630–632).—Treatment of seed with Fungicide 1991 (0'05-2% of seed WI.) or application as a soil drench (0·15 mg-1·2 g per 4-in pot) effectively controlled powdery mildew of squash and cucumber in the glasshouse. Primary development of scattered colonies on plants previously

treated with effective dosages indicated a resistant form of fungus. A. H. CORNFIELD.

Inhibitory effects of juices of various fungi on tobacco mosaic virus. D. N. Wiggs *(Pl. Dis. Rep/r,* 1968, 52, 528-529).-Aq. extracts of certain fungi (particularly of the genera *Lepio/a* and *Amanita)* had an inhibitory effect on tobacco mosaic virus when tested by the local lesion technique on detached leaves of *Nico/iana glutinosa.* A. H. CORNFIELD.

Effect of soil flDDigation on incidence of soil-borne wheat mosaic and wheat yield. R. P. Pacumbaba, E. A. Addison, W. H. Sill, jun. and 0. J. Dickerson *(Pl. Dis. Rep/r,* 1968,52, 559-562).-Soil fumigation with, e.g., 98% MeBr-2% chloropicrin, IIb per 48 ft2, greatly reduced the incidence of soil-borne wheat mosaic and increased grain yields of Pawnee, a susceptible variety; Ottawa, a resistant variety, was not affected. (17 ref.) A. H. CORNFIELD.

Biological control of a stem-rot patbogen affecting sugar-cane. G. Roth *(Proc. S. Afr. Sug. Technol. Ass., 42nd Ann. Congr., 1968,* 154–156).-Laboratory and field experiments have shown that the stem-rot disease, caused by a fungus that has been isolated, can be effectively checked by inoculation with spores of the hyperparasite *Trichoderma lignorum,* which forms substances toxic to the pathogen. P. S. ARUP.

Thermogravimetric study of zineb and some of its decomposition products. Yu. S. Lyalikovand M. I. Kitovskaya *(Ukr. khim. Zh.,* .1969,35,. 719-725).- T.g.a. showed that the first stage of decomp. IS the splttmg off of one SH group from the radical of ethylenebis-dithiocarbamic acid. It is suggested that wI. loss by t.g.a. at 150–170° and determination of SH groups liberated on thermal decomp. can be used to determine the active material in zineb. On extraction of l ineb with pyridine vapour, Zn ethylenebisdithiocarbamate is decomposed giving ZnS and pyridinium salts. Zineb is stable to air sunlight and atm. moisture. (From summary.) (II ref.) R. J. M.

Photodecomposition of tbe acaricide *N'-(4-chloro-o-tolyl)-N,N*-dimethylformamidine [Galecron]. C. 0. Knowles and A. K. Sen Gupta *(J. econ. En/.,* 1969, 62, 344-348).-The photodecomposition of Galecron (I) and of 4-chloro-o-toluidine (n) in various solvents and on silica gel t.l.c. plates is discussed. The major decomposition product of I Irradiated with u.v. light (254 and 364 om) or sunlight is N-formyl-4-chloro-o-toluidine; products from IT were not characterised. C. M. HARDWICK.

Temperature and toxicity of insecticides. M. Abdullah *(Sci. Ind., Pakistan,* 1967,5, 528- 541).-The temp. coeff. of an insecticide depends on chemical composition, mode of action, insect species, elc. Tabular presentatIOn of literature surveys shows the following to have negative coeff. of actIOn: DDT, pyrethrum, rotenone derris, lethane, lindane, nicotine, methoxychlor, terpenol, lauryl thiocyanate, chloropicrin, ethylene dichloride, dinitrophenol, Ca arsenate, baSIC Cu arsenate, Pb arsenate and synthetic cryolite. A positive temp. coeff. of actIOn IS reported for lime sulphur, extracts of derns, CS2, micoune sulphate, ethylene oxide, nicotine, BHC, toxaphene, chlordane, heptachlor, parathion dieldrin aldrin cryolite, sodium azide and rotenone. (98 ref.) , , , E. G. BRICKELL.

Effects of diazinon on the microflora of submerged soils. N. Sethunathan and I. C. MacRae *(Pl. Soil,* 1969 30, 109-112)-Application of diazinon to a flooded clay (pH 6.'6) at 2-20 kg per ha increased thc actinomycete population and caused a brown pigmentation in the upper (oxidised) soil layer. The treatment also increased the algal population in the water above the soil. A. H. CORNFIELD.

Absorption, translocation and distribution of phorate in loblolly pine seedlings. R. A. Werner and E. W. Clark *(J. econ Em.,* 1969,69, 436-437).-When 10% phorate (I) granules at 2'6-'10' 5 g were applied at the base of pine seedlings, I was translocated to the whole plant. At all doses tested, 100% mortality of the weevils, *Hylobius pales* and aphids, *Cinara watsoni* was obtained in 15 days and lasted for < 300 days. There was no phytotoxicity. (22 ref.) C. M. HARDWICK.

Metabolism of diazinon-^{14}C in western corn roohl'Orm beetles. C. C. Conaway and C. 0. Knowles *(J. econ. Ent.* 1969 62, 286-289). -The major degradation route of 14C-ethoxy and "C-ring labelled .diazmon in *Diabrotica virgifera* was the cleavage of P–O-pyrimidinyl bond of dlazinon and breakdown to a P-contamng aCid and 6-hydroxy-2.isopropyl-4-methylpyrimidine. C. M. HARDWICK.

Effects of dimetbyl sulphoxide [DMSOl on the biological activity of selected miticides and insecticides. L. D. Olinger and S. H. Kerr *(J. econ. En/.,* 1969, 62, 403-407).-DMSO at 100 and 1000 ppm had no effect on root absorption, translocation and toxicity of oxydemeton-methyl (I), disulfoton and demeton in cotton growing in nutrient solutions. 1% DMSO did not affect the action of I applied as a dip to the cotyledons of squash, nor did it affect the rate of absorption of other insecticides. When applied topically (used as a solvent in place of acetone) to *Epilaclma varives/is,* it increased the LT values for carbaryl, decreased them for endosulfan and did not affect those for malathion. C. M. HARDWICK.

Toxicity of DDT and related compounds to certain lepidopteran cotton insects. O. A. Wolfenbarger and W. L. Lowry (1. *econ. En/.,* 1969, 62, 432-435).- Deutero-DDT [1,I,I-trichloro-2,2-bis-(p-chlorophenyl)ethane-2d2] as a *LV* spray was the most effective against several species of the many compounds tested. (17 ref.) C. M. HARDWICK.

Physiological factors influencing toxicity of carbamate insecticides to insects. Shawky Abd El-Aziz, R. L. Metcalf and T. R. Fukuto *(J. ecol/. En/.,* 1969, 62, 318-324).-The LOs. of 6 carbamates, alone or with synergists, were evaluated against susceptible and resistant strains of *Musca domestiea, Apis mellifera* and *Blal/ella germanica.* Susceptibility, rale of development of resistance, and the relation of increased toxicity with age to levels of detoxifying enzymes are discussed. (16 ref.) C. M. HARDWICK.

Genetics of cyclodiene-insecticide resistance in the seed-corn maggot. D. G. R. McLeod, C. R. Harris and G. R. Driscoll *(J. econ. Em.,* 1969, 62, 427-432).- The toxicity of aldrin, diazinon and ODT to susceptible and homozygous resistant strains of *Hylemyapla/ura* was recorded. (22 ref.) C. M. HARDWICK.

Susceptibility of eggs and young adults of *Crypto!estes ferrugineus* and C. *turcieus* to hydrogen phosphide. P. S. Barker *(J. eeon. En/.,* 1969, 62, 363-365).- Two strains each of C. *turcicus* and C. *ferrugil/eus* adults were fumigated with PH_3. 50% mortality was obtained with doses of 0·03–0·076 mg/l for 5 h at 24°c. Eggs of C. *ferrugineus* were far more tolerant and required 2'5 mg/1. (14 ref.) C. M. HARDWICK.

Relative toxicity of certain flDDigants to *Trogoderma granarium* G. K. Punj and G. K. Girish *(J. stored Prod. Res.,* 1968, 4, 339-342).-Ethylene dibromide was the most toxic fumigant to larvae and pupae both at LDs. and LO_3. levels, followed by CS_2, ethylene dichloride (l) and I-CCI. (3 : I) mixture. The fumigants were tested at 65°, 80° and 95°F; LOs. and LD_3. values decreased considerably as the fumigation temp. increased. (17 ref.) P. P. R.

Response of overwintered boll weevils to reflected light, odour and electromagnetic radiation. H. M. Taft, A. R. Hopkins and H. R. Agee *(J. econ. Ent.,* 1969, 62, 419-424).-Overwintered *All/honomus grandis* were attracted to light of 500–525 om, but not to light of 600–680 om. They did not respond to the odour of growing plants or macerated tissue, but could detect (in one experiment) the difference between electrically earthed and unearthed plants. It is suggested that the weevil may be able to distinguish the electrostatic field radiated by a field of cotton from that generated by other plants. (20 ref.) C. M. HARDWICK.

Relationship between *P!odia interpunctella* and stored-grain fungi. H. A. Abdel-Rahman, C. M. Christensen and A. C. Hodson (1. *stored Prod. Res.,* 1968, 4, 331-337).-Development of *P. interpunc/ella* in shelled maize slightly raised the moisture content of the grain but discouraged the growth of fungi. On mouldy maize, development of the insect was severely hindered; the relationship between *P. in/erpunctella* and most stored-grain fungi appeared to be antagonistic but a plate culture of *Aspergillus !alophilicus* attracted egg laying by the adult insect and appeared to provide a satisfactory diet for development of normal larvae. (10 ref.) P. P. R.

Nitrogen in sugar-cane and the fecundity of *Numicia viridis* Muir. R. H. G. Harris *(Proe. S. Afr. Sug. Technol. Ass., 42nd Anl/. Congr.,* 1968, 163-166).-The leaf-sucking insect reared on sugar-cane grown at four different levels of N showed greater fecundity on plants grown at high than at low N levels. (II ref.) P. S. ARUP.

The tomato fruitworm. J. Wilcox and A. F. Howland *(Leaft., U.S. Dep. Agric.,* 1969, No. 367, 6 pp.).-The control of *Helio/his zea* by insecticides as dusts and sprays and by cultural methods is described. E. G. BRICKELL.

The meadow spittlebug. Anon. *(Leafl., U.S. Dep. Agrie., 1968,* No. 514, 4 pp.).-Control of *Philael/us spumarius* on legumes by insecticidal sprays is described. E. G. BRICKELL.

Locusts in relation to sugar-cane. J. A. Whellan *(Proc. S. Afr. Sug. Technol. Ass., 421ld Ann. Congr.,* 1968, 167-171).-An account is given of the history of attacks by locusts on sugar-cane (chiefly by the red locust *Nomadacris seplemfasciala* Servo and the African migratory locust *LOCUSla migraloria migralorioides* Reich. and Fairm.) in countries of southern Africa. P. S. ARUP.

Laboratory evaluation of malathion as a protectant for stored walnuts. G. H. Spitler and P. L. Hartsell *(J. ecoll. EII/.,* 1969, 62, 305-307).-Malathion (dust or emulsifiable concentrate) was applied to walnuts at 5-50 ppm and the effect on *Plodia ill/erpUllclella* and on *Oryzaephilus mercalor* was noted after 1-12 months. No beetles developed from eggs in any dust treatment. Dust residues of 6·2-6·8 ppm prevented infestation by all stages of both species for 6 months. The emulsifiable concentrate treatment was relatively ineffective. C. M. HARDWICK.

Field studies on chemical control of the stem borer *Chilo partellus* on hybrid sorghum in India. S. M. Ahmed and W. R. Young *(J. econ. Enl.,* 1969, 62, 478-482).-BHC, endrin, carbaryl, trichlorfon and lindane as sprays or granules reduced borer damage and increased yields; however, some phytotoxic effects occurred with trichlorphon. Cost of treatment was soon covered by the value of increased yields. C. M. HARDWICK.

Systemic activity of UC-21149 against the citrus red mite, citrus thrips, California red scale and spirea aphid on nonbearing orange trees. H. Tashiro, D. L. Chambers, J. G. Shaw *el 0l.* (1. *ecoll. EII/.,* 1969, 62, **443–447).—UC-21149** [2-methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime] (I) granules were applied to soil around young orange trees at 1·5–40 g/tree. The 2 higher doses controlled field populations of the red mite for 44 weeks, the aphid for 29 weeks and the thrips for 10 weeks. Laboratory tests with I showed it to be active against red scale also. No phytotoxic effects were noted. (11 ref.) C. M. **HARDWICK.**

Codling moth control with carbofuran. I. A. Rammer and E. A. Kurtz *(J. econ. Enl.,* 1969, 62, 356-357).- In experiments over 3 years in California, sprays of 0·125-0·5 lb/100 gal controlled *Carpocapsa pomonella* on apples, pears and walnuts.
C. M. HARDWICK.

Yield reduction of oats caused by the cereal leaf beetle. D. L. Merritt and J. W. Apple *(J. ecoll. EII/.,* 1969, 62, 298-301).–Granular carbofuran (3 lb/acre) applied broadcast gave complete control of *Oulema melanopus.* C. M. HARDWICK.

Control of four species of aphids on deciduous fruit and nut trees with carbofuran. I. A. Rammer, E. A. Kurtz and P. E. Primer *(J. econ. EII/.,* 1969, 62, 498-500).-Sprays of carbofuran gave good control of *Dysaphis plonlaginea, Aphis pomi, Chromaphis juglandicola* and *Hyaloplerus pruni* at dosages of 0·25-0·5 lb/100 gal.
C. M. HARDWICK.

Practical trial of **pyrethrins-in-oil** surface sprays for protection of bagged grain against infestation by *Cadra cautella* in Kenya. J. A. McFarlane and N. K. Sylvester *(J. slored Prod. Res.,* 1968, 4, 285-293).-Two formulations of synergised pyrethrins were tested monthly on fumigated, bagged wheat; results were assessed after 18 weeks storage and 5 applications. Both treatments were quite effective against the tropical warehouse moth, C. *caulella,* but were not satisfactory against *Silophilus oryzae* or *Tribolillm caslalleum.* No taint was detected in bread made from treated grain.
P. P. R.

Production of concentrated pyrethrum extract. S. Prasad and Rajkumari Jamwal *(Chemy Illd.,* 1969, 756-757).- Methanol (I) and ethylene glycol monomethyl ether (0) are the most effective solvents for separation of pyrethrins (III) from the initial oleoresin, the impurities being pptd. when the solution is centrifuged and cooled. Addition of 5-10% H20 to I and II improves their refining capacity, the content of m being increased from ~21% to ~40% at _20°. Lowering the temp. to - 45° increases the m content to ~58% with I and to ~70% with II-H20 (9 : 1). The biological activity is unaffected by this concn. procedure.
W. J. BAKER.

Rôle of insecticide synergists in resistance problems. C. F. Wilkinson *(Wid Rev. Pesl COlllrol,* 1968, 7, 155-168).- A review. (96 ref.) P. P. R.

Use of *Bacillus thuringiensis* as a microbial insecticide. T. A. Angus *(Wid Rev. Pesl Conlrol,* 1968, 7, 11-26).-An illustrated review, including tables of: sources of varieties of *B. Ihuringiellsis* (I), comparative toxicities of these I to *Bombyx mori* larvae, and some economically important insect pests susceptible to I. (35 **ref.)** **P. P. R.**

Development of the bait principle for boll weevil control: Calco Oil Red N-1700 dye for measuring ingestion. R. J. Daum, G. H. McKibben, T. B. Davich and R. McLaughlin *(J. eCOll. Enl., 1969,* 62, 370-375).- The dye is not metabolised by *Anlhollomus grandis* and is easily recovered. (27 **ref.)** C. M. **HARDWICK.**

Attractants for the Japanese beetle. P. H. Schwartz, jun. and D. W. Hamilton *(J. econ. EII/.,* 1969,62, 516-517).–None of the 46 compounds selected from food, flavour and perfume catalogues was as attractive to *Popillia japonica* as was the standard phenethyl butyrate-eugenol (9 : 1). C. M. HARDWICK.

Hexalure, an insect sex attractant discovered by empirical screening. N. Green, M. Jacobson and J. C. Keller *(Experientia, 1969,* 25, 682-683).- The sex attractants emitted by the virgin females of several species of *Lepidoplera* are CI2_16 alkenol acetates. The cis-7-hexadecen-1-ol acetate is an outstanding attractant for the male pink bollworm moths *Pec/illophora gossypiel/a* (Saunders). This has been named Hexalure; the attractiveness is highly unusual since propylure, the natural attractant, is a CI6 alkadienol acetate which has a branched chair and *Iralls* configuration. The efficacy is described and it was found that the synthetic lure at all test dosages was superior to either the natural one or to live caged virgin moths. Hexalure is now being used by the U.S. Dept. Agric. Plant Pest Control Division to combat the pink bollworm in Florida. C. V.

Sex pheromone specificity: Lepidoptera. W. L. Roelofs and A. Comeau *(Sciellce, N. Y.,* 1969, 165, 398-400).- The taxonomic and evolutionary aspects are discussed and the attractiveness of *Irans*- and cis-9-tetradecenyl acetate and cis-11-tetradecenyl acetate to the various species is discussed. (11 rer.) C. V.

Reproducing capacity of gamma-irradiated adult males of tbe confused flour beetle. A. Vereecke *(J. ecoll. Enl.,* 1969, 62, 357-359).-Adult male *Tribolill/ll c0llfusum* were exposed to 3·5-5·5 krad. Fecundity of untreated females was not affected but fertility was initially considerably reduced. The sex ratio was also affected. C. M. HARDWICK.

Effects of gamma-radiation on the tobacco budworm. I. Irradiation of pupae. II. Irradiation of moths. m. Irradiation of eggs and larvae. E. I. El Sayed and J. B. Graves *(J. ecoll. Enl., 1969,* 62, 289-293; 293-296; 296-298).- 1. ***Heliothis*** *virescells* pupae were exposed to up to 40 rad/min from 60CO. Between 1-3 days, this resulted in pupal mortality, adult malformation and reduced egg viability. Irradiation of older male pupae caused reduced longevity and decreased egg viability while irradiation of female pupae greatly reduced egg no. (11 rer.)

II. When males or females were exposed to 35-50 krad, only a few viable eggs were produced and there was considerable larva and pupal mortality amongst eggs that hatched. (11 rer.)

III. Early-stage eggs were very susceptible to irradiation, and there was high mortality amongst any that hatched. Irradiation of larvae also resulted in high subsequent mortality. (15 rer.)
C. M. HARDWICK.

Transfer of chemosterilant by tepa-sterilised Mexican fruit flies. M. W. McFadden *(J. ecoll. Enl.,* 1969, 62, 511-512).–Treatment of female *Allaslreplja ludells* with tepa caused complete sterility. The reduced hatch from the 2nd mating suggests that treated females contaminated the males. C. M. HARDWICK.

Chemosterilisation of the two-spotted spider mite. I. Effect of chemosterilants on biology of the mite. II. Histopathological effect of apholate and 5-fluorouracil on the reproductive organs. III. Effect on host plant. IV. Effect on population. M. Jalil and P. E. Morrison (1. *ecoll. EIII.,* 1969, 62, **393–400;** 400-403; 506-507; 415-419).- (A total of 37 ref.) C. M. HARDWICK.

Apparatus (or subsurface application of nematocides to nursery plants in containers. A. L. Taylor and J. H. O'Bannon *(PI. Dis. Replr,* 1968, 52, 218-222).–The use of the apparatus with a number of org. P nematocides successfully controlled root-knot, spiral, and burrowing nematodes in a number of species. Subsurface application was more effective than surface drenching or soaking the containers. A. H. CORNfIELD.

Iodometric determination of dimethoate. F. de A. Bosch Arioo and F. Bosch Reig *(Revla Agroqui/ll. Tecnol. Alimenl.,* 1969, 9, 262-265).- The method described depends on pptn. of AS2S3 by reaction of dimethoate solution with excess of AS203 in HCl medium followed by iodometric determination of the separated AS2S3. The washed ppt. of AS2S3 is then dissolved in 2 N-NaOH and the solution mixed with a measured amount of 0·1 N-I_2 in dil. HCl, and after neutralisation with NaHC03 the excess I_2 is back

titrated with O·I N-As,03 solution and the dimethoate content is calculated from the I_2 consumed. The method is less time consuming and more reproducible than the conventional gravimetric procedure. E. C. APLING.

Residues on sugar-cane tissues twenty-four hours after application of different formulations of azinphos-methyl. L. Davis, F. Bonner and S. D. Hensley *(J. econ Ent.,* 1969, 62, 505-506).-Azinphos-methyl was applied to sugar-cane as granules, *EC* spray or *LV* spray at 1 lb/acre. Residues were higher from spray application for whole plant samples, most of the residues being on the leaves. C. M. HARDWICK.

Malathion, methyl parathion, diazinon and endosulfan residues in sunflower seeds. N. M. Randolph, H. W. Dorough and G. L. Teetes *(J. econ. Ent.* 1969,62, 462-464).- Sunftowers were sprayed up to four times with the insecticides, the last spray being ≮ 14 days before harvest. Endosulfan gave the highest residues (by g.C. analysis) but these were well below the 2 ppm tolerance level; diazinon was the most persistent of the organo-P compounds, but none of the insecticides appeared to leave excessive residues in the seeds. C. M. HARDWICK.

[Herbicidal] thiocyano-aryl carhamates. Roussel-Vclaf (B.P. 1,159,507, 15.1266. Fr., 15.12.65).-The compounds have phenylene or naphthylene rings with a 4-SCN and a 1- or 2-O·CONRIR' group or have a quinolinediyl ring with a 5-SCN and an 8·0·CONR'R2 group, R' and R' are alkoxy. aralkyl, or aryl or (substituted) alkyl or NR'R2 is heterocyclic. In an example, 1-(N-methylcarbamoyloxy).4-thiocyanonaphthalene, of m.p. 161-162', is prepared by reftuxing a mixture of 4-thiocyano-I-naphthol, MeNCO and Et3N in Pr'20 and working up the reaction mixture. S. S. CHISSICK.

Alkyleneiminourea compounds, preparation and use. *V.S.* Borax & Chemical Corp. (RP. 1,158.858, 26.4.68. *V.S.. 19.5.67).*- Compounds of the formula o-CN·C.H4NH·CO·NHR wherein R is C4. ,-alkyleneimino are herbicides. In an example, a mixture of I-aminopiperidine, o-CN·C.H4·NCO and ether is boiled for 3 h, to give 1-(piperidino)-3-(o-cyanophenyl)urea. m.p. 118·5' (hexane). F. R. BASfORD.

3-Chloropropyl N,N-di-isopropylthiolcarbamate. Monsanto Co. (Inventors: J. J. D'Amico and M. W. Harman) (B.P. 1,158,502, 5.1.67).- The title compound (I) is prepared from di-isopropylamine which is reacted with COS in aq. alkali to yield a salt of di-isopropylthiolcarbamic acid; this is then treated with a 3-chloropropyl halide. I is claimed as a herbicide. S. S. CHISSICK.

[Herbicidal) substituted quinazolines. Badische Anilin- & Soda-Fabrik A.-G. (Inventors: G. Scheuerer, A. Zeidler and A. Fischer) (B.P. 1,159,543, 28.10.66. Ger., 30.10.66).- I-Rl-3-R'-2,4-dioxo-decahydroquinazolines, where R' is an optional acyl group and R^2 is alkyl, optionally substituted with Cl or MeO, are prepared from the (corresponding) 2-aminocyclohexanecarboxylic acid (I) by heating with an isocyanate and cyclising the product in presence of acid. E.g., Pr'NCO is added to I in PhMe with cooling; the resulting solid is dissolved in EtOH and cone. HCI added. The mixture is reftuxed for I h and worked up to yield 3-isopropyl-2,4-dioxodecahydroquinazoline. S. S. CHISSICK.

Cycloalkenecarboxanilides. H. Schwartz (B.P. 1,145,333, *25.5.66. V.S.,* 27.5.65).-Vsed in post-emergence herbicidal compositions, the compounds X.C.H4·NH·CO·R (where R is cycloalkene of 3-8C and X. is halogen, lower alkyl, lower alkoxy or halogenated lower alkyl of 1-7C, except CF3 alone or with I or 2 halogen atoms when R is I-cyclopentene or I-cyclohexene and n = I, 2, or 3) are prepared by reacting a cycloalkenecarboxylic acid halide with the corresponding aniline deriv. A typical compound is 3'-chloro-l-cyclopentenecarboxanilide, m.p. 123-124'. The herbicidal properties of the compounds, applied as 10% emulsion concentrates, are listed in tables. S. D. HUGGINS.

Thiazole derivatives. May & Baker Ltd. (Inventors: M. S. Barber, D. R. Broad and B. J. Heywood) (B.P. 1,145,822, 10.3. and 3.12.65).-Herbicidal 2-NHCOR-4RI-5X-thiazoles (and their salts) are claimed, where R is 2-5C aliphatic group, R' is H, halogen or 1-3C (halogeno)-aliphatic group, and X is Cl, Br or 1. They are prepared by halogenation of a 2-acylaminothiazole or by acylation of a 2-amino-5-halogenothiazole. The compounds, e.g., 5-bromo-4-methyl-2-propionamidothiazole, are active against mono- and di-cotyledonous weeds. S. S. CHISSICK.

Halophenylpyridine derivatives. Monsanto Chemicals Ltd. (Inventors: F. Long and K. N. Ayad) (B.P. 1,147,438, 6.7.66).- The title bases have the formula $R_mC5H4\text{-}_mN·C.H5\text{-}.X.$, where R is halogen, CN, OH, mercaptoor aliphatic-oxy group, m = I, 2 or 3, X is halogen, and n = 3, 4 or 5, provided that where the pyridine nucleus contains both CI and CN, the halophenyl group is present in an **α-** or **β-position** in the pyridine nucleus. An Rnrsubstituted pyridine sulphonyl halide is heated with an Xn-substituted benzene to give, e.g., 2-chloro-5-(2',4',6'-trichlorophenyl)pyridine, m.p. 82-84'. This compound, or its N-oxide, is a highly effective herbicide (0'25-1 lb per acre) against, e.g., crabgrass, pigweed, wild oat, smartweed. S. D. HUGGINS.

[Herbicidal] **halocyanoimidazole.** Shell Internationale Research Mij N.V. (Inventors: K.-H. Buchel and A. Conte) (RP. 1,147,555. 6.2.67. Ger., 8.2 and 12.10.66).-I-Cyano-2,4,5-trichloroimidazole (I), useful against broad- and narrow-leaved plants, is prepared by reacting 1,2,4,5-tetrachloroimidazole with $Cu(CN)_2$ at ≮ 110' or by reacting 2,4,5-trichloroimidazole with NaOEt in presence of an inert polar solvent (MeCN), with exclusion of moisture, and treating the resulting salt solution with cyanogen chloride or bromide at **0–30°**. The latter process can give I (m.p. 115-116' from hexane) in 80% yield. S. D. HUGGINS.

Amine salts of herbicidal organic acids. Farm Protection Ltd. (Inventors: W. Furness, J. L. Forryan and P. Wainwright) (B.P. 1,148,387, 29.6.65).-Emulsifiable formulations of water-insol. amine (≮ 8 C and preferably di- or tri-amines) salts of, e.g., dicamba, are claimed as selective herbicides against weeds such as *Chrysanthemum segetum, Rumex* spp. and *Pteridium aquilinium.* S. S. CHISSICK.

Herbicidal compounds and compositions. E. Lilly & Co. (Inventor: Q. F. Soper) (RP. 1,149,139, 6.10.67).- The herbicides, viz., 4,3,5,I-NRRIC.H2(NO,),·S02NR"RIII, are especially effective **against** ***Datura stramonium, Ambrosia arlemesiifolia, Hibiscus trionium,*** and *Abutilon theophrasti,* wherein R-R' are C3-4-alkenyl or C3-.-alkyl or NRRI is pyrrolidino, piperidino, or morpholino, or R is H; R"_RIII are H, C'-5-alkyl, allyl, Ph, C3-5-cycloalkyl, or NR"_RIII is pyrrolidino or aziridino. An example is 3,5-dinitro-4-dipropylaminobenzene sulphonamide, m.p. 137-138' (acetone-light petroleum), prepared by boiling 4,3,5,I-NPr_2·C.H,-$(NO_2)_2SO_2Cl$ in cone. aq. NHa for 3 h. F. R. BASfORD.

Herbicidal substituted a-halogenoacetanilides. Monsanto Co. (B.P. 1,149,843, 1.3.66. *V.S.,* 14.10.65).- Compounds claimed have the formula C.H3_.RIII.RIR"·N(COCH2X)·CH2·A'CR'v-RVRvl wherein RI and R" (in the 2 o-positions to N)are C'-lo-alkyl or alkoxy; Rill is halogen or as Rl; *n* is **0–3;** A is 0 or S; X is CI, Br, or I; and RIV_Rvl are H, alkyl, alkenyl, or alkynyl of 1-18 C, aryl of 6-24 C, heterocyclyl **of** ≯ 24 C atoms and 1-3 hetero atoms, or CR,vRv is a carbocyclic radical of 2-7 C, and any of RIV_RVI may carry non-interfering substituents. An example is *N*-2-methoxymethylchloroacet-6'-t-butyl-2'-toluidide, m.p. 77-78', made by interaction of 2,6,I-C.HaMeBut.N(CH2CI)·COCH,CJ with a 25% solution of NMe3 in MeOH. Its action on a variety of plants is tabulated. F. R. BASFORD.

Quaternary ammonium salts. Farbenfabriken Bayer A.-G. (Inventors: G. Zumach, H. Scheinpflug and E. Kuhle (B.P. 1,138,434, 22.2.67. Ger., 26.3.66).-Bactericidal compositions active against *Xanthomollas* and *Pseudomonas* spp. in cotton, tomatoes, tobacco and rice, comprise, (together with carriers or diluents containing a surface-active agent) as active ingredient, a quaternary ammonium compound [R'X·CHRt. NR'R2Ra)+z - in which X is 0 or S; R is alkyl (1-4C), cycloalkyl (with **5–7** ring members) or aralkyl which may be substituted by halogen, nitro and/or chloroalkoxy, or, if X is S, aryl which may be similarly substituted; R' is H or alkyl (I-4C); R' is **1–4C** aliphatic (methyl) or cycloalkyl radical; R3 is alkyl **(10–18C)** and Z is an anion. These compounds are prepared by reaction of ethers R.X.CHR'Hal with tertiary amines NR2R'R3. J. M. JACOBS.

[Fungicidal] eyanoethyl phenylcarhamate compounds. Sumitomo Chemical Co. Ltd. (RP. 1,143,894, 22.9.67. Fr., 22.9.66).-a-Cyanoethyl N-phenylcarbamates in which the Ph group is substituted with 1-5 CI and/or Me groups are prepared from (i) the corresponding CJ- and/or Me-substituted aniline and a-cyanoethyl chloroformate, or (ii) the corresponding CI- and/or Me-substituted N-phenylcarbamoyl chloride or phenyl isocyanate, and a-cyanoethyl alcohol. The compounds (e.g., the 2-chloro-, 2,3-, 2,5- or 3,5-dichloro-, and the 3-methyl-2,4,6-trichloro- deriv.) are effective against several plant diseases and are less toxic to mammals than organo-Hg fungicides. S. S. OUSSICK.

Control offungal diseases in trees. Thomson Research Associates ltd. (Inventor: A. W. Thomson) (B.P. 1,145,496, 29.6.65).-A method for **treating** elm trees to combat Dutch elm disease com-

prises treating the tree trunk with a 0·1-10 wt.-% **solution** of (i) tris(hydroxymethyl)nilromethane (0 or (*ii*) cetyl pyridinium bromide and citral, in an aq. or non-aq. carrier. In an example, elm trees are treated with a 1·5% solution of I in an acetone **(44·3%)–castor** oil {44'3%)-DMSO (9'9%) mixture; out of 35 infected trees so treated, one died and the remainder were in an improved condition. S. S. CHISSICK.

Triazine derivatives. Nippon Kayaku K.K. (Inventors: Y. Sakurai, T. Ishizawa, S. Ishida and R Sekine) {B.P. 1,145,620. 1.9.67).-2-NHR'-4-NHRII-6-SNa-s-triazines (R' and R" are H or alkyl of **1–4C)** are reacted with CH2:CX·CH2X' (X and X' are halogen) to give **6-SCH₂CX:CH₂** deriv. with fungicidal properties (especially against *Pirieularia oryzae)*. An example is 2,4-di-{ethylamino)-6-{2-chloroallylthio)-s-triazine (prep. is described). F. R. BASFORD.

Agents for control of phytopathogenic **fungi** [on fruits and vegetables]. Farbenfabriken Bayer A.-G. (Inventors: H. Goeldner and F. Grewe) {RP. 1,148,140, 16.11.67. Ger., 20.12.66 and 19.7.67). –A synergistic combination of zinc propylene-I,2-bis-(dithiocarbamate) (I) and N-trichloromethylthiophthalimide (II), active against phytopathogenic fungi including *Batrytis* spp., is claimed. The II: I ratio is in the range 0'25-2: 1. S. S. CHISSICK.

Trichloromethanesulphenyl-N-anilide derivative. Farbenfabriken Bayer A.-G. (Inventors: P.-E. Frohberger, E. Kuhle and E. Klauke) (B.P. 1,149,937, 31.1.68. Ger., 2.3.67). –The compound *N*-trichloromethanesulphenyl-N-trifluoromethyl-2-methyl- 5- nitroaniline (I) which is strongly fungitoxic and has good plant compatibility is prepared from N-trifluoromethyl-2-methyl-5-nitroaniline and ClS·CCl3 by reaction in presence of a t-amine, e.g., Et3N, in an inert solvent (benzene, acetone, etc.) at 0-80 (10-50'). I is particularly useful for protecting cotton (seed or crop). S. S. CHISSICK.

Carhamyl hydroxamate pesticides. E. 1. du Pont de Nemours & Co. (Inventor: J. R Buchanan) (RP. 1,138,347-9, 21.12.65. o and Cdiv. out of A).-The title compounds are especially effective against insects, acarids, and nematodes, and have the formula NRIIRIII. C02' N:CR'X'R wherein RII and RIII are H or Me; R is, e.g., alkyl, Ph, C,H,Cl-p; X' is [A,C) S, [0) O; and R' is [A) alkyl or halogenoalkyl, cycloalkyl, CN2Ph (which may contain halogen), I- or 2-ClOH1, or Ph, [0) H, alkyl or halogenoalkyl, alkenyl, alkynyl, carbalkoxy, cycloalkyl, CH2Ph (which may contain halogen), 1- or 2-ClOH1, Ph, alkoxyalkyl, dialkylamino, or alkylthioalkyl, or dialkylaminoalkyl or carbalkoxyalkyl, [c) H, carbalkoxy, alkenyl, alkoxyalkyl, dialkylamino, or a1kylthioalkyl, or alkynyl, dialkylaminoalkyl, or carbalkoxyalkyl, or [A, 0) R' may be 3,4-methylenedioxyphenyl or substituted phenyl. As an example of method of prep., [A) p-Cl-C,H"C{SMe):NOH is added to a suspension of 50% NaH-mineral oil in tetrahydrofuran (I) then the resulting Na salt, suspended in I, is added gradually to ether containing phosgene at 0-5'. Excess of the latter is distilled off / < I atm. and the residue is stirred with NH3 in MeCN at 0_10'. The filtered solution is extracted with hexane to give Me O-carbamyl p-chlorothiolbenzhydroxamate. Formulations containing it are active against *Macrosiphum euphorbiae* and *M. rosae.* Many compounds are noted, also many pests against which they are effective. F. R. BASFORD.

Tri- and tetro-thiophosphate esters. Pechiney-Progil {B.P. 1,158,632, 17.6.66. Fr., 18.6.65).-Antiparasitic agents (insecticides, pesticides, fungicides) of the formula SR'{SRII)PY'SR are claimed wherein Y is 0 or S; RI and RII are alkyl; and R is [CH2]IIC.H,M (M is halogen, alkyl, etc., *n* is I or 2), A·C02RIII (A is CH2 or **[CH₂]₂;** RIII is alkyl), [CH2kCONR2IV (RIV is H, alkyl, alkenyl, etc., or NR2'V is heterocyclyl), [CH2]IINR2'V, **[CH₂]ₙ**-PO{OR')(OR") or [CH2kZPO{ORI)(ORII) (Z is 0, S or NH). In an example, a mixture of **$(CH_3S)_3PO$** and a 28% solution of NMe3 in acetone is heated at 95' during 8 h, and ether is added to the cooled mixture with pptn. of tetramethylammonium *S,S*-dimelhyltrithiophosphate, m.p. 132', in 85% yield. F. R. BASFORD.

Stabilised microbial insecticides. International Minerals & Chemical Corp. (B.P. 1,159,137, 11.1.67. U.S., 19.1.66). –The title products comprise (*i*) a water-insol. parasporal cryst. material produced by an insect toxin-producing, spore-forming microorganism *(Bacillus thuringiensis)* and (*ii*) a stabilising mixture of an antioxidant (e.g., an aromatic amine) and a radiation **(300–400** nm) absorber, e.g., 2-(2'-hydroxy-5'-methylphenyl)benzotriazole. S. S. CHISSICK.

[Pesticidal) organophosphorus compounds. Imperial Chemical Industries Ltd. (Inventors: F. L. C. Baranyouits and R. C. Hinton) {B.P. 1,140,721, 1.2.65).-Esters of the formula RI{XIRII)PX'SR are highly effective against, e.g., *Aedes aegypti, Aphis labae, P/utel/a maeulipennis, Phaedon eoeh/eariae* and *Musca domes/iea* (R is 5-RIII-2-oxotetrahydrofuryl; R' and RIII are alkyl of 1-7C or RIII is H or halogen; X is 0 or S and the P-containing group is attached either to the /l-position of R, in which case Xl is 0 or S and **R^II** is alkenyl, branched-alkyl, aralkyl, or cycloalkyl, or to the a-position, in which case XIRII represents heterocyclic or arylamino, or Xl is **O** or Sand R" is alkenyl, aralkenyl, halogenoalkenyl, aryl, aralkyl [which may contain halogen or OMe), alkyl optionally containing heterocyclyl, alkylthio, NH2 or carbalkoxy, or a [CH2]nR'" group; *n* is 2 or 3; and RI Vis 5-methyl-2-oxotetrahydrofur-3-ylthio-[methyl)phosphinolhioyloxy). In an example, a mixture of MeP(OCH2Ph)S2Na, benzene, and y-valerolactone is heated at 70' during 30 min, with formation of O-benzyl-S-(5-methyl-2-oxotetrahydrofur-3-yl) methanethiolothionophosphonate, n_D^{23} = I·3782. F. R. BASFORD.

[pesticidal) substituted **nitriles.** CIBA Ltd. (B.P. 1,140,979, 6.10.66. Switz., 7.10.65).-Compounds of formula CN'CZ:CXY or CN,CYZ,CXY2 are especially useful as molluscicides, wherein X is halogen, SR, or NR'R"; R is Cl-,-alkyl, alkenyl, aryl, or aralkyl; R'-R" are H, aryl, saturated or unsaturated aliphatic radical of 1-12C, or NR'R" is heterocyclyl; Y is halogen or SRIII; RIII is Cl-,-alkyl; and Z is CN, C02RIV, or CONRvRv, (RIV is Cl-,-alkyl, cycloalkyl, alkenyl, or alkoxyalkyl; RV_RvI are H, Cl-,-alkyl, optionally substituted Ph, or NRvRvI is 5- to 7-membered heterocyclyl). In an example, **SO_2Cl_2** is added dropwise at room temp. to CH2Cl2 containing C02Me'C(CN):C(SMe), then after 30 min at the boil the mixture is distilled, to give Me 1,2,2-trichloro-l-cyano-2-methylthiopropionate, b.p. 103-105'/0'15 mm, m.p. **38–41°,** as well as Me 2-chloro-I-cyano-2-methyllhioacrylate, b.p. 110-115'/0'15 mm, m.p. 94-96'. F. R. BASFORD.

Combating soil nematodes. N. V. Philips' Gloeilampenfabriken (RP. 1,141,707, 16.2.66. Neth., 18.2 and 24.4.65).-The soil is treated with a 4-halopyridine (or an acid addition salt) or a 4-halopyridine N-oxide, 10-80 kg/ha. The 4-chloro- or -bromo-compounds in a solid or liquid carrier are dispersed 5-14 days before planting or sowing and may be combined with soil fungicides or insecticides, another nematocide and/or an artificial fertiliser. A typical composition consists of 45% 4-chloropyridine N-oxide, 1·5% emulsifier spreader (alkylarylpolyglycol ether) and 53·5% distilled water. S. D. HUGGINS.

Pesticides. Hercules Inc. (B.P. 1,142,170, 24.5.66. U.S., 25.5.65 and 4.4.66).-Used to kill pests by direct or indirect contact, Ihe compounds are of formula m-R·C.H,·OCO·NMe·COCX3, where R is lower alkyl and X is H or CI, at least one being CI. They are made from the corresponding chloroacetyl chloride and m-alkylphenyl N-methylcarbamate. E.g., m-Me·C,H,·OCO·NHMe is heated with CH2CI·COCI at 130' until no more HCI is evolved. The product melts at 94-96' (benzene). The compounds are active against aphids, Mexican bean beetles, mosquitos, mites, etc. S. D. HUGGINS.

Esters of ***O,O*-di-isopropyl** phosphorodithioic acid. American Cyanamid Co. (B.P. 1,142,340, 23.6.66. U.S., 27.7.65).-The S-ethylsulphinylmethyl and S-ethylsulphonylmethyl esters **(n_D^{25}** 1·5232 and 1,5135, respectively) are claimed; they are highly effective against mites, aphids, etc., while having a **10–100-fold** safety margin over closely related compounds (low toxicity to mammals). They are prepared by reacting O,O-di-isopropyl-S-ethylthiomethyl phosphorodilhioate with I or 2 equiv. of monoperphthalic acid at - 10 to + 10' in an inert solvent. F. R. BASFORD.

Insecticidal compositions. Richardson-Merrell Inc. (Inventors: P. D. Harwood and D. M. Burkhart) {B.P. 1,143,053, 1.2.67).- The claimed composition contains 0·5-50 pt. of piperonyl butoxide to one pt. of a Lobelia alkaloid (lobeline, lobelanine or lobelanidine obtained by solvent extraction of, e.g., *Lobelia siphili/iea),* and is active against ants and several types of fly. S. D. HUGGINS.

Alkylthioethyl [phenyl]carbamates. Farbenfabriken Bayer A.-G. (Inventors; G. Schrader, G. Unterstenhofer, and I. Hammann) (RP. 1,143,144,6.10.67. Ger., 8.11.66).-The alkyl group contains **1–4C;** the Ph contains 1-3 CI, and the products are acaricides. An example is 2-(methylthioethyl) p-chlorophenylcarbamate, m.p. 67' (EtOAc-light petroleum), prepared (60%) by adding p-NCO·C.H,CI in benzene to a mixture of **SMe[CH₂]₂OH,** benzene, and NEt3, then working up after 20 h at 60'. F. R. BASFORD.

Insecticides. Stauffer Chemical Co. (Inventors: K. Szabo and D. J. Broadbent) (B.P. 1,143,726, 5.9.67).-Carbamates of formula m-RCONHC.H,OCO·NHMe (where R is 1-6C alkyl) are prepared from the appropriate m-alkylamidophenol and MeNCO by reaction in, e.g., boiling **CHCl₃** in presence of EtaN. They have superior insecticidal activity, compared with p-substituted carbamates. S. S. CHISSICK.

Substituted phenyl carbarnates. Stauffer Chemical Co. (B.P. 1,145,207, 27.9.66. U.S., 29.9.65).- Used as insecticides, the carbamates RC.H,·OCO'NHMe (where R is m-substituted lower alkyl-thioalkoxy group, a p-substituted cyanomethyl or cyanoethyl group or is a 2,3-substituted ethylenedioxy or 2,3-oriented lower alkyl ethylenedioxy group) are obtained by reacting Me isocyanate (I) with the appropriately substituted hydroxyphenyl compound in presence of a t-amine. As an example, m-(methylthiomethoxy)phenol in **CHCl₃** is reacted with I, EtaN being added as catalyst, and the mixture is cooled to room temp. over I h. The volatile materials are then removed *in vacuo,* leaving the product N-methyl-(m-methylthiomethoxy)phenyl carbamate, m.p. **70–72°** (MeOH). S. D. HUGGINS.

Aziridine derivatives. Esso Research & Engng Co. (B.P. 1,145,760, 19.12.66. U.S., 3.1.66).-Aminohydrin compounds of formula YCR'R'OH, where Y is N or N.(CH')nNH, *n* is 2,3, or 4, RI is a (per)halo-alkyl/-aryl group and R' is H or an org. radical, preferably alkyl, aryl or RI, having pesticidal (e.g., nematocidal) properties and a process for their prep. from aziridine (I) and/or its deriv., are claimed. lor certain of its N-(w-aminoalkyl) deriv. are reacted with halo-ketones or -aldehydes. E.g., 2,2,2-trichloro-l-(N-aziridyl)ethanol of m.p. 88-89' (acetone) is prepared from chloral which is reacted with I in dry ether at **0°** under N,. S. S. CHISSICK.

Thiol-phosphorlc (-phosphonlc) and thionothiol-phosphoric (-phosphonic) acid esters. Farbenfabriken Bayer A.-G. (Inventors: G. Schrader, I. Hammann and W. Behrenz) (RP. 1,146,125, 30.8.67. Ger., 25.1O.66).-The esters, viz., RI(OR")PX'S'CH-(SR)'CN have insecticidal and acaricidal properties (R-RII are alkyl of I-4C or RI is Ph or alkoxy of I-4C; X is 0 or S). An example is *a,a-Me,* S-cyano(methylthio)methyl thiothionophosphate, b.p. 94'/0'01 mm, obtained (45%) by interaction of **(OMe)₂PS₂NH₄** with CICH(SMe)CN in MeCN at **50–60°**. F. R. BASFORD.

Thio-phospboric (-phosphonic) and dithio-phosphoric (-phosphonic) acid esters. Farbenfabriken Bayer A.-G. (Inventors: H. Timmler, I. Hammann and R. Wegler) (B.P. 1,149,159, 16.11.67. Ger., 8.12.66).-The esters have the formula RI(ORII)PX'SR and are active against sucking and biting insects, especially spider mites (RII is alkyl of 1-6C; RI is Ph, RII, or ORII; R is -triazol-3-yl optionally substituted in the 4- and 5-positions by RII or in the 4-position by Ph, optionally substituted; X is 0 or S). In an example, a mixture of the Na salt of 3-SH-4-Ph-I,2,4-triazole, acetone and **(OEt)₂P(O)Cl** is boiled overnight with formation of S-(4-phenyl-I,2,4-triazol-3-yl) *a,a-Et,* thionophosphate, m.p. **65°** (aq. MeOH). F. R. BASFORD.

Insecticidal composition containing a substituted dialkyl sulphide. Vsesoyuznyl Nauchno- Issledovatel'skil Institut Khimicheskikh Sredstv Zashchity Rasteni! (Inventors: N. N. Mel'nikov, Va. A. Mandelbaum and S. A. Roslavtseva) (RP. 1,146,616, 18.3.66).-The insecticidal effect of **(OR)₂PX₂[CH₂]ₙSR¹** (R is alkyl; X is 0 or S; ***n*** is 1-3; RI is alkyl) is synergised by **SR[CH₂]ₙ·XR¹¹** (RII is aryl which may contain a neutral polar substituent). A typical synergist is 2-(p-nitrophenoxy)ethyl ethyl sulphide, b.p. 160-164'/0'7 mm, prepared (73%) by slowly adding SEt·**[CH₂]₂Cl** at **40°** to a solution of p-NO,·C.H,·OH in aq. NaOH. F. R. BASFORD.

Nematocidal **2,4-dihalophenylsulphonates.** Shell Internationale Research Mij N.V. (RP. 1,/47,035, 27.7.66. U.S., 29.7.65).-The compounds ROCH,CH,SOaC.H,XX'-2,4, which are highly effective as nematocidal seed-treating agents and relatively non-phytotoxic to germinating plants, are claimed, where R is H, I-6C-alkyl, -alkenyl or -hydroxyalkyl, 2-6C-alkanoyl or -alkoxyalkyl or a-heterocyclic-substituted alkylene; X and X' are F, Cl or Br. Prep. is, e.g., by reacting an appropriate 2,4-dihalopheno! with an alkanesulphonyl chloride in presence of an acid acceptor. E.g., 2,4-dichlorophenol-2-hydroxyethanesulphonateacetate,m.p.31-32', is prepared from 2,4-dichlorophenol which is mixed with EtaN and added to isothionylchloride acetate in ether at **10–20°** over 1·5 h and then stirred for a further 2 h. S. S. CHISSICK.

[Insecticidal] imidazole derivatives. Badische ADilin- u. Soda-Fabrik A.-G. (Inventors: H. Adolphi, A. Steimmig and H. Spaenig) (B.P. 1,148,103, 21.7.66. Ger., 22.7.65).-I-RI_2-RII_5-Rlll-imidazoles are synergists for pyrethrin, carbamate and phosphorus ester insecticides, wherein RI is H or aliphatic hydrocarbon radical of 1-I3C, optionally substituted by, e.g., Cl, OH, NH" morpholino, alkoxy, Ph or CH(OMe)OPh; R" is H, Ph, or CI-3-alkyl which may contain OH; and Rlll is H or Et which may contain OH or Cl, at least one of RI_Rlll not being H. An example is dodecyl-imidazolylacetaldehyde-*a,* N-acetal[I-(1-dodecoxyethy!)imidazole], b.p. 171-174'/1'7 mm, prepared in 73'5% yield by heating a mixture of imidazole, CH,:CH'OC12H'5 and quinol for 5 h at 180'. Many more products are described and their synergistic effects tabulated. F. R. BASFORD.

2,2,2-Trichloroethylideneanilines. Farbenfabriken Bayer A.-G. (Inventors: H.-G. Schmelzer, E. Degener, G. Unterstenhofer, H. Tarnow and I. Hammann) (B.P. 1,149,138, 5.10.67. Ger., 28.10.66).-These compounds, having strong acaricidal activity, are prepared by interaction of NSO'CoH5-X-y-z'RzXx(CFa)y with CClaCHO (I) (R is alkyl and/or CN; X is F, CI or Br; *x* is *0-5; y* is 0-2; *z* is 0-3, *x* or *y* being ⩽ I and $x + y + z$ being ⩾ 5. Thus, *m-C.H,*·CI·NSO boiled with I affords (2,2,2-trichloroethyl-idene)-m-chloroaniline, b.p. 123-125'/0'4 mm. F. R. BASFORD.

Fluorinated allenes. Allied Chemical Corp. (B.P. 1,149,450, 19.12.67. U.S., 22.12.66).-Compounds of formula CF,X'C-(CF'Y)'C:C:CF(Z), where X and Yare H, F or CI and Z is H or CI (if X and Yare H, Z is also H), are useful for controlling nematodes and as fumigants against stored product insects. Prep. is by heating under pressure SF, and CF,X'C(CF,Y)(OH)'C,CZ (I : I) at 0-30 for 2-20 h. E.g., 1,1,I-trifluoro-2-trifluoromethyl-3-butyn-2-ol is cooled to **−78°** in a steel reactor, which is then evacuated. SF, is condensed into the reactor, which is then left to stand for 16-18 h at **20–25°;** the contents are worked up to yield I,I,l-trifluoro-2·trifluoromethyl-4-fluoro-2,3-butadiene, b.p. **34°.** S. S. CHISSICK.

Animal Husbandry

1st and 2nd nutrition conference for feed manufacturers. Eds. H. Swan and D. Lewis *(Proe. Can! Univ. NOl/inghom Seh! Agrie.,* 1967, 99 pp.; 1968, 192 pp.).-I. Conference held at Sutton Bonington, Loughborough, Leics., 3-5 Jan. 1967. Food processing and nutrient availability. A. A. Woodham (6-18).-(65 reL) Synthetic amino acids in animal diets. D. Lewis (19-29). Metabolisable energy and ruminant diets. K. L. Blaxter (30-36). Net energy and ruminant rations. B. P. Cardon (37-55). Practical feed quality control. H. E. Bechtel (56-57). Toxic materials in animal feedingstuffs. K. W. G. Shillam and A. N. Worden (68-84). -(89 ref.) Problems in mineral nutrition. J. T. Abrams (85-96).
II. Conference held at University Park, Nottingham, 20-22 Mar. 1968. Principles of fat utilisation. D. G. Armstrong and I. P. Ross (2-21).-(40 ref.) Selection of commercial fat sources for use in livestock and poultry feed. H. D. Hathaway (22-42).-(45 ref.) Possible adverse effects of oxidised fat in feeds. K. J. Carpenter (54-67).-(16 ref.) Milk problems of fat inclusion. J. D. Wilson (75-86). Use of non·protein nitrogen in animal feeds. A. W. Broome (92-113).-(23 ref.) Protein quality and animal feeds. G. D. Rosen (114-138).-(19 ref.) Consequences of mineral imbalance. N. F. Suttle (150-168).-(98 ref.) Effect of mill processing on vitamin levels in diets. J. R. Pickford (175-184). Discussions. (43-46,47-51; 68-71, 72-74; 87-88, 89-90; 139-141, 142-147; 169-170, 171-174; 185-186). P. C. W.

Rôle of stubble in the survival of ice-covered forages. S. Freyman *(Agron. J.,* 1969, 61, 105-107).-Pot tests with 22 species and varieties of forages which were encased in ice and stored for 60 days at **−4 ± 1°** showed that reed canarygrass, Kentucky bluegrass, and orchardgrass were injured to a much smaller extent when stubble protruded through the ice during the frozen period than when no stubble protruded. Two lucerne varieties also showed somewhat less damage when the stubble protruded. Meadow foxtail, smooth bromegrass, timothy and red fescue were not injured even when entirely encased in ice but legumes usually showed considerable damage. (21 ref.) A. H. CoRNFIELD.

Pasture productivity of crested wheatgrass as influenced by nitrogen fertilisation and alfalfa [lucerne]. G. A. Rogier and R. J. Lorenz *(Tech. Bull., U.S. Dep. Agrie. Res. Serv.,* 1969, No. 1402, 33 pp.).-A Io-yr study on the pasture performance of *Agropyron deserlorum* (Fisch. ex Link) Schult. is reported. Average beef production from the 1st to the 10th year increased on pastures fertilised with 40-80 lb of N/acre, decreased slightly in those bearing crested wheatgrass-lucerne mixtures, and decreased sharply

in unfertilised pastures. Org. C and total N increased consistently with fertilisation. Soil pH increased slowly in surface soils under the fertilised treatment. P losses were less under fertilised treatments and greatest under grass mixtures. (26 reL)
E. G. BRICKELL.

Crude protein content of eleven grasses as affected by yearly variation, legume association, and fertilisation. J. R. Johnson and J. T. Nichols *(Agron. J.,* 1969, 61, 65-68).- In a dry season, the crude protein % of forage from II grasses was increased to a greater extent by application of N than by growing the grasses in association with lucerne. In a wet season, there was on average no significant difference in crude protein % between applying N and growing the grasses with lucerne. Although crude protein % varied with species, the grasses generally maintained the same relative ranking in protein % irrespective of season and treatment. (10 reL)
A. H. CORNFIELD.

Effect of N, P and K levels and clipping frequency on forage yield and protein, carotene, and xanthophyll content of Coastal Bermudagrass. G. W. Burton, W. S. Wilkinson and R. L. Carter *(Agron. J.,* 1969, 61, 6O-63).-Forage yields of Bermudagrass were lower where N was applied without P + K, but protein *(P),* carotene *(C),* and xanthophyll *(X)* contents were not affected. Increasing the ratio of P and K to N above a **4–1–2** (N-P205-K20) ratio failed to increase *P,* C and *X* contents except at a very high N rate (1008 kg/hal. (12 ref.)
A. H. CORNFIELD.

In vitro digestibility of two **tropical** *legumes-Phaseo/us afrO-purpureus* and *Desmodium intortum.* R. J. Jones *(J. AUSI. Insl. agric. Sci.,* 1969, 35, 62-63).–At all stages of growth, the *in vitro* O.M. digestibility of *D. in/orlum* was lower than that of *P. alro-purpureus.* The difference between the legumes at the 16-week growth stage was apparent for all plant parts; stems had the lowest digestibility and petioles the highest in both legumes.
E. G. BRICKELL.

Effect of fertiliser nitrogen, variety and maturity on dry matter yield and nitrogen fractions of corn [maize] grown for silage. R. G. Gonske and D. R. Keeney *(Agron. J.,* 1969, 61, 72-76).-The effects of N (100-300 kg per hal on dry matter *(DM)* yields, total N, EtOH-insol. N, EtOH-sol. N were studied on a sandy loam with 3 single-cross varieties harvested at the early and late dent stages. There were considerable differences among varieties in *DM* yields and contents of the various N fractions. Fertiliser in excess of that required for max. *DM* production increased N03- and total EtOH-sol. contents. Harvesting at the late dent stage resulted in higher *DM* and protein yields and lower N03- and total EtOH-sol. N levels than did harvesting at the early dent stage. (18 ref.)
A. H. CORNFIELD.

Relationship of corn [maize] silage yields to maturity. J. N. Rutger *(Agron. J.,* 1969, 61, 68-70).-Although fresh silage yields of late maize hybrids were considerably higher than those of early hybrids, there was little difference in dry matter silage yields between hybrids. Since harvest and storage costs per unit of dry matter silage are greater for late than for early hybrids, and because early hybrid silage has the greater feeding value, it is suggested that hybrids used for silage should be as early as the best adapted grain hybrids for the region.
A. H. CORNFIELD.

Comparative nutritive value of silages made from high-sugar male sterile hybrid corn [maize] and regular starchy corn [maize]. T. W. Perry and D. M. Caldwell *(J. Dairy Sci.,* 1969, 52, 1119-1121).- The results of trials with four bullocks showed that the digestibility of the crude fibre and crude protein fractions of silage made from male sterile maize was significantly greater ($P < 0.01$) than that of silage made from regular starch maize. Chemical analyses showed that the male sterile maize silage contained more crude protein, crude fibre and ether extract and less N-free extract and ash than the regular starch maize silage.
M. O'LEARY.

Dairy cattle feeding. [Symposium]. Group feeding of concentrates to dairy cows. G. E. Stoddard *(J. Dairy Sci.,* 1969, 52, 844-847).- Group feeding of grain to dairy cows apart from the milking area is advocated instead of the more common practice of individual feeding during milking. Group feeding is claimed to be more economical than individual feeding, mainly because of an improvement in milking rate.
M. O'LEARY.

Dairy cattle feeding. [Symposium]. Problems associated with all corn [maize] silage feeding. C. E. Coppock *(J. Dairy Sci., 1969,* 52, 848-858).-A review of problems associated with all maize silage feeding to dairy cattle is presented. It is concluded that the main problem is the limited knowledge available about the type, most effective route, and most economic level of supplementation necessary. (76 ref.)
M. O'LEARY.

Dairy cattle feeding. [Symposium]. Supplementing corn [maize] silage. D. Hillman *(J. Dairy Sci.,* 1969, 52, 859-870).–A review of the literature on supplementing maize silage is presented. Practical guidelines for supplementing maize silage rations with energy, protein, minerals and vitamins are suggested. (27 ref.)
M. O'LEARY.

Dairy cattle feeding. [Symposium]. Complete rations for dairy cattle. A. H. Rakes (J. *Dairy Sci.,* 1969, 52, 870-875).- The practice of and problems associated with the feeding to dairy cows of complete feeds consisting of a blend of concentrates and roughage are discussed. Areas requiring further research are indicated. (49 reL)
M.O·LEARY.

Dairy cattle feeding. [Symposium]. Feeding dairy **cows** in drylot. D. L. Bath *(J. Dairy Sci.,* 1969, 52, 876-878).- The advantages and disadvantages of drylot feeding of dairy cows are discussed.
M. O'LEARY.

Biological treatment of molasses [stock feed] meal to reduce stickiness. G. Roth *(Proc. S. A/r. Sug. Technol. Ass., 42nd Ann. Congr.,* 1968, 57-64).-A search for gum-destroying organisms led to the selection of 19 bacteria, 17 fungi and 5 actinomycetes for the production of a free-flowing meal. Successful factory trials were made by mixing the meal with a pure suspension of *Penicillium nolalum* spores. Some other organisms also appeared promising in laboratory tests. (18 reL)
P. S. ARup.

Pelletting small amounts of purified diets. W. A. Dewar *(J. agric. Sci., Comb.,* 1969, 72, 325-326).–The substitution of alcohol for water in the prep. of pelleted experimental poultry feeds prevented formation of mixes too sticky for pelleting. The addition of pellet binding agents 'Totanen' and 'Wafolen' gave pellets superior to those obtained with Na bentonite or dextrin.
M. LONG.

Nonprotein nitrogen in the nutrition of ruminants. J. K. Loosli and I. W. McDonald *(F.A.a. agric. Slud.,* 1968, No. 75, 94 pp.).- A review on the use of urea, biuret, ammonium salts and ammoniated feeds. (319 ref.)
E. G. BRICKELL.

Effeet of N supplementation on *in vitro* digestibility of corn [maize], sorghum, and lucerne. A. R. Schmid, G. C. Marten and L. S. Roth *(Agron. J.,* 1969,61, 20-21).- The *ill vilro* digestibility *(D)* of shelled maize and maize fodder was greatly increased by supplementation with urea. Sorghum D was increased slightly, whilst that of lucerne was unaffected. The optimum level of supplementation, that which gave max. *D* without inhibiting digestion of anyone crop material, was 0·04 g per g of substrate dry matter.
A. H. CORNFIELD.

Biological evaluation of selected wheat fractions from nine different wheat samples for energy and protein quality. J. D. Summers, S. J. Slinger, W. F. Pepper and E. T. Moran, jun. *(POIIIII. Sci,* 1968, 47, 1753-1760).-Wheat bran, shorts, red dog and germ from hard and soft red winter, hard red spring, and white wheat samples were assayed for metabolisable energy, metabolisable dry matter, protein efficiency ratio, and net protein utilisation, using the growing chicken.
A. H. CORNFIELD.

Metabolisable energy of 'Opaque-2' and 'Floury-2' corn [maize] for the chick. W. F. Gipp, T. R. Cline and J. C. Rogier *(POIIIII. Sci.,* 1968, 47, 2018-2021).-The metabolisable energy for **2–4** week-old chicks was 3·70, 3·66 and 3·20 Cal. per g for normal, Opaque-2 and Floury-2 maize samples. (10 ref.)
A. H. CORNFIELO.

New carotenoids from *Streptomyces media/ani* n. sp. F. Arcamone, B. Camerino, E. Cotta, G. Franceschi *el 01. (Experiell/ia,* 1969, 25, 241-242).-Of several compounds produced by this species, 3-hydroxyisorenieratene improved the colour of egg yolk when used as a feed additive for the laying hen. (II ref.)
C. V.

Dietary effects on beef composition. I. Quantitative and qualitative carcass traits. R. R. Garrigus, H. R. Johnson, N. W. Thomas, N. L. Firth, R. B. Harrington and M. D. Judge. II. Quantity and distribution of fat. H. R. Johnson, R. R. Garrigus, R. D. Howard, N. L. Firth, R. B. Harrington and M. D. Judge. III. Weight, composition and potassium-40 content of fat-free muscles. M. D. Judge, N. L. Firth, H. R. Johnson, W. V. Kessler, R. B. Harrington and R. R. Garrigus *(J. agric. Sci., Comb.,* 1969, 72, 289-295; 297-302; 303-309).–1. Feeding with hay led initially to suppressed development of the edible portion of the carcass, followed by compensatory growth. Cattle fed hay had less carcass wt., less fat cover and more reticulo-rumen wt. than did those fed maize silage. When maize silage was fed initially, followed by maize concentrate, the

edible portion was no greater than when silage was fed continuously. During the final period of higher energy feeding, there was a reduction in edible portion where maize had been fed in the intermediate period. Callie fed silage in the early period had higher marbling scores than those fed hay, and those fed silage during the intermediate period had lower colour and firmness scores in the *longissimus dorsi* muscles than those fed maize. The treatment effects are explainable on the basis of age differences. (10 reL)

II. Feeding hay initially led to less fat deposition than did silage.

111. An early period hay diet led to lower fat-free wt., reduced the dry mailer content and increased the ash content of the muscles compared with the silage diet. These effects were reversed when higher energy rations were fed and the animals slaughtered at a higher wt. .oK emission data confirmed the existence of age or wt. association decreases. The trends in these data indicated that low energy feeding in the early period delayed a decline in muscle K normally accompanying maturing. (15 reL) M. LONG.

Contribution of carcass weight gain to body weight gain of cattle. J. G. Morris *(J. Aust. Inst. agric. Sci.,* 1969, 35, 6O-61).- Six experiments on the intensive fmishing of steers are described and the mean carcass Wl. is tabulated as a % of the body wt. gain for each experiment. As a 'rule of thumb', 62% of body wt. gain may be taken as equal to carcass wt. gain, but this value must not be interpreted as a const. as it can vary with age, class and degree of finish of the callie. (II ref.) E. G. BRtCKELL.

Distribution of potassium, as determined by whole-body .oK counting, and its relation to carcass composition in steers. T. G. Lohman *(Diss. Abstr., B,* 1967,28, 1297).- In a factorial experiment which included 105 steers belonging to four breed types, the animals were divided into 4 wt.-groups, using 2 diets and a stilboestrol treatment, and the distribution of body-K was measured by use of .oK. It is concluded that carcass-K mass can estimate carcass composition within 3%, allowing for differences in carcass mass and breed-type, and for experimental errors. Whole-body-K estimated carcass composition almost as precisely as carcass-K where a low-radioactivity diet was fed prior to whole-body counting. Thus, .oK radioactivity measurement provides a satisfactory method for determination of carcass composition. A. G. POLLARD.

Dairy herd management research in a changing world. J. L. Albright and W. M. Dillon *(J. Dairy Sci.,* 1969, 52, 808-809).- Aspects which should receive priority arc discussed. M. O'LEARY.

InOuence of roughage-tn-concentrate ratios on *ad libitum* consumption by lactating cows. G. M. Ward and P. L. Kelley *(J. Dairy Sci.,* 1969, 52, 1017-1019). –The results of feeding trials, in which rations with lucerne hay: concentrate ratios ranging from 5·5 : I to I : I were fed to Holstein cows, showed that total feed consumption increased consistently with increase in ration concentrates. M. O'LEARY.

InOuence of three ratios of silage and grain and corn [maize] versus beet pulps on voluntary intake by dairy heifers. M. E. McCullough and L. R. Sisk *(J. Dairy Sci.,* 1969,52, 102o-I024). –Feeding trials were carried out in which silage (wheat and ryegrass mixture) was fed either alone or in ratios of 80 : 20, 60 : 40 or 40 : 60 (dry mailer basis) with either maize or beet pulp to dairy heifers and fistulated steers. With maize, dry matter intake was not improved over the 80 : 20 ratio by higher levels of substitution. A similar result was obtained with beet pulp except that the max. level of 40 : 60 reduced intake to the level obtained with silage alone. Because of increased ration digestibility, similar and consistent increases in estimated net energy intake occurred at each level of substitution of both maize and bcet pulp. Multiple regression analysis indicated a complex relationship between ration composition and intake, involving a delicate balance between cellulose, N-free extract, rate of rumen fermentation and bulk density of the ration. M. O'LEARY.

Nutrition of the dairy heifer. VIII. Effect on milk production of level of feeding at two stages of the lactation. W. N. Broster, V. J. Broster and T. Smith *(J. agric. Sci., Comb.,* 1969, 72, 229-245).- 80 Friesian lactating heifers were randomly allocated to two levels of intake containing 7·9 and 6·2 kg starch equiv./day with adequate protein. After 9 weeks they were re-allocated for a further 9 weeks. The higher level of feeding increased yields of milk and milk solids and *SNF* content in each period but had a smaller effect in mid than in early lactation. Body reserves were also conserved. A residual effect from the first period persisted into the second. The absolute output of milk per unit of food over the 18 week period was greatest for the group which had received the lower level of feeding throughout. This was achieved at the expense of body reserves. (51 reL) M. LONG.

Production losses in dairy cattle due to days open. A. Louca Louca *(Diss. Abstr., B,* 1967,28, 1300).- Production by 756 cows up to the end of the lactation period terminating at the nearest to 48 months after first calving was recorded. Regression analysis within herd and year of first calving with age at calving, total days dry and total days in milk, revealed a quadratic relationship between days open and production. An average decrease of 5·3 lb of milk and 0·25 1b of fat was found for each additional day open. A second and similar test was carried out over shorter production intervals. On the basis of results obtained, a max. interval of 13 months for first calvers and 12 months for second and later calvers is indicated for max. production. The correlation between 9O-day production and days open was higher for high- than for low-producing cows. A. G. POLLARD.

Oxytocin in dry period inhibits lactation. E. W. Swanson and J. E. Claycomb *(J. Dairy Sci.,* 1969,52, 1116-1119). –Intravenous injections of Holstein cows with 5 *IV* of oxytocin twice daily during a period beginning 14 days after drying off and ending on parturition caused a significantly lower ($P < 0,01$) milk yield in the subsequent lactation. Oxytocin did not significantly affect the composition of mammary secretions before parturition and there was no evidence of delayed involution. The results suggest that prolonged milking with either short or no dry periods reduces milk yields by an effect of frequent oxytocin release accompanying milking on subsequent lactogenesis. M. O'LEARY.

Protein from wasteland. W. F. Macfarlane *(Aust. J. Sci., 1968-*69, 31, **20–30).—The** breeding and selection of animals capable of making the most efficient use of water are the important factors of physiological ecology in a country such as Australia. The use of tracts of dcnse grassland is improving and animals are being found that can live on what was formerly unproductive country. (18 ref.) C. V.

Domestic animals of China. H. Epstein *(Commonwealth Agric. Bllr.,* 1969, 166 pp.). –205 illustrations of cattle, sheep, goats, pigs, horses, ponies, etc., are shown. (177 ref.) C. V.

Effect of urea on the utilisation of ground, pelleted roughage by penned sheep. I. Food intake, live-weight change and wool growth. J. B. Coombe and G. K. Preston. II. Utilisation of organic matter, nitrogen and minerals. J. B. Coombe and K. R. Christian *(J. agric. Sci., Comb.,* 1969, 72, 251-259; 261-269).- 1. Merino wethers were fed for 16 weeks on a basal diet of ground, pelleted oat or *Phalaris* straw. Urea was supplied *ad lib.* as salt-urea blocks or incorporated in the pellets. Wt. loss was reduced from a mean of 14·5 kg for the control group to a mean of 8·7 kg for the urea-supplemented group. Food intake for all groups increased at first and then fell. The intake of the supplemented groups rose thereafter, whereas the control groups remained low. Wool production was not related to urea intake but was closely related to food intake and live-wt. change. (43 ref.)

II. On the same diets, org. matter and cellulose digestibilities were generally increased. Urea did not affect water intake, but increased N intake, faecal and urinary N excretion and N balance. S balances were generally positive and did not improve in the second half of the trial when more S was supplied. An increase in the P intake from 0·5 to 2·0 g/day brought nearly all sheep into positive P balance. With the urea diets, a significant negative relation existed between urinary N excretion and P balance. Na, K, Ca and Mg intakes appeared satisfactory. An increased supply of minerals rather than an adaptation appeared to be the reason for the improved response to urea in the second half of the trials. (36 reL) M. LONG.

Utilisation of surplus protein by sheep. Y. Dror and A. Bondi *(J. agric. Sci., Comb.,* 1969, 72, 327-330).-When highly increased amounts of protein were ingested, protein degradation in the rumen and removal of urea by the kidneys were limited. Four levels of protein intake were investigated: 100, 140, 180 and 220% of the theoretical maintenance requirement. M. LONG.

Summer nutrition of weaner sheep: voluntary feed intake, body weight change and wool production of sheep grazing the mature herbage of sown pasture in relation to the intake of dietary energy under a supplementary feeding regime. W. G. Allden *(Aust. J. agric. Res.,* 1969, 20, 499-512).-Two field studies with Merino sheep were carried out in a Mediterranean environment. For each 100 g of supplement (up to levels of 400 g of dry matter/day), the intake of herbage decreased by **65–69** g. The daily digestible energy intake needed to maintain wool-free body Wl. was 170 kcal of

digestible energy/$W^{0.75}$ ($\equiv \sim$ 144 kcal of metabolisable energy/$W^{0.75}$ where W = avo net body wt. in kg. The production of each additional g of clean wool was associated with an increased intake of 52 g of digestible dry matter although in practice 86 g of digestible energy is actually required because herbage intake is reduced when a supplement is fed. (27 ref.) E. G. BRICKELL.

Efficiency of conversion of food to wool. IV. Comparison of sheep selected for high clean wool weight with sheep from a random control group at three levels of dietary protein. V. Comparison of the apparent digestive ahility of sheep selected for high clean "'001 weight with that of sheep from a random control group. L. R. Piper and C. H. S. Dolling *(Aust. J. agric. Res.,* 1969, 20, 561-578; 579-587).-IY. Diffcrences were significant for clear wool wt. *(W),* efficiency of conversion of gross energy to wool *(W/l),* fibre no. per unit area of skin *(N),* and mean body wt. *(B),* the clean wool group having higher values for *W, W/l* and *N,* but being lighter in *B.* (37 ref.)

Y. Differences between groups were small, variable and in no instance significant. Enhanced efficiency of conversion of food to wool is probably related to improved metabolism of nutrients after absorption from the alimentary tract. (14 ref.)
E. G. BRICKELL.

Growth and composition of wool. V. Stimulation of wool growth hy abomasal administration of varying amounts of casein. P. J. Reis *(Aust. J. bioi. Sci.,* 1969, 22, 745-759).-Mature Merino or English Leicester x Merino castrate male sheep, with an abomasal cannula ncar the pylorus, were kept in metabolic cages and fed on one of several diets at different levels with supplements of casein (60-200 g/day) in the diet or via the abomasum. Animals were weighed weekly and wool growth was studied on defined areas of each sheep. In the diet, casein had little effect, but by the abomasum, the wool growth rate could be increased threefold. The improvement was independent of diet or energy level intake. The S content of the wool was increased as much as when S-containing amino acids were supplied per abomasum. There was also a significant increase in body wt. (24 ref.) J. B. WOOF.

Performance of Merino ewes deprived of drinking water. J. J. Lynch *(Aust. J. Sci.,* 1968-69,31, 369-370).-Drinking water was withheld for two years and the findings were compared with the Agric. Res. Council's (1965) results and those found in Nova Scotia. The considerable cost of providing drinking waler for grazing sheep can sometimes be avoided. C. V.

Percent caesium-134 and strontium-85 in milk, urine and faeces of goats on normal and verxite-containing diets. D. G. Hazzard *(J. Dairy Sci.,* 1969, 52, **990–994).—The** addition of 3% of vermiculite(verxite flakes) to the diets of goats receiving O·6 μCi of 134CS per day resulted in an increase from 62 to 83 in the % of the total body clearance of 134CS occurring during a 5-day dosing period. Excretion and secretion data (expressed as % of the daily ingested 13'CS) for animals receiving and not receiving vermiculite were, respectively: milk, 0·3 and 2'3; urine, 1·7 and 11·2; faeces, 64·6 and 24·6. No consistent differences were detected between treated and untreated animals receiving **0·6 μCi** of *8* Sr per day. (11 ref.)
M. O'LEARY.

Verxite Oakes for *in ,i,o* binding of caesium-134 in **cows.** D. G. Hazzard, T. J. Withrow and B. H. Bruckner *(J. Dairy Sci., 1969,* 52, 995-997).-Incorporation of 0,82,0'54 and 0·27 kg/day/cow of verxite into the morning grain ration of cows receiving O'2 μCi/day/cow of 13'Cs in the afternoon grain ration resulted in 88, 84 and 68% reductions, respectively, in the amount of 13'CS in the milk. M. O'LEARY.

Digestibility of fats differing in glyceride structure and their effects on growth performance and carcass composition of bacon pigs. R. H. Davis and D. Lewis *(J. agric. Sci., Comb.,* 1969, 72, 217-222). -The digestibilitics of natural lard (I), interesterified lard (II) and beef tallow (III) were compared. The digestibility of I was higher than that of II and, in tum, higher than that of III, the differences arising from differences in the digestibilities of the saturated fatty acids. Absorption of palmitic acid was highest when it was esterified in the p-position. The positional distribution of stearic acid did not appear to affect its digestibility. No differences due to the different fats were found in growth performance or carcass composition. Improvements in both these could be brought about by the addition of fat and careful regulation of nutrient.
M. LONG.

Nutritional significance of the essential fatty acids in swine diets. G. Mojisola Babatunde *(Diss. Abstr., B,* 1967, 28, 1302).-The need for essential fatty acids *(EFA)* in diets for maintaining normal growth, skin condition, efficient food utilisation and carcass quality in pigs from weaning (10 kg) to slaughter (90 kg) and their effects on various biochemical parameters were examined. The *EFA* diet contained 3% of safflower oil (2'4% linoleate), other dietary compounds being added for comparative purposes. Semi-purified diets (16% protein) suitably fortified with minerals and vitamins were fed *ad lib.* to weanlings; no significant effects of *EFA* on growth rate, blood characteristics, fresh wt. of liver and heart or carcass quality were found. In one trial, a fat-free diet resulted in skin lesions. Feeding safflower oil, 3% hydrogenated coconut oil or maize-soyabean diets did not cause skin lesions in the fat-free group. Oils rich in polyunsaturated fatty acids may be beneficial in lowering serum- and tissue-lipids and -cholesterol, but for normal healthy growth, fats of low *EFA* content are adequate. Growth rates and skin lesions are not reliable indices of *EFA* deficiency.
A. G. POLLARD.

Determination of caloric and nitrogen content of excreta voided by birds. C. Blem *(Poult. Sci.,* 1968,47,1205-1208).-
A. H. CoRNFIELD.

Evaluation of a low-gossypol glandless cottonseed meal in broiler diets. P. W. Waldroup, E. G. Keyser, Y. E. Tollett and T. E. Bowen *(Poult. Sci.,* 1968, 47, 1179-1186).-The effect of replacing all or part of the soyabean meal *(SM)* in practical maize-SM diets with solvent-extracted low-gossypol (0'044% total and 0'009% free gossypol) cottonseed meal *(CM)* was studied. *CM* replaced part or all of the *SM* (31'4% in the diet) without adversely affecting broiler performance. Diets containing both *SM* and *CM* supported somewhat better performance than did those fed either alone. Lysine supplementation (0'12%) was necessary only when > 75% of the *SMwas* replaced by *CM.* A. H. CoRNFIELD.

Influence of calcium in laying rations on shell quality and interior quality of eggs. C. Y. Reddy, P. E. Sanford and R. E. Clegg *(Poult. Sci.,* 1968, 47, 1077-1083).-When dietary Ca ranged from 2·25 to 5'05%, max. shell wt., as % of egg wt., was obtained with 5·05% dietary Ca in one test and 3·85% in another. Increasing dietary Ca had no effect on egg quality (Haugh units), but increased egg wt. slightly; > 3'85% was detrimental to egg production, which was max. wilh 3'05-3'85%. (20 ref.)
A. H. CoRNFIELD.

Influence of vitamin D3 on the utilisation of soft **phosphate** by broiler chicks. I. Motzok, D. Arthur and S. J. Slinger *(Poult. Sci.,* 1968, 47, 2021-2023). –Wt. gains of chicks to 10 weeks of age receiving 750 I.C.U. vitamin D3 per kg of feed were somewhat lower where P (0'32% in the diet) was added as soft phosphate as compared with CaHPO.. At the 1800-3600 I.C.U. vitamin D3 levcl, wt. gains of birds receiving soft phosphate were as good as those receiving CaHPO,. A. H. CoRNFrELD.

Effects of high levels of dietary EDTA, zinc, or copper on the mineral contents of tissue of turkey poults. P. Yohra, G. D. Gottfredson and F. H. Kratzer *(Poult. Sci.,* 1968, 47, 1334-1343).-The effects of high levels of EDTA, Zn and Cu, alone and in combination, on the Zn, Cu, Mn and Fe contents of heart, liver, kidney, testes, feathers and ash of tibias and beaks, of poults fed purified diets were investigated. A. H. CoRNFIELD.

Effect of zinc deficiency on bone mineralisation and plasma proteins of turkey poults. P. Yohra and F. H. Kratzer *(Poult. Sci.,* 1968, 47, 1135-1140). –Weight gains of poults over 3 weeks were greatly increased by addition of 15 ppm of Zn or EDTA (0'684 mmole per kg) (which improved availability of zinc already present in the diet) to a Zn-deficient purified diet (17 ppm of Zn). The treatments had little effect on tibia ash, Ca/P ratio and Mn % in the bones, plasma proteins as determined by electrophoresis, or stabilily const. of plasma proteins for Zn. A. H. CoRNFIELD.

Importance of the bursa of Fabricius in resistance to disease. 1. Resistance to virus diseases. R. Sadler and S. A. Edgar *(Poult. Sci.,* 1968, 47, 1224-1230).-Removal of the bursa of Fabricius of chicks at I or 5 days of age had no significant effect on resistance or development of immunity to fowl pox virus. Chicks bursectomised at 14 days of age were more susceptible to fowl pox virus than non-bursectomised birds or those treated at I day of age. Removal of the bursa at 5 days of age did not prevent development of immunity to Newcastle disease virus. (16 ref.)
A. H. CoRNFIELD.

Effect of pyridoxine deficiency on lipid metabolism in the chick. N. J. Daghir and J. M. Porooshani *(Poult. Sci.,* 1968, 47, **1094–**1098).-Addilion of 1% cholesterol (I) to a low-fat, low-I pyridoxine (II) semi-purified diet increased serum- and liver-I in 3-week-old chicks. "-deficient birds had higher serum-I values

than II-adequate birds except when both fat and I were added. (I9 ref.) A. H. CoRNFIELD.

Bee research directory: A world guide to bee research workers and institutions. Ed. E. Crane. 1966, 141 pp. (London: Bee Research Association). C. V.

Honey bee recruitment to food sources: olefaction or language. A. M. Wenner. P. H. Wells and D. L. Johnson *(Science, N. Y.,* 1969, 164, 84-86).- The many facets of this problems are briefly reviewed. (17 ref.) C. V.

Weather factor in foot-and-mouth disease epidemics. L. P. Smith and M. E. Hugh-Jones *(Nature, Land.,* 1969,223,712-715).- Evidence based on study of four separate outbreaks suggests that wind and rain strongly influence the spread of the disease. Of 280 farms affected, only 15 were outside a rain-wind danger zone at times which would provide an acceptable incubation period. Initially, the disease invariably spread downwind by airborne transport and subsequent deposition of the virus. An increasing rate of new outbreaks correlated with onset of wet weather, and ~90% of all secondary infections occurred in the sector of prevailing wind and rain. Wet, windy weather can change an **ap**parently small local outbreak into a serious epidemic. The amount of virus released, as well as the amount or duration of rainfall (especially at night when u.v. inactivation of the virus is min.) are determining factors. Calculation of directional 'danger indices', based on infection rating and rainfall, is explained. W. J. BAKER.

Pre-natal and post-natal mortality in cattle. *(Notn. Acad. Sci.,* 1968, Publication No. 1685, 130 pp.).–The genetic and nutritional aspects of calf losses are examined, also losses due to disease. C. V.

Treatment of traumatic gastritis [hardware disease) in cattle. N. Vlachos *(Bull. Hellenic Vet. med. Soc.,* 1966, 17, 99-109).- Diagnosis, pre-medication with mineral oil and antibiotics, and the introduction of the electromagnet are discussed. The technique is based on the work of various workers (Cooper *et al., J. Am. Vet. med. Ass.,* 1954, 125,301; 1955, 126, 473; 1956, 129, 376; 1957, 131, 473). Of the 50 cases treated, 47 were cured **(94%).** (In Greek, English summary.) S. C. **HAWORTH.**

Treatment of sterility In cattle by vaginal douche. C. Tsamis *(Hellenic Vet. med.,* 1969, 12, 7-12).–A douche based on chloramphenicol, neomycine sulphate, nitrofurazone, ethynyloestradiol and polyvinylpyrrolidone was used on a series of 430 cows suffering from endometritis, cervicitis, irregular oestrus cycles, etc.; results show that treatment was successful. (10 ref.) (In Greek with brief English summary.) S. C. **HAWORTH.**

Parasitology for veterinarians. J. R. Georgi. 1969, 237 pp. (Philadelphia, etc.: W. B. Saunders Co.). S. C. H.

Boron cbemosterilants against screw-worm flies: **structure–activity** relationship. J. A. Setlepani, M. M. Crystal and A. B. Borkovec *(1. econ. Ent.,* 1969, 62, 375-383).-Alkoxy- and aryloxy-boranes were active chemosterilants for *Cochliomyia hominivorax* because of their breakdown to boric acid. Aminoboranes and deriv. of boronic acid were more resistant to hydrolysis. C. M. HARDWICK.

New agent for treatment of liver fluke infection (Fascioliasis). H. Mrozik, H. Jones, J. Friedman, G. Schwartzkoff, *et 01. (Experientia,* 1969, **25,** 883).-Earlier work that suggested that salicylic deriv. were specially effective led to the synthesis of phenoxysalicylanilides which possessed high potency against mature and miniature fluke infections in sheep. Over 200 compounds have been prepared for evaluation. 3,5-Di-iodo-3'-chloro-4'-(*p-chloro*phenoxy)salicylanilide showed exceptional potency and greatly improved safety in the treatment of early infections. The method of synthesis is described and the *in vivo* findings are discussed. Observations relating to experimental infections with *Haemonchus contortus* in sheep, *Hymenolepis nana* and *H. diminuta* in rodents and *Schistosoma nwnsoni* in mice are also recorded. C. V.

Hypoglycaemia in piglets. C. B. Tarlatzis, P. N. Dragonas, E. N. Stoforos and L. E. Eftstalthiou *(Bull. Hellenic Vet. med. Soc.,* 1966, 17, 161-168).–This is the first occasion that this condition has been noted in Greece; 500 animals died. Food had been of low grade quality containing 4'5% total protein instead of the required 14-16% and no attention had been paid to mineral or vitamin content. Deaths ceased upon the administration of 50% glucose injections together with raising, and maintaining, the ambient temp. to ~24'. (13 ref.) (From Greek, with English and Italian summaries.) S. C. HAWORTH.

Requirements for investigation of new **drugs** and feed additions for animal usage-a symposium. *(Poult. Sci.,* 1968, 47, 1745-1753).- The papers include: New drug research and development. R. R. Chalquest (I745-1748). Food and drug regulations and evaluations. F. G. Sperling (1748-1751). Application through manufactured feed. E. I. Robertson (1751-1753). A. H. CORNFIELD.

Potential biological control of poultry lice. C. F. Meinecke *(Poult. Sci.,* 1968, 47, 2017-2018).-Spraying pheasant breeders with a culture of *Bacillus thuringiensis* ('Biotrol BTB 25-W') controlled body lice. A. H. CoRNFIELD.

Detoxification of pesticidal residues in fish and shellfish. A. H. Hallab *(Diss. Abstr., B,* 1968, 29, 649).–Oysters and shrimps were used as experimental animals. Special plexiglass aquaria were built for the *in vivo* studies. Sublethal doses of endrin, dieldrin, *p,p'-DDT* and toxaphene were established, and endrin was the most toxic. Aminopyrine, orinase and pyralgin were used in I and 10 ppm concn. as detoxification agents. Orinase was the most effective. 'oCo irradiation showed highly significant effects ($P < 0{\cdot}01$) in degrading the pesticide mol. at 0·2 and 1·0 Mrad. V.v. light showed highly significant effects ($P < 0{\cdot}01$) in the degradalion of endrin and dieldrin. F. C. SUTTON.

3,6-Pyridazine-diol derivatives useful for preparing nutriments. Chinoin Gyogyszer es Vegyeszeti Termekek Gyara Rt (B.P. 1,157,820, 29.11.66. Hungary, 17.12.65).-These products increase the appetite of domestic animals and their food utilisation. A fodder or water composition is mixed with 3,6-pyridazine-diol (I) or an organotropic salt thereof, particularly a double salt of! with salts of amino acids. Thus, a fodder composition for cattle is prepared by mixing wheat bran with the reaction product of aq. NaOH, glycine and I, then adding other suitable fodder components. S. S. CHISSICK.

Substituted benzoic acid lactones having oestrogenic properties. Commercial Solvents Corp. *!B.P.* 1,158,879, 8.6.67. V.S., 29.6.66, 23.5.67).–Compounds claimed have the formula 4,6,3,5,1,2-(ORhC,YXR'RIl wherein R is H, CH2Ph or alkyl or acyclic acyl; X and Yare N02, NH2, CN, OH, aryl, etc., or one of them is H; and RL RII together represent CO'0 'CHMe'[CH2]s'B'ICH2]s'A; A is CH:CH or [CH2]2 and B is CH2, CH(OR), or CO, but is CH2 when A is $[CH_2]_2$. They are useful as growth promoters in animal feeds. F. R. BASFORD.

2.-FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Possibilities and limitations of the falling number method. L. Tunger, R. Heckel, V. Erhardt and H.-J. Siebert *(Ernüihrungsforschung,* 1969, 14, 93-116).-A review. (114 ref.). J. B. WOOF.

Calorimetric determination of freezable water in dough. R. J. Davies and T. Webb *(Chemy Ind.,* 1969, 1138-1139).-The energy change when ice in the frozen sample (~5 mg) melts is measured in a differential scanning calorimeter of a d.t.a. apparatus. The calorimeter is cooled to -50' and then heated (I0'/min) to +50'; the amount of freezable water *(W)* is obtained from the endothermic peak area and a calibration factor. The graph of *W (0'2-0'6)* vs. total water **(0·6–0·9** mg/mg) is rectilinear, and *W* for any particular moisture content is the same for hard- and soft-wheat flours, the unfreezable water being ~0'33 gig of dry flour, i.e., ~25% moisture, for each dough. Presence of salt does not affect the values. W. J. BAKER.

Bakery products with non-wheat flours. Review. J. C. Kim and D. de Ruiter *(Baker's Dig.,* 1969, 43 (3), 58-63).-Preliminary investigations were carried out on tuber flours, including those derived from cassava, yam, sago and arrowroot, to test their suitability for making bread when combined with protein concentrates obtained from soyabean. groundnut, cottonseed and fish meal. Mixtures of cassava and soya flours were studied in detail to develop formulae and procedures for the production of bread. Satisfactory bread baking results were obtained with mixtures of cassava and groundnut flours, maize and soya flours, and of maize, cassava and soya flours. The limits and conditions of adding non-wheat flours to wheat flours were also studied. I. DICKINSON.

Vitamin and mineral contents of mixed bread. L. Tunger and W. Borchmann *(Erniihrungsforscllung,* 1969, 14, 83-92).- Analytical values for vitamins B, and B2, niacin, Ca and Fe are presented

for a large number of products made from rye and wheat flour in different proportions. (17 ref.) J. B. WOOF.

Some quantitative aspects of bread staling. G. A. H. Elton *(Baker's Dig.,* 1969, 43 (3), 24-29, 76).- The author's work over the last three years is reviewed. It is concluded that for a particular breadmaking process, factors which lower the specific vol. increase the staling rate, and factors which raise the specific vol. lower the staling rate. The use of d.t.a. for studying changes in the starch fraction during staling is discussed. I. DICKINSON.

Succinylated monoglyceride, a new processing aid for bakers. D. Meisner *(Baker's Dig.,* 1969, 43 (3). 38-41).- Succinylated monoglyceride (SMG) is the product obtained by combining equal molar quantities of purified glycerol monostearate and succinic anhydride. It strengthens dough structure effectively, improves loaf vol., produces bread with a soft crumb and prolongs the shelf life of baked bread. Doughs which contain SMG are more tolerant to the variables of mechanical processing and to variables normal to the baker's ingredients, particularly flour. Since SMG has the function of bread softening and dough conditioning, it affords formula simplification and ingredient economies. I. DICKINSON.

Flavour enhancement of bakery goods with citrus products. H. E. Swisher and D. E. Pritchett *(Baker's Dig.,* 1969, 43 (3), 53-55, 77).- For best flavour retention, liquid citrus oils should be incorporated in the fatty portion of the batter formula (3 oz per 100 lb of batter). Among the types of flavours available are spray-dried, microencapsulated and those entrapped in maize syrup solids. Citrus peels are available in different forms, including pasteurised, frozen and preserved as well as dehydrated products. (II ref.) I. DICKINSON.

Food product. Clover Club Foods Co. (B.P. 1,158,549, 1.9.66. U.S., 3.9.65).-A puffed food product is made by: (i) forming a slurry of a mixture of 60-90 % by wt. of water, foodstuff and at least one added starch, the solid in the slurry including $\leq$ 20 % by wt. of amylose; (ii) heating the slurry at above the glutinisation temp. of the starch; (iii) forming the gelatinised mass into pieces and *(iv)* drying to a moisture content that causes the pieces to puff and expand on cooking. E.g., a mixture of presoaked cracked wheat, whole wheat flour, maize starch, maize flour, salt and water is passed through a heat exchanger (I min) at 185°F and extruded into a ribbon. The moisture content of the ribbon is reduced to 70–75 % by exposure to warm air, prior to cutting and further dehydration at 160°F to a final moisture content of 10–12%. After frying (385°F, 20 sec) a crisp product with a fresh wheat flavour is obtained. S. S. CHISSICK.

Sugars and confectionery

Dissolved starch in mixed [cane] juice. P. A. Prince *(Proc. S. Afr. Sug. Technol. Ass., 42nd Ann. Congr.,* 1968, 53-54).- The concn. of dissolved starch in the juice varied (probably seasonally) from early Dec. to late Jan. At the max. average concn. ($\sim$ 15 %), the sol. starch interfered appreciably with the efficiency of the vac. flotation starch removal process. P. S. ARUP.

Enzymatic hydrolysis of starch in cane juice. J. Bruijn and R. P. Jennings *(Proc. S. Afr. Sug. Technol. Ass., 42nd Ann. Congr.,* 1968, 45-52).-Amylolytic enzymes from *Bacillus subtilis* were used for hydrolysing starch in mixed juices at pH 6·5 and at temp. near but $\gtrsim$ 80°. In 10 ppm concn. the enzyme hydrolysed> 85% of the starch (including sol. starch) within 30 min; degree of hydrolysis of boiled mixed juice at 70° was as high as 94 % after 15 min. Application to syrups and clear juices appeared promising. (15 ref.) P. S. ARUP.

Cuha raw sugar composition. N. A. Arkhipovich and B. A. Kutsenko *(Pishch. Tekhnol.,* 1968, No.5 (66), 12).—(In Russian.) P. P. R.

Distribution of impurities during crystallisation. R. P. Jennings *(Proc. S. Afr. Sug. Technol. Ass., 42nd Ann. Congr.,* 1968, 74-77).- A method for calculating inclusion ratios for gums, starch and ash in sugar crystals, from processing data, is illustrated by data observed for a worked sample. Factors relating to changes in working techniques (extraction, clarification, etc.) and in raw material quality are considered. P. S. ARUP.

Paper chromatographic method of detection and estimation of individual sugars present in foodstuff in presence of one another-milk. P. K. Gupta and T. V. Mathew *(J. Instn Chem. India, 1969,* 41, 57-59).-The milk, after treatment with Pb acetate and K oxalate, is filtered and the filtrate is subjected to descending order chromatography using Bu^nOH/AcOH/H20 as solvent and aniline hydrogen phthalate as reagent. 0·10% of sucrose can be detected in the presence of lactose and up to 1% estimated by an extraction and colorimetric technique. E. G. BRICKELL.

Hydrogenated milk fat as an inhibitor of the fat bloom defect in dark chocolate. L. B. Campbell, D. A. Andersen and P. G. Keeney *(J. Dairy Sci.,* 1969,52, 976-979).—Under accelerated test conditions involving controlled temp. cycling during storage, chocolate coatings with 2·5 % of hydrogenated milk fat (I) remained free of fat bloom two to four times longer than chocolate containing an equal amount of unhydrogenated I. Fully hydrogenated I was shown to be more effective in preventing the fat bloom defect than partially hydrogenated I. The results of trials in which hydrogenated I concn. was varied up to 3% showed that bloom susceptibility decreased as the amount of additive in the coating increased. (10 ref.) M. O'LEARY.

Abnormal spoilage of jams by osmophilic yeasts. Determination of heat resistance. E. Hernandez and A. Feria *(Revta Agroqulm. Tecnol. Aliment.,* 1969,9, 296-298).- Detection of an unusual type of spoilage in 65° Brix strawberry jam in a local cannery, consisting of product fermentation with evolution of gas, is reported. The micro-organism responsible was identified as an osmophilic *Saccharomyces* yeast. Thermal death time (determined with 100,000 cells in 2 g of 65° Brix jam pcr tube) was very high, 2 min at 90° and 14 min at 80°. E. C. APLING.

Fermentation and Alcoholic Beverages

Separation of hop extracts. G. Krauss and H. U. Fiildner *(Mschr. BrOil.,* 1969, 22, 182-183).- Standard and super hop extracts were stored in plastic containers for up to 53 days at 0, 32,40 and 50° and at the end of this time they were chilled and samples taken from the upper and lower regions for analysis of total resin and a-acid by normal methods. Considerable separation was observed in the extracts above 32° with concn. occurring in the upper phase. Rates of separation increased with temp. J. B. WOOF.

Yield of bitter substances during the preparation of Hopflx. G. Krauss *(Mschr. BrOil.,* 1969, 22, 154-156).-Hopfix was prepared from three different batches of Hallertau hops under impartial supervision. Frcsh hops, dried hops, Hopfix and the spent hops were analysed for moisture, *a-* and *β*-acids and hard and soft resin by standard procedures in several laboratories. In Hopfix containing 6'5 % moisture and about 10% a-acid, the recovery was 93 %. The 4-5 % found in the spent hops indicated that 2-3% had been converted to hard and soft resin during preparation. J. B. WOOF.

Yield of bitter substances in the preparation of Hopfix. K. Karnbach and G. Krauss *(Mschr. Brau.,* 1969, 22, 186).-Further analyses of spent hops remaining after Hopfix prep. showed that no a-acid remained in them (cf. *ibid.,* 1969, 22, 154). J. B. WOOF.

Direct analysiS of individual analogues of alpha-acids, beta-acids and 4-desoxyhumulones in hops using gas-liquid chromatography and nuclear magnetic resonance spectroscopy. P. V. R. Shannon, R. O. V. Lloyd and D. M. Cahil(J. *Inst. Brew.,* 1969, 22,376-382).- Hops dried to 5-10 % moisture were extracted by repeated maceration in benzene and quickly filtered. An aliquot was taken for quant. g.J.c. and the Me,Si deriv. prepared by reaction with hexamethyldisilazane at 50° in DMSO/dioxan. Chromatographic separation was carried out on a silanised column of 1% DC 560 with temp. programming from 150 to 250° at 3'/min. Peak areas were measured by the cut and weigh method and referred to standards. Dual peaks were obtained for humulones (I), lupulones (II) and 4-desoxyhumulones (01) which were identified by n.m.r. spectroscopy. By following changes in the developing hop cone, the relative initial enrichment of II and 01 was demonstrated. I reached a max. later but eventually predominated at the expense of 0 and III. The proportions of the co-homologues in each group increases during the growth of the hop. (16 ref.) J. B. WOOF.

Accuracy of the Wijllmer analysis. G. Krauss and H. U. Fiildner *(Mschr. Brau.,* 1969, 22, 152-154).-Aged Hallertau hops (1966) were analysed 17 times by the Wöllmer method. The results were statistically analysed, but were not satisfactory when compared with newer conductometric and spectrophotometric methods. J. B. WOOF.

Continuous alcoholic fermentations. I. Effect of antibiotics in the control of contamination. I. Mubakami, N. Futai and Y. Takahara *(Rep. Fermel/t. Res. [I/st.,* 1968, No. 33, 51-58).- The growth-inhibiting powers of penicillin (I) and aureomycin (II) for each group of bacteria in media containing tomato juice, peptone and malt extract were studied. Both I and II inhibited lactic acid-forming bacteria at 5 μg/ml; with acetic acid-forming organisms, II inhibited but I did not. Below a concn. of 2 μg/ml, both I and II repressed acid production but did not inhibit the growth of yeast. Similar results were obtained with a 20-day continuous alcoholic fermentation run. It is considered that the use of I is industrially reasonable on account of its high solubility and low cost. (From English summary.) C. V.

New methods for detecting wild yeasts in the brewery. II. A. Scherrer, E. Schlienger and H. Pfenninger *{Brallwissel/schaft, 1969,* 22, 273-274).- Comparative tests in six laboratories showed that, at the level I wild yeast per 100,000 culture yeasts, a combination of crystal violet agar and lysine agar provides a detection system superior to those tested previously. J. B. WOOF.

Protein composition of highly kilned malts. II. V. Karel *(Brallwissel/schaft,* 1969, 22, 274-278).- Paper chromatograms of the high mol. wt. proteins in worts prepared from malts obtained by normal kilning, and ones heated to 120' for 20-30 min subsequently were compared. The differences in the silica gel-adsorbed proteins in these worts demonstrated the increased pptn. caused by heating. Protein-tannin complexes were observed in infusion worts prcpared from kilned but not from green malts. (II reL) J. B. WOOF.

Controlling malting losses with anoxia, carhon dioxide and sulphur dioxide. I. D. Ponton and D. E. Briggs *(I. II/st. Brew.,* 1969, 75, 383-39I).- Using micro-maltings with gibberellic acid added at the rate of O'25 mg/kg, the effects of known partial pressures of CO, and the application of measured amounts of S02 on the quality of the resulting malt were assessed. With periods of increasing anoxia and C02 accumulation as in the Kropff process, the reduced malting loss was accompanied by reduced hot water extract. With various partial pressures of C02 during germination, the malt was also of inferior quality. S02 reduced malting loss by 3% and increased hot water extract by 3 Ib/Qr when applied 3 days after casting and could produce a high yield of malt in I day less than the control. Worts from treated malt fermented normally but had lower pH and higher sol. N. (23 reL) J. B. WOOF.

Development of a new small-scale apparatus for experimental brewing under reproducible conditions. L. Narziss and H. Heissinger *{Brallwissel/schaft,* 1969. 22, 331-338).- The beaker method of mashing, which resembled the Congress procedure, was found to be satisfactory. A wide range of temp. variations could be carried out and the wort separation by filtration, and the boiling and filtration stages could be closely controlled. However, the vol. handled were small and a 'jar' system was developed. The whole grist was mashed in a flask and the spent grain removed and sparged in a lauter tun. Boiling was carried out on electric mantles with powdered hops and break removed by sedimentation. Rapid cooling on a plate cooler gave a good cold break. Fermentation with pure yeast culture was maintained at 10'. Conditioning was carried out in 21 bottles for 4 weeks in a series of refrigerators according to a rigidly defined scheme. The vol. were convenient and the reproducibility was good. (23 ref.) J. B. WOOF.

Effect of yeast pitching rate on fermentation and beer quality. K. Wackerbauer *{Mschr. BrOil.,* 1969,22, 211-216).- The effects of time and rate of pitching on yeast growth and fermentation were studied. Yeast behaviour was followed through 20 consecutive fermentations in a commercial brewery at the normal O'51/hl pitching rate and one of 2·Ol/hl. The age distribution of cells, assessed by counting bud scars, remained constant, as did the % of dead cells. Respiration-dcficient cells did not increase. With the later fermentations, the beers were fermented to the attenuation limit and treated so that O'8% of the fermentable extract was avaHable for the secondary fermentation. In the later fermentations, the level of diacetyl decreased whilst acetoin tended to be variable. Beers stored for 8 weeks showed no significant analytical variations and remained biologically stable. (14 reL) J. B. WOOF.

Effect of fermentation temperature on beer quality in top fermentation. G. Sommer *{Mschr. BrOil.,* 1969, 22, 183-185).- A standard wort was fermented in the miniature brewery with the same yeast strain at 12, 16 and 20'. As expected, fermentation time decreased with increasing temp. As the temp. increased, the chill stability improved as the total and formol-N contents decreased, the pH fell slightly and the production of some volatiles increased. The bitterness and head retention were adversly affected by raising the temp. Tasting trials indicated that beers fermented at 16° were preferred. (12 ref.) J. B. WOOF.

Effect of gypsum or calcium chloride additions at different stages of wort production on the pH of wort and beer. P. Kolbach and W. Rinke *(Mschr. BrOil.,* 1969, 22, 145-151).-Ca salts in brewing liquor reduce pH of worts and beers without affecting flavour. This effect was examined in more detail in miniature brews. Using a multi·temp. mash system with distilled water as a control, Ca was added up to 19 or 38° hardness to mashing liquor, sparge liquor, total brewing liquor and to wort in copper. The beers and worts produced were then analysed. In general, pH was reduced by as much as 0'16, the total· and MgS04-pptd.·N were increased and the colours were decreased to an extent depending on the degree of hardness and the point of addition. Addition to the total or mashing liquor had greatest effect but changes were relatively small when the additions were made later. J. B. WOOF.

Some changes in beer flavour during ageing. S. Engan *(J. II/st. Brew.,* 1969, 75, 371-376).- Four pale ales and two dark beers, all bottom fermented in the same brewery with the same yeast and with malt extracts ranging from 6 to 17%, were stored at 0, 25 and 40° for up to 6 months. At intervals, the flavour components were examined by g.C., the more volatile ones by headspace analysis and the higher boiling ones after extraction with ether. At 40', ethyl and isoamyl acetate decreased in concn. whilst ethyl formate increased and acetaldehyde decreased after an initial increase. At 0°, differences in volatile composition were hardly detectable. Differences in wort strength caused differences in storage behaviour even when the same yeast was used for fermentation. (10 reL) J. B. WOOF.

Glycerin, pyruvate, citrate and malate contents of various types of beers. B. Mandl, F. Wullinger, A. Fischer and A. Piendl *{BrOil' wissel/schaft,* 1969, 22, 278-284).-5 pale lagers, 2 dark lagers, 7 pale export, 2 dark export, 5 Pilsner, 2 March, 2 wheat, 2 pale strong and 2 dark strong beers were analysed for these fermentation products. Glycerin was estimated by determining the ADP formed in reaction with glycerokinase. Pyruvate was estimated from the amount of NAD+ formed on reaction with lactate dehydrogenase. Citrate was converted to oxaloacetate with citrate lyase and this was converted to malate and the NAD+ formed was assayed. Malt dehydrogenase was used to catalyse conversion of malate to oxaloacetate with the formation of NADH. Average levels found for these 9 types of beers, were, respectively, for glycerine 1647, 1443, 1823, 1495, 1622, 1609, 1870, 1852 and 1832; for pyruvate 59, 61, 66, 79, 59, 60, 53, 51 and 66; for citrate 185, 174,203, 187, 194, 199, 151,266 and 279 and for malate 80, 54,98,54, 85,74,59, 136 and 57 mg/1. (18 ref.) J. B. WOOF.

Gel chromatographic determination of oligosaccharides in wort and beer by means of an automatic analysis system. H. Dellweg, G. Trenel, M. John and C. C. Emeis *{Mschr. BrOil.,* 1969, 22, 177-181).-Separation of mono· and oligo·saccharides and their quant. determination were carried out on a column of Bio-gel P-2. In order to improve resolution, -400 mesh gel was used and this was further classified by sedimentation. A 200 x 1·7-cm column was packed with the swollen gel and elution was carried out with water at 65'. The eluate was reacted continuously with orcinol-H2S04 reagent, and the absorption at 420 nm was continuously recorded. Wort (25 μl) or beer (75 μl) can be applied directly and in a 7-h run, glucose polymers with a degree of polymerisation up to 15 can be resolved. Iso-sugars are resolved also. (37 ref.) J. B. WOOF.

Gas chromatograpbic determination of volatile sulphur compounds [in beer). B. Drews, G. Barwald and H.-J. Niefind *{Mschr. BrOil.,* 1969, 22, 140-144).-Quant. g.c. headspace analysis was carried out on the volatile S compounds in beer. The sample was chromatographed on a steel column packed with Triton X-305 isothermally at 40° initially and then programmed to 150'. A Micro-Tek flame colour detector was used with an interference filter at 394 nm. Components were identified by relative retention times and peak height was found to be linearly related to concn. up to 100 μg/l, H2S, alkyl mercaptans, dialkyl sulphides, dialkyl disulphides and S02 were determined in this way. (20 ref.) J. B. WOOF.

Colour changes in extracts of skins and stems of white grape, variety Ohanes. M. de Miguel and I. Mareca *{Revta Agroqllim. Teel/ol. Alimel/t.,* 1969,9, 285-291).-A study of the colour changes, as measured by determinations of optical *d* at 425 om, of extracts

of skins and stem of Ohanes grapes and the influence of the oxidation catalysts Fe, Cu and Mn in the presence of air, H_2O" SO, and ascorbic acid are reported. Colour changes were very similar to those occurring in wine under the same conditions. (12 ref.)
E. C. APLING.

Tea cider [and vinegar]. R. L. de Silva and T. V. Saravanapavan *(Tea Q.,* 1968, 39, 37-41).-Prep. of the products is described, using a ferment supplied by the Tea Research Institute. The most important organisms involved are probably *Saccharomycodes ludwigii* and *Bacterium xylinum.* P.P.R.

'Brennwein' according to Micko and to WUstenfeld and proposals for improvement. I. Official directions for the Micko distillation and comparison with technical distillation. K.-G. Bergner and H.-A. Meemken *(Dt. LebensmillRdsch.,* 1969,65, 199-208).-The official analysis of 'Brennwein' (imported wine for distillation fortified with spirits distilled from wine) based on the fractional distillation according to Micko was investigated by means of temp.-time diagrams and g.c. of the fractions. Separation of fractions I and II, II and III, and IV and V was carried out without taking into account the boiling temp. curve in addition to the composition of the distillate. Only the distinction between fractions III and IV corresponded to boiling characteristics. G.l.c. indicated that the concn. of volatiles, higher alcohols and higher esters were higher in the Micko fractions than in ones taken from a brandy distillery. A correlation between first, middle and last runnings of redistilled brandy with Micko fractions was therefore not possible. (32 ref.) J. B. WOOF.

Gas chromatographic investigation of brandies. C. Reinhard *(Dt. LebensmittRdsch.,* 1969,65, 223-228).- Samples of 15 Cognacs, 4 Armagnacs, 5 Napoleon brandies, 3 Italian, 3 Spanish, I Russian and 18 German brandies in different price ranges were analysed by g.c. Using a 2-m column of 15% trimethylol tripelargonate, it was possible to determine quant. MeOH, EtOAc, PrnOH, BunOH, BujOH, Bu'OH, Et propionate, Et butyrate, 2- and 3-methylbutanol-I and hexanol-I. The ranges of concn. in different kinds of brandies differed considerably. Cognacs were low in MeOH whilst Napoleon brandies were high in MeOH and BunOH. (30 ref.) J. B. WOOF.

Isomerising and purifying hop extracts. Miller Brewing Co. (B.P. 1,158,370, 16.9.68. U.S., 21.9.67).-A process is claimed for treating hop extract to make it simultaneously free of oils, waxes and non-acidic compounds, and to render it useful in the production of beer with improved light stability. A two-phase liquid system is obtained by adding the hop extract dissolved in a water-immiscible solvent (I), e.g., C_6H_{14}, to an alkaline aq. phase optionally containing $NaBH_4$. The mixture is heated, the I removed, fresh I added and the resulting system acidified. The second lot of I is removed and the hop acids are recovered. S. S. CHISS[CK.

Cyclopentadione derivatives. Brewing Patents Ltd. (Inventors: P. R. Ashurst and D. R. J. Laws) (B.P. 1,159,039, 29.11.65).-Compounds claimed comprise 2-CORv-4-0R,v-5-RV'-cyclopenta-1,3-diones further substituted in the 4-position by $C{:}C'CH{:}CRIIRIII$ wherein R^{II}-R^{III} are H or alkyl, RIV is H or acyl, RV is alkyl, and RVIjs alkyl or alkenyl. They may be converted (by treatment with a Hg^{++} salt) into isohumulones, and are prepared by cyclising a keto ester derived from a β-keto ester and a substituted lactic acid halide. F. R. BASFORD.

Fruits, Vegetables, etc.

Determination of the main substrates responsible for enzymic browning and of polyphenoloxidase activity in apples grown in Chile. Use of some chemical inhibitors. H. Schmidt-Hebbel, I. Pennacchiotli, M. Scheel and C. Vergara *(Revta Agroquim. Tecno/. Aliment.,* 1969, 9, 276-284).-Determinations of 'acutal' and 'potential' browning (colour developed in 20 min at pH 6·7 in homogenate and homogenate + excess catechol) and of contents of total phenols, chlorogenic acid and ascorbic acid are reported for seven apple varieties widely grown in Chile, and determinations of polyphenoloxidase activity are reported for three of them (Winesap, White Winter and Delicious). Results showed very great inter-varietal differences, and Winesap is considered to be the best adapted to processing. Comparative studies of the efficiency of browning inhibitors showed that cystine was more effective than glutathione, cysteine, cupferron or $NaBO_3$. (18 ref.) E. C. APLING.

Storage of apples and pears. C. A. S. Padfield *(Bull. N.Z. Dep. scient. indo Res.,* 1969, No. 111, 117 pp.).-A review of experimental work dealing with pre-storage conditions, storage conditions, and physical disorders and fungal rots of stored fruit. (127 ref.) E. G. BRICKELL.

Treatment of bananas with thiabendazole. C. Beaudoin, J. Champion and R. Mallessard *(Fruits d'outre mer,* 1969, 24, 89-99).-Before treatment with the fungicide (I) the bananas must be washed with water [o which 500 ppm of $Ca(OCl)_2$ may be added with advantage. Immersion for 2-3 min in an aq. suspension of I, containing ~ 400 ppm of active material, destroyed all infections of *G/oeosporium musarum* that were ≯ 3 days old, and prevented development of any subsequent infections. I appeared to be valuable for the prevention of post-harvest fungal infections.
P. S. ARUP.

Influence of tetramethylthiuram disulphide on potato properties. A. L. Fel'dman, S. M. Kobeleva and E. N. Bespal'ko *(Pishch. Tekhno/.,* 1968, No. 5 (66), 15).-(In Russian.) P. P. R.

Suitability of some kinds of kitchen-garden bean for commercial processing. B. M. Vladimirov *(Pishch. Tekhno/.,* 1968, No.5 (66), 19).- (In Russian.) P. P. R.

Determination of nitrate in spinach by means of a nitrate-selective electrode. P. Voogt *(Dt. LebensmittRdsch.,* 1969,65, 196-198).-The electrode exhibited Nemst behaviour over a concn. range of 10^{-1}-10^{-2} moles of NOs/l. Other anions present in spinach had no direct effect on the accuracy of the results. The NO_3^- activity can vary with ionic strength, and, to minimise this, measurements were made in 1% Na_2SO_4 solution. The standard error was ± 2% and 96-102% recovery was recorded with added NO_3^-. Levels were rather higher than those determined by the xylenol chemical method. J. B. WOOF.

Non-alcoholic beverages

Essential oil contained in citrus fruit juices. R. Huet *(Fruils d'outre mer,* 1969,24, 129-136).-The essential oils responsible for the characteristic aromas of the juices of the different fruits were shown by centrifugal experiments to be associated mainly with the suspended matter (pulp) of the juices. The oils are derived (probably entirely) from the glands of the rind. G.l.c. analyses of the headspace vapours, formed when four different juices were (separately) agitated in an atm. of N, at 25° for 45 min, detected the presence of Et formate, acetate and butyrate, MeCHO, EtOH, MeOH and various terpenes. P. S. ARUP.

Detection of blood orange juice in fresh and browned orange juice concentrate. E. Benk, M. Hass and G. Krein *(Dt. Lebensmill-Rdsch.,* 1969, 65, 193-195).- During storage of concentrates containing blood orange juice, the red anthocyanin pigments, cyanidin-3-glucoside and delphinidin-3-glucoside, are decomposed under the action of heat, light and O_2. The hydrolysis products give rise to brown coloration and hence the anthocyanin test is useful only for detecting the presence of blood orange before or in the early stages of browning. This is done by lead acetate pptn. and paper chromatography. Anthocyanin may be determined quant. after purification on a column of Sephadex G-50 from the absorption at 520 nm. In browned juice, pectin is first removed enzymically and a portion chromatographed on a column of Sephadex G-25. The coloured fraction is hydrolysed with HCI and paraldehyde is added. Caramel browning can be distinguished from anthocyanin browning in that it gives a fine brown ppt. after 1-2 days. J. B. WOOF.

Sodium, potassium and calcium contents of industrially produced orange juice. H. Rother *(Dt. LebellsmillRdsch.,* 1969, 65, 233-235).-The levels of Na, K and Ca in juices produced in three commercial plants were determined. In order to determine whether the pressing method had any effect on these levels, each factory used high and low pressure procedures which were compared with manual pressing. The procedure was found to have little effect, but the Na/K ratio was altered if pulp or peel conponents were used. J. B. WOOF.

Cbanges [found) in concentrated mango juice. D. Manolkidou and H. Haralambaki *(Aristoteleioll Panepistemioll Thessalon. Geopon. Das%g. Seha/e Epistem. Epet.,* 1967, 201-224).-Alcoholic fermentation in conc. Egyptian mango juice, 60° Brix, was studied and the organisms responsible were identified. *Saccharomyces fructul/!* (I), *Rhod%ru/a glutillinus* var. *rubeseens* and *Calldida myeoderma* were found. Only I was capable of producing fermentation in the conc. state. (In Greek, with English and French summaries.) S. C. HAWORTH.

Tea, **coffee, cocoa**

Quality and flavour of tea. IU. Gas chromatographic analysis of the aroma complex. T. Yamanishi, R. L. Wickremasinghe and K. P. W. C. Perera *(Tea Q.,* 1968,39, 81-86).-Aroma concentrates of four black teas were prepared by steam distillation and extraction of the volatiles *with* ether. G.c. analysis showed that high-grown tea contained more Iinalool, linalool oxides, geraniol and *cis*-jasmone than low-grown tea, but the latter contained more highly volatile components than the former. P. P. R.

Milk, Dairy Products, Eggs

Flavour of milk and dairy products. [Symposium). Flavour of dairy products: **review** of recent advances. D. A. Forss *(J. Dairy Sci.,* 1969, 52, 832-840).-New flavour isolation techniques are reviewed together *with* current knowledge on the flavour of butter, Cheddar cheese and several low-fat dairy products. (66 ref.) M. O'LEARY.

Flavour of milk and dairy products. [Symposium). **Flavour.** J. Tobias *(J. Dairy Sci.,* 1969, 52, 81O). –The development of flavour evaluation into an exact science from its current position as an empirically acquired art is discussed. M. O'LEARY.

Flavour of milk aod dairy products. [Symposium). Sensory perception. D. G. Moulton *(J. Dairy Sci.,* 1969, 52, 811-815).-A review is presented of reccnt progress of research into the properties of the chemical senses and the measurement and analysis of response. (15 ref.) M.O'LEARV.

Flavour of milk and dairy products. [Symposium]. Chemical aod instrumental methods of flavour analysis. R. Teranishi *(J. Dairy Sci.,* 1969, 52, 816-823).-A review is presented of recent advances in chemical and instrumental methods of flavour analysis which enable chemical structure determinations to be carried out on submilligram quantities. (45 rer.) M. O'LEARY.

Flavour of milk and dairy products. [Symposium). Acceptance aod **consumer** preference testing. B. H. Ellis *(J. Dairy Sci., 1969,* 52, 823-831).-The various techniques for acceptance and consumer preference testing are reviewed. (38 ref.) M. O'LEARV.

6-trans-Nonenal: an off-flavour componcnt of foam **spray-dried** milks. O. W. Parks, N. P. Wong, C. A. Allen and D. P. Schwartz *(J. Dairy Sci.,* 1969,52, 953-956).-The 0 3-induced olT-flavour of fresh spray-dried milks, manufactured in urban areas in warm weather, was shown to be caused by *6-trans-nonenal* (I). The flavour threshold of this compound was found to be less than 0·07 ppb (American) in fresh whole milk. I may be formed by trace ozonolysis of minor lipid components on the surface of the dried product. (14 rer.) M. O'LEARY.

Quantitative determination of [odd-numbered) n-methyl ketones and ***o*-aminoacetophenone** in sterilised concentrated milk. R. G. Arnold and R. C. Lindsay *(J. Dairy Sci.,* 1969, 52, 1097-1100).-Mter 13 weeks storage at 27°, sterilised conc. milk was found to contain sufficient quantities of odd-numbered n-methyl ketones and o-aminoacetophenone to contribute significantly to its stale flavour. (14 ref.) M. O'LEARY.

Effects of hydrogen peroxide on growth of ***Pseudomonas*** */ragi* and shelflife ofpasteurised half-aod-half. E. B. Collins and H. A. Dirar *(J. Dairy Sci.,* 1969,52, 962-967).-The shelf life of commercial half-and-half (10% milk fat and 11% *SNF)* at 11° was almost doubled by the addition of **0·005–0·009%** of H20,. Addition of H202 tripled the shelf life of half-and-half containing 1·0 × 10' *Pseudomonas fragi* per ml at the beginning of the storage period. This concn. of H202 in the product was not detected by a sensory evaluation panel. (20 rer.) M. O'LEARY.

InOuente of mastitis on properties of milk. I. Curd tension, P. T. Tallamy, H. E. Randolph and C. W. Dill (1. *Dairy Sci.,* 1969, 52, 980-983).-With individual quarter samples, an inverse relationship was observed between the Wisconsin Mastitis Test and **curd** tension. The curd tension of Wisconsin-positive samples was 35% lower than that of negative samples. Statistically significant coelT. ($P < 0,01$) were obtained when the Wisconsin Mastiiis Test was correlated to curd tension and to the reduction in curd tension associated with increasing leucocyte counts. (13 ref.) M. O'LEARY.

Evidence for losses of free fatty acids in heated milk. D. A. Withycombe and R. C. Lindsay *(J. Dairy Sci.,* 1969, 52, **1100–**1104).-By means of g.l.c., the loss of free fatty acids in heated milk was shown to exceed 13% at all levels of commercial thermal processing and to increase with increase in the severity of heating. M. O'LEARY.

Casein pellet solvation aod heat stability of individual **cow's** milk. M. P. Thompson, R. T. Boswell, V. Martin, R. Jenness and C. A. Kiddy *(J. Dairy Sci.,* 1969, 52, 796-798).-A method of determining casein pellet solvation is described. A linear relationship ($r = 0·89$) was demonstrated between casein pellet solvation and heat stability at 135° of individual cow's milk. M. O'LEARY.

Caseinate–phosphate–calcium complexes as they exist naturally in milk. G. A. Decelles, jun. *(Diss. Abstr., B,* 1967, 28, 1295-1296). –Fractions of decreasing micelle size were centrifugally separated from skim-milk at 0°. The whey-sol. casein fraction was pptd. isoelectrically from the supernatant, each fraction being washed with water, repptd. and freeze-dried. With decrease in micelle size there was a decrease in Ca, Mg and total P, and an increase in total N contents. The whey-sol. casein fraction contained a low org. P : casein-N ratio. Values for the relative amounts of a-, fJ- and **κ-caseins** in the fractions, obtained by moving boundary and urea-starch-mercapto-2-ethanol electrophoresis, were in fair agreement. A. G. POLLARD.

Changes in casein-bound water during rennet action. W. L. Keyser *(Diss. Abstr., B,* 1968. 29, 422).-Casein dehydration was measured using an isotope dilution technique *with* lactose-**1-^{14}C,** using both rennin and rennet. Dehydration during the primary phase of rennet action was not detected. No change in casein·bound water was detected when the incubation temp. was 5°. After 4 h incubation at 35°, ~ 300 mg of bound water per g of casein was released by 2 **μl** of rennet or 0'81 mg of rennin per ml of raw skim-milk or 10% casein micelle solutions. This was interpreted as a secondary phase phenomenon. No differences between the sedimentation rates of raw skim-milk proteins and *HTST* pasteurised skim-milk proteins were detected. F. C. SurrON.

Flavoured bultermilks. F. V. Kosikowski *(J. Dairy Sci., 1969,* 52, 799-800).-A description is given of a procedure for pilot scale production of flavoused buttermilk by combining cultused buttermilk with tart fruit Oavours. M. O'LEARY.

Consumer acceptance of peanut [groundnut)·flavoured frozen desserts. I. Preferred flavour level and type of peanut [groundnut) ice cream. J. H. Martin and P. E. Swenson *(J. Dairy Sci., 1969,* 52, 1129-1133).-The results of a consumer acceptance study showed that a significant demand exists for groundnut.Oavoured ice cream. Overall preference ratings were in favour of a 5% level of nut butter in 46% of the cases and a 7·5% level in 30% of the cases, *with* 22% expressing no preference between these two levels. A fairly even preference was found to exist for three types of groundnut-flavouring material- plain nut butter, nut butter ripple, and nut particles. M. O'LEARY.

Chemical changes in the fat aod protein of Limburger cheese duriog ripening. S. Singh *(Diss. Abstr., B,* 1968, 29, 650). –The 'complete spectrum' of free fatty acids (C2:0 to C,,: 2) was determined by g.l.c. and mass spectroscopy. The changes in the major casein components (as- and fJ-casein) were determined by polyacrylamide gel electrophoresis. The results showed that milk fat is not only an essential component of cheese, but it must be present at a certain level for normal Limburger flavous to develop. The best cheese flavour was obtained using milk con·taining 3·5% of fat. Three different strains of *Debaryomyces* yeast did not differ significantly in their abilities to hydrolyse fat and protein in cheese, and no significant Oavour differences were produced in the cheeses. High levels of acidic carbonyls in ripened Limburger cheese suggested an extensive degradation of amino acids resulting from protein hydrolysis. The balance of the fat and protein hydrolysis finally determined the overall Oavour production in the cheese. F. C. SUTION.

Application of titanium dioxide to whiten Mozzarella cheese. F. V. Kosikowski and D. P. Brown *(J. Dairy Sci.,* 1969, 52, 968-**970).—0·02–0·05** wt.-% of TiO, in the original milk was shown to be an acceptable and effective whitener for Mozzarella cheese. M. O'LEARY.

Proteolytic acitivity of *B,.,ibacte,ium* ***linens*** during ripening of Trappist-type cheese. G. L. Ades and J. F. Cone *(J. Dairy Sci.,* 1969, 52, *957-961).-Brevibacterium linens,* when present on the surface of Trappist-type cheese, was shown to be capable of elaborating proteolytic enzyme systems, though not to the same degree as the yeasts which are also normally present. (10 rer.) M. O'LEARY.

Semi-soft skim-milk cheese. Pilot plant **procedure.** H. E. Walter, A. M. Sadler and W. A. Mattingly *(J. Dairy Sci., 1969,* 52, I133-1136).-The procedure is described. M. O'LEARY.

Carbohydrate metabolism in Cheddar cheese. I. N-Acetylneuraminic acid, **2-deoxy-D-ribose** and pbosphorylated sugars. R. A. Sullivan and D. G. Infantino *(J. Dairy Sci.,* 1969,52,761-767).- The concn. of N-acetylneuraminic acid in Cheddar cheese was shown to be unchanged during ripening and was not affected by pasteurisation of the milk used. A sugar, tentatively identified as 2-deoxY-D-ribose, was found in some cheese samples and was found to originate in milk of which it is a normal constituent, previously undetected. The concn. of phosphorylated metabolites in cheese was found to be high in samples from which naturally occurring deoxyribose had disappeared. This indicates that the disappearance of free galactose is no justification for stating that the cheese is free of fermentable materials. (30 ref.)

M. O'LEARY.

Cooling rates of green Cheddar cheese under normal conditions and under immersion in brine. A. H. Miah, G. W. Reinbold, E. R. Vedamuthu and E. G. Hammond *(J. Dairy Sci.,* 1969, 52, 971-975).-A single 4-h pressed 18·2 kg Cheddar cheese block was shown to take 65 h to cool from 32 to 8° when held in a curing room at **7·2°.** Blocks pressed for 20 h required 150 h to cool to 8°. Cooling times for **4-** and 2Q-h pressed blocks immersed in a brine tank (held between 4·4 and 7,2°) were 25 and 45 h, respectively. Similar sized blocks stacked together two or more high in the curing room required a min. of 165 h to cool to 8° for both 4- and 2Q-h pressing. 9·1 kg cheese blocks required only 120 h in the curing room and 20 h in the brine tank to cool to 8°.

M. O'LEARY.

Direct gas chromatographic measurement of acetic, propionic and butyric acids in milk serum and aqueous extracts of cheese. R. A. Ledford *(J. Dairy Sci.,* 1969, 52, 949-952).-A description is given of a g.C. technique in which uncoated porous polyaromatic polymer beads are used as the column packing, for quant. determining acetic, propionic and butyric acids in milk and cheese.

M. O'LEARY.

Quantitative determination of certain sbort-chain acids in frozen wbole eggs by gas-liquid chromatography. J. E. Steinhauer *(Diss. Abstr., B,* 1968, 29, 650).-G.l.c. and A.O.A.C. analytical procedures and recoveries obtained are described. Analysis of whole eggs which had undergone natural bacterial decomposition showed that lactic acid was present in the highest concn., followed by acetic, succinic, formic and propionic acid. No butyric acid was detected. The high total, coliform, and salmonella counts indicated that a relationship exists between the types and numbers of micro-organisms present and the quantity and kind of acids produced in decomposed eggs. F. C. SUTTON.

Autoxidation products of cbolesterol in aerated sols and irradiated spray-dried egg yolk. E. Chicoye *(Diss. Abstr., B,* 1968, 29, 649).-The prep. and purification of cholesterol autoxidation products, for use as reference compounds in the following studies, are described. Samples of fresh (I) and spray-dried egg yolk (II) were studied by t.l.c. and g.l.c. to assess the presence of autoxidation products of cholesterol. None were detected in I. Unirradiated II stored for ~ I yrcontained trace amounts of 7a- and **7β-hydroxy-**cholesterols. Preparative t.l.c. with a double development of the chromatoplates was used to separate the hydroxycholesterols and cholesterol oxide fractions of the non-saponifiable matter of irradiated yolk solids. After purification of these fractions, Lr. spectroscopy was employed to confirm the structure of the epimeric diols and the oxide. F. C. SUTTON.

Milk and cream products. A. Bratland (D.P. 1,158,577, 16.8.66. Nor., 31.8.65).-Long-life, sterilised milk and cream products are prepared by mixing milk or cream with a refractionated low fat milk fraction, e.g., buttermilk, and heat sterilising either before (preferably) or after mixing. Homogenisation may be carried out before or during heat sterilisation. S. S. CHISSICK.

Edible Oils and Fats

Analysis of vegetable oil mixtures by gas chromatography. M. **Jernejčič** and L. Premru *(J. Oil Colour Chern. Ass.,* 1969, 52, 623-627).- The Me esters of the fatty acids present in maize, linseed, castor, dehydrated castor, tung, rapeseed, sunflower, arachidic, coconut and soyabean oils were analysed by g.c. The relatiye retention times were determined on an LAC 446 column with temp. programming from 150 to 200° using Ar as carrier gas. The contents of the different fatty acids present were determined by comparing their retention times with the Me ester of stearic acid. The reproducibility of the results was studied; there was a relative error of about 10% for the main constituents. (14 ref.)

W. E. AUSEBROOK.

Isolation and characterisation of methyl oleate hydroperoxides. I. M. Piretti, P. Capella and M. Taddia *(Riv. ital. Sostanze grosse,* 1969, 46, 324-326). – Methyl oleate was autoxidised at 80° and the methyl oleate hydroperoxide mixture was separated by chromatography on a column of silicic acid, slightly deactivated (5% H20) with a solvent mixture of hexane, Et20 and MeOH (94 : 5 : I). The hydroperoxides had a purity of ⩾98% (iodometric) and the absence of by-products was shown by t.l.c. (18 ref.) (In English.) L. A. O'NEILL.

Fats in human nutrition: olive oil. P. Viola *(Riv. ital. Sostanze grasse,* 1969, 46, 287-323).-A review. (384 ref.)

L. A. O'NEILL.

Examination of phenolic substances in olive oil. G. Montedoro and C. Cantarelli *(Riv. ital. Sostanze grasse,* 1969,46,115-124). – Polyphenols (I) were extracted from virgin olive oils obtained from different cultivars and at two stages of maturation, from rectified and sansa olive oils, from the fruit and from vegetation water. Total I were determined and their nature was examined by t.l.c. on silica gel. The antioxidant effect of I was demonstrated by comparing the stability of oils before and after removal of I by washing with MeOH. (24 ref.) L. A. O'NEILL.

Pro- and anti-oxidants in fats. XXV. Their localisation in seeds, fruits and potatoes. H. P. Kaufmann, A. W. El Baya and D. Meinsen *(Fette Seifen AnstrMittel,* 1969, 71, 537-542).-The antioxidative activities of groundnut and linseed oil seed extracts, together with extracts from potatoes and gladioli bulbs, were compared by determining the peroxide values of a purified linseed oil before and after suitable incubation. Extracts from different parts of the plant or seed showed different effects, and were further related to the storage conditions and variety. Tests were also carried out with a flavanol glycoside which occurs in onion flesh. Good protection occurred with an extract obtained from potato peel, but a similar extract from the pulp did not show such a good effect. The general storage of potato products is considered with respect to the lipid content and the lipid distribution between the starch and protein constituents. The possible use of potato skin extract as a preservative is discussed. G. R. WHALLEY.

Meat and Poultry

Sausage and small goods production. F. Gerrard, 1969, 5th ed., 245 pp., 63/-. (London: Leonard Hill Books).- S. C. H.

Effect of temperature and sample location on the penetration of minimal curing chemicals in ham. A. K. Chatterjee *(J. Fd Sci. Technol.,* 1968, 5, 190-192).-Hams were studied under different environmental conditions using minimal curing chemicals (salt, sugar and NaN03). Four methods were used: (i) dry cure, (ii) 4% pickle and cover cure, (iii) 4% pickle and brine cover, and *(iv)* 8% pickle and cover pickle. After treatment, two-thirds of the hams were cured at 3'3-4'4° and the rest at 14'5° for 35 days (R.H. 65-85%). After curing, the hams were aged for 5 weeks. The first phase of ageing lasted for 5 weeks, and during this phase all the hams cured at 14'4° plus one sample cured at 3·3-4·4° were aged in a 14·4° environment, while the rest were aged at 22'2°. The final phase lasted 5 weeks during which time all hams were aged by keeping them for 16 h/day at 25'6° and others at **36·7°.** The dry cure method was found to be the most desirable curing technique, the other cures being inadequate to produce a preserved country-style ham under elevated temp. and R.H.

I. DICKINSON.

Fish

Shelf life and sensory evaluation of fish sausage manufactured on a pilot plant scale. M. A. **Krishna** Swamy, J. D. Patel, S. Dhanaraj. V. S. Govindarajan and S. Yunus Ahmed *(J. Fd Sci. Technol.,* 1968, 5, 186-189).-Sausages made from freshly caught croaker and shark with sorbic acid and Na benzoate as preservatives were microbiologically safe. They were free from coliforms, yeasts and moulds, coagulase-positive staphylococci and pathogenic anaerobes including *Clostridium* types. The product had a shelf life of 3 weeks at 25°. Total lysine, available lysine, methionine, cystine and tryptophane in fish sausages were 10'3, 10'0, 2,2, 0·9 and

1'0%, respectively. The sausages had good consumer acceptability. (13 ref.) I. DICKINSON.

Trimetbylamine oxide **content** of Norwegian sbrimps and its degradation to methylamines and fonnaldebyde. I. Introduction. **Presence** of formaldehyde in canned shrimps. III. Effect of storage conditions on the development of fonnaldebyde and otber degradation products of trimetbylamine oxide in canned sbrimps. O. C. Sundsvold, B. Uppstad, G. W. Ferguson, T. MacLachlan and D. Feeley *(Tidsskr. Hermetlnd.,* 1969,55,94-97; 131-136).-1. The occurrence and formation of HCHO in canned shrimps from trimethylamine oxide (I) was studied. The amount of. HCHO formed increased with increasing pH; little change took place at low temp. (-37°), at 4° considerable Fe was dissolved from the tinplate with the formation of medium quantities of HCHO, and at 18-37° the amount of Fe dissolved was less and the quantity of HCHO formed was lower, but the amounts of di- and tri-methylamine (II, ill) and other volatile bases increased. Scoring the tinplate, independent of depth, increased the decomp. of I and the action of Al was less than that of tinplate, but Al was not as inert as a catalyst. It is concluded that in canned shrimps, the breakdown of I takes place as the result of simple fission, hydrolysis, and also oxidation-reduction caused by either catalytic or electrolytic action. (19 ref.)

III. Storage at any temp. or with different types of containers or lacquer led to greater formation of volatile bases, other than II and III in the first place, but as the length of storage time progressed there was a tendency towards increased breakdown to II and IU compared with other volatile bases. The quantity of III formed under all conditions was about twice that of II. At 4°, the amount of Fe dissolved into the shrimps and brine was about 150 ppm after 4 months storage, compared with only 35-40 ppm at **20–37°.** The depth of the scoring line on the tinned Fe plate had little effect on the quantity of Fe dissolved, and the type of lacquer employed did not appreciably affect the solution of Fe. The amount of HCHO formed corresponded to the quantity of Fe dissolved, but there was no parallelism with the quantities of II, ill or other volatile bases formed. (In English.)

I. DICKINSON.

Post mortem degradation of fish muscle proteins: **rôle** of proteolytic *PserulomolUls* spp. and their mechanism of action. J. R. Chung *(Diss. Abstr., B,* 1968,29, 1056).-The development of dominance by *Pseudomonas* spp. (I) can be explained from the action of proteolytic I. During early spoilage, I can outgrow other groups because of their greater oxidative capabilities, less exacting nature nutritionally, and their ability to utilise many NPN components. However, as the concn. of NPN decreases below a critical level, the activity of proteolytic I replenishes NPN through protein breakdown. Thus I have an added advantage for growth over non-proteolytic bacteria, resulting in eventual dominance by I among the total microflora. F. C. SUTTON.

Spices, Flavours, etc.

Monosodium L-glutamate: phannacology and **rôle** in 'the Chinese restaurant'syndrome. H. H. Shaumburg, R. Byck, R. Gerste and J. H. Mashman *(Science, N. Y.,* 1969, 163, 836-828).-This compound can, when injected, produce headaches and in appropriate doses even cause a burning sensation, facial flushes or chest pain. There is a considerable variation in the oral threshold dose amongst individuals. (14 ref.) C. V.

Preservatives

Simultaneous occurrence of preservatives in citrus juices and drinks. Detennination of sulphur dioxide and benzoic and sorbic acids. J. Royo Iranzo, C. Cervell6 and A. Aranda *(Revta Agroqulm. Teenol. Aliment.,* 1969, 9, 292-295). –A study of appropriate methods for the determination of each of the three preservatives in the presence of the other two, necessary because of the frequent use of mixed preservatives, is reported. Best results were achieved by determining SO, by the Monier-Williams method, benzoic and sorbic acids together by the official AOAC method and sorbic acid alone by the method of Floyd. E. C. APLING.

Pesticides in Foods

Formation of ethylene bromohydrin in flour and wheat during treatment with ethylene oxide. S. G. Heuser and K. A. Scudamore *(Chemy Ind.,* 1969, 1054-1055).-Experimental evidence is advanced for the anomalous and preferential formation of ethylene bromohydrin (I) during fumigation with ethylene oxide (II), the necessary Br being derived either from naturally occurring inorg. bromide or, in higher concn., from pre-fumigation with MeBr. Thus, in flour 08% H,O, 230 ppm Br- before II treatment) the concn. of I was a max. (330 ppm) **24–48** h after II treatment, although the concn. of ethylene chlorohydrin (III) continued to increase for the first 7 days. Concn. of I, III and II after 28 days were 175,640 and 1 ppm, respectively. Complete conversion into I in presence of excess of II in acid solution should provide a simple g.l.c. method of determining inorg. bromide in foodstuffs. Mammalian toxicity off is discussed briefly. (13 ref.) W. J. BAKER.

Detennination of residual morpholine in the skins of citrus fruits. E. Kroller *(Dt. LebellsmillRdseh.,* 1969, 65, 232-233).-The peel sample is homogenised in Me,Co-HCI and washed. After distilling and extracting the morpholine, it is converted to dithiodicarbamate by reaction with CS,. This is converted to the Cu complex which is extracted with **$CHCl_3$.** The absorption is determined at 435 om against a suitable blank and the conen. is obtained by reference to a standard curve. Some types of orange peel contain a base which reacts in a similar manner to morpholine and this source of interference has not been eliminated.

J. B. WOOF.

Contamination by chlorinated pesticides. II. Detennination of residues of insecticides in a sample of cod liver oil. G. Baluja, M. Dabrio, J. M. Franco, M. A. Murado and M. E. Pereiro *{Revta Agroq/llm. Teenol. Aliment.,* 1969, 9, 266-275).-Methods applied in the identification and determination of insecticidal residues and their metabolites in a sample of cod liver oil are reported. Extraction and purification was based on hexane-acetonitrile partition, and adsorption on Florisil and elution with hexanc-ether. Residues were identified and determined by g.l.c. on Pyrex glass columns filled with Chromosorb G AW-DMSC **(80–100** mesh) and 3% methylsilicone (DC-2oo) or 7·5% trifluoropropylmethylsilicone (QF-I) with 5% DC-2oo as stationary phases. The oil was found to contain traces of a-BHC, 0·1 ppm of lindane, 0·3 ppm of aldrin, 0·2 ppm of *p,p'-DDE,* 0·8 ppm of *p,p'-TDE* and 0·7 ppm of *p,p'-DDT.* (19 rer.) E. C. **APLING.**

Food Processing, Refrigeration

Dehydration processes for convenience fonds. R. Noyes *(Fd Process Rev.,* 1969, No.2, 367 pp.).-Detailed, descriptive process information is presented, based on 236 U.S. patents issued between Jan. 1960 and May 1968. Topics covered include dry milk products, cheese and yoghurt, eggs, fruit and vegetable juices, fruits, potatoes, vegetables, coffee and tea. P.C.W.

Effect of y-radiation on maturing of apricots after harvesting. S. Guelfat-Reich, R. Ben-Arie, R. S. Kahan and E. Eisenberg *(Fruits d'o/ltre mer,* 1969, 24, 137-142). –The degree of softening undergone by Canino apricots was proportional to the dose. In no case did irradiation improve the quality or storage life of the fruit, and irradiation at 200 krad caused excessive softening and browning of the interior tissue. Green fruits were somewhat less sensitive to damage than the more mature fruits; their ripening was moderately accelerated by doses of 25 krad. Respiration was generally increased by irradiation. (13 ref.) P. S. ARUP.

Packaging

Multiwall paper sacks as possible harriers against entry of insect pests of copra in storage. K. Mathen, J. Mathew and C. Kurian *(J. Fd Sci. Teclmol.,* 1968, 5, 195-197).- Containers used were 6-ply 20 x 15-in paper bags. Some bags had a layer of Pybuthrin (pyrethrins piperonyl butoxide) and the inner ply was wax-laminated. The bags were sealed at the bottom with Repellex tape and thread. The results showed that all paper bags could keep copra free from infestation for up to 3 months, and those treated with Pybuthrin up to 9 months. I. DICKINSON.

Miscellaneous

Nutrition, proteins, amino acids, vitamins

Interactions of nutrition and infection. N. S. Scrimshaw *et 01.* 1968, 329 pp., 60 pp. bibliog. {Geneva: World Health Organisation).-W.H.O. Monograph Series No. 57. S. C. H.

Importance of the chemical composition of food proteins in human nutrition. R. J. Tannous *(Wid Chern., Beirut,* 1969, I, 53-49).-Discusses the production of protein-rich food mixtures based on chemical composition and the inclusion of essential amino acids. A suitable food for infants and children suffering from protein malnutrition has been developed from parboiled wheat (60%) and chickpea flour (28%), mixed with dried skim-milk, bone-ash and sucrose. Its nutritive value is higher than that of wheat or chick-peas alone and approaches that of milk. (In English.)
W. J. BAKER.

Protein for human nutrition (Torula food yeast). R. J. H. Bisanz *(Chern. Age India,* 1969, 20, 143-146).-A plant and the material requirements for growing and processing *Torula utilis* fermented with molasses are briefly outlined. The black liquor waste from paper making would also be a suitable nutrient. K. GRAUPNER.

Protein-enriched cereal foods for **world** needs. Ed. M. Milner. 1969, 343 pp., $7.50 [U.K.). (St. Paul, Minnesota: Am. Ass. Cereal Chem.).-31 papers divided into 8 parts. I. Economic aspects: Quantitative **rôle** of cereals as suppliers of dietary protein. Q. M. West (2-12) (18 ref.); Economic factors affecting distribution of world food protein resources. J. C. Abbott (13-29). II. Public health implications: Infant and child nutrition and the need for meeting protein needs. W. H. Sebrell, jun. (32-36). Ill. World food and nutrition crisis: Nutritional value of cereal proteins in relation to human needs. D. M. Hegsted (38-48) (38 reL); Formulation and testing of weaning and supplementary foods containing oilseed proteins. R. Bressani (49-66) (35 reL); FAO/WHO/UNICEF guidelines for safety evalnation and human testing of supplementary food mixtures. E. M. DeMaeyer (67-73) (24 ref.); Factors in8uencing adequacy of dietary proteins for young children: lysine enrichment of wheat 80ur. G. Graham (74-79) (10 ref.). IV. Protein resources: Low cost foods: fortified cereals and protein beverages. A. M. Altschul (82-96) (27 ref.); Status of development and use of some unconventional proteins. M. Milner (97-104) (13 ref.); History and status of specific protein-rich foods-bulgur, Wurld wheat and wheat protein beverages. J. W. Pence (105-116) (34 rer.); Wheat protein concentrates and related food products. E. J. Bass (117-128); Protein foods of India based on cereals, legumeS and oilseed meals. H. A. B. Parpia (129-139); History and status of specific protein-rich **foods—extrusion-processed** cereal foods. O. B. Smith (140-153) (17 ref.); Edible rice bran foods. L. Lynn (154-172) (31 ref.). V. Bread: Protein fortification of bread-Baladi bread. G. Dalby (174-180); Enrichment of cereal foods in Chile with fish protein concentrate. J. Santa Maria (181-184) (25 ref.); Bread from non-wheat 80urs. J. C. Kim and D. de Ruiter (185-198) (15 ref.). VI. Amino acids, vitamins and plant genetics: **Practical** problems of amino acid fortification. M. A. Beigler (200-207) (15 rer.); New approaches to amino acid and vitamin enrichment in Japan. H. Mitsuda (208-219) (27 ref.); Aspectsofvitamin and mineral enrichment. S. H. Rubin and W. M. Cort (220-233) (41 ref.); New approaches to amino acid and vitamin improvement of cereal products-protein improvement by breeding. P. J. Mattern (234-244) (40 rer.). VII. Programmes and products: Formulated cereal foods in the U.S. Food for Peace programme. F. R. Senti (246-254); History and status of specific protein-rich foods---FAO/WHO/UNICEF protein food programme and products. G. D. Kapsiotis (255-265) (13 ref.); Development, production and marketing of high-protein foods H. J. H. De Muelenaere (266-277); Faffa-a supplementary cereal-based weaning food in Ethiopia. G. Agren, Y. Hofvander, R. Selinus and B. Vahlquist (278-287) (14 ref.); Protein-rich cereal foods in Peru. A. Bacigalupo (288-304) (22 rer.); Development of 'Laubina'-infant food mixtures for the Middle East. J. W. Cowan and P. L. Pellett (305-314) (30 ref.). VIII. Acceptability and marketing: Planning tbe introduction of low-cost, protein-enriched cereal foods in Africa and the developing countries. T. L. V. Blair (316-319); Incapurina in Central America. R. L. Shaw (320-333) (II ref.); Experiences in marketing a supplementary food mixture for children in Ethiopia. B. Wickstrom (334-340); Incaparina in Colombia. A. Dimino (341-343). P. C. W.

Development of pre-digested protein-rich food based on Indian oil seed cakes and pulses. I. N. Narayana Rao, C. T. Dwarakanath and T. N. Ramachandra Rao *(J. Fd Sci. Technol.,* 1968,5, 198-201).-A standardised procedure for the prep. of this product on a laboratory scale and its chemical analysis is presented. Mixtures of soyabeans with Bengalgram dhal and other oil seed cakes were used as substrates for fermentation, to improve nutritive value, texture and acceptability. The final product had an increased digestibility, a marked increase in the sol. N fractions and also in amino N. The reducing sugars increased as the fermentation proceeded, which indicated that during the course of fermentation the amylolytic and proteolytic enzymes were activc on the sub-strate. Freeze-drying was uneconomic and other methods of dehydration will have to be found as the wet miso has a high moisture content and a very short shelf life. I. DICKINSON.

Alternative methods to the Kjeldabl estimation of protein nitrogen. E. R. Cole *(Rev. pure appl. Chern.,* 1969, 19, 109-130).-A wide variety of procedures for the estimation of protein N is reviewed, including separation of intact protein by formylation of defatted material followed by selective pptn. with solvents, volumetric methods (Kjeldahl methods, digestion with alkali, Kofranyi distillation and formol titration), spectrophotometric methods (u.v. absorption and fluorescence measurements), colorimetric methods using the Folin-Ciocalteu phosphomolybdic-phosphotungstic reagent or procedures based on the formation of the Cu-biuret complex, dye binding methods and turbidimetric methods. Applications to products such as cereals, herbages, dairy products and meats are discussed. (131 ref.) J. M. JACOBS.

Suggested topical outline for a course in organoleptic evaluation of foods. E. L. Thomas, F. Shipe, W. L. Dunkley, W. L. Slatter and J. Tobias *(J. Dairy Sci.,* 1969,52, 841-843).-Suggestions for a 10-14-week course in organoleptic evaluation of foods are presented. (25 ref.) M. O'LEARY.

Polycyclic hydrocarbon contents of European yeasts. VII. Hydrocarbons in the human environment. G. Grimmer and G. Wilhelm *(Dt. LebensrnittRdsch.,* 1969, 65, 229-231).-Baker's yeast from different sources was hydrolysed at 85° for 8 h with IN-KOH and extracted with cyclohexane. After extraction into a dimethylformamide phase and filtration on a SiO, column, pre-parative paper and column chromatography were used to estimate phenanthrene, anthracene, pyrene, f1uoranthene, benzo[a]anthra-cene, chrysene, benzo[a]- and -[e]-pyrene, perylene, anthanthrene, benzo[ghijperylene, dibenz[a,h)anthracene and coronene contents. The ranges found in dry yeast were considerable, e.g., benzyprene varied from 1·8 to 40·4 μg/kg. Dietetic and fodder yeasts grown on mineral oil had lower hydrocarbon contents. A major part of undesirable hydrocarbons could be removed by suitable washing. (16 rer.) J. B. WOOF.

Toxicological aspects of the migration of polyvinyl chloride stabilisers into foodstuffs. H. Woggon and W. J. Uhde *(Plaste Kautsch.,* 1969, 16, 88-91).-The extent of migration of various stabilisers from PVC containers into edible oils has been compared, and toxicity hazards are discussed. L. A. **O'NEILL.**

Applications of plastics in foods. XII. Migration of monomeric plasticisers from PVC tubes into cleansing solutions. G. Wildbrett, K. W. Evers and F. Kiermeier *(Fette Seifen AnstrMittel,* 1969, 71, 330-335).- Analytical methods are described for the quant. determination of several monomeric plasticisers in the presence of simple cleaning solutions, and such tests are used to study the migration of such materials from PVC tubing into the wash solution. Certain detergent mixtures are more prone than others to this effect. Milk tubing whose internal surfaces have become roughened by plasticiser migration is very difficult to clean, thereby causing **an** adverse effect on the milk being transported. (17 rer.)
G. R. WHALLEY.

Susceptibility of the mushroom mite to phosphine and ethylene dibromide. P. S. Barker *(J. econ. Ent.,* 1969, 62, 145-146).-Eggs of *Tyrophagus putrescelltiae* were 86-fold as tolerant to ethylene dibromide (I) as were the mobile stages; as they aged, the eggs became more susceptible. *T. putrescentiae* was highly tolerant to PH,; long exposure times were needed to kill 50% of the mobile stages. The high tolerance of the eggs to I suggests a limitation to its use for eradication of mites in stored foods. (15 ref.) C. M. HARDWICK.

Quantitative method for the estimation of aflatoxins in peanuts [groundnuts] and peanut products. K. Mayura and V. Sreenivasa-murthy *(J. Ass. off. analyt. Chern.,* 1969, 52, 77-81).-Toxins are extracted with CHCl" defatted by partition between water and petroleum ether and cleaned up by passage through Al_2O_3 beds. An aliquot of the purified extract, dissolved in $CHCl_3$–MeOH–Me,CO (I : I : 8), is passed into an Al_2O_3 column and washed with the same solvent. G, and G, are retained in the column whilst B, and B, are eluted and their absorbances measured at 360 nm. Treatment of the B, + B, residue with 10% HNO, destroys B, and allows B, to be extracted with $CHCl_3$ and determined. Total aflatoxins are determined on the residue from the original purified solution, and subsequent treatment of this with 10% HNO, destroys B, and G, and allows B, + G, to be extracted

and determined. Recoveries of individual and combinations of toxins from groundnuts containing ≤ 20 μg were 90–100%. M. BARNETT.

Analysis of **cocoa** beans for allatoxins. P. M. Scott *(i. Ass. oI[. analyt. Chem.*, 1969, 52, 72-74).-Ground, defatted, air-dried beans are dispersed in 25% AgNOa and extracted with **CHCl3**. This is cleaned up in a silica/Na2S0. column which is washed with hexane and Et20, and the aflatoxins are eluted with MeOH-**CHCl3** (3 : 97). The residue from evaporation of the solvent is applied to a silica gel t.l.c. plate and developed with acetone-**CHCl3** (I : 9). Under long-wave u.v. light, a min. of I ng of aflatoxin BI or GI could be detected, and recoveries of BI and GI added to beans at the 2·5 ppb (American) level were 80-90%. M. BARNETT.

Collaborative study on the determination of allatoxins in cottonseed products. W. A. Pons, jun. (I. *Ass. oI[. analyt. Chem., 1969,* 52, 61-72).-The ground meal or seed is extracted with 0'8% HOAc in aq. acetone (I) and the extract clarified with **Pb(OAc)2** and celite. Aflatoxins are extracted with **CHCl3** and cleaned up in a silica gel/Na2S0. column. After elution with **CHCl3–I** (4 : I) and evaporation of the solvent, spots are applied to silica gel t.l.c. plates and developed with **CHCl3–I** (17 : 3) solvent. Aflatoxins BI and B2 are estimated visually under u.v. light or by a densitometer fitted with a u.v. source. Recoveries in either case are 80-100%, with coeff. of variation of ~ 20% for the former method and 9-17% for the latter. M. BARNETT.

Some guidelines for the safe use of fillings, toppings and icings. J. H. Silliker *(Baker's Dig.*, 1969, 43, No. I, 51-54).-The organisms capable of causing food poisoning are discussed, and of these only *Salmonella* and *Staphylococcus* spp. are periodically traced to bakery products. Cooking normally destroys these organisms but with some icings the heating is not sufficient to kill even non-spore formers. In most cases the water activity is lowered by the **high** sugar content so that spoilage is more likely to be caused by moulds than bacteria or yeast. Spoilage is usually inhibited at pH < 4·5. Additives effectively control contamination except where it is excessive. Merchandising conditions are important and unless the product is adequately stabilised by one or more of the preceeding factors it must be stored below 45°F. The application of these results to icings, butter-cream fillings, synthetic cream fillings, toppings and meringues is discussed. J. B. WOOF.

Low temperature **growth** of salmonella. J. R. Matches and J. Liston *(i. Fd Sci.*, 1968, 33, **641–645).—The** pattern of survival or growth was followed by inoculating the organisms into tubes of broth and incubating the tubes in a polythermostat over the range 1·1to 12'3°. Min. growth temp. obtained after 19 days' incubation for S. *heidelberg*, S. *fyphimurium* and S. *derby* were 5·3, 6·2 and 6'9', respectively. Results indicate a growth temp. shift, during extended incubation of salmonellae at low temp. This phenomenon and the low temp. growth capability of salmonellae could be significant in foods stored for long periods of time at temp. > 5'. I. DICKINSON.

Effect of gamma radiation on *Staphylococcus aureus* in seafood. B. M. Slabyj *(Diss. Abstr., B*, 1968, 29, 863-864).-Commercially processed Dungeness crabmeat was examined for incidence of bacteria before and after exposure to y-radiation and storage at I and 5'5°. Immediately after exposure to 100 and 200 krad of y-radiation or after subsequent storage at I or 5·5° until spoiled, coagulase-positive staphylococci, salmonellae, shigellae, *E. coli* or coliforms were never detected in fresh crabmeat. Enterococci survived radiation exposure, increased in viable count on storage at 5·5' and decreased in viable count on storage at 1°. S. *aureus* grew well in sterilised crabmeat at **12°** but was greatly inhibited in irradiated and non-irradiated meat. It grew rapidly in sterilised meat at 22' but grew poorly in the non-sterile meat. This inhibition was due to competition by saprophytic bacteria, and this was largely eliminated by the irradiation. Repair of radiation damage by staphylococci was optimum at 37° and pH 5·5, and survival of staphylococci depended on the relative rates of DNA repair and replication. F. C. SUTTON.

Characterisation of the microflora of the **raw** milk supply. R. N. Pal *(Diss. Abstr., B*, 1968, 29, 862-864). –The types of organisms in 320 raw and pasteurised milk samples were divided into groups which have different significance, e.g., total population and coliform and spore counts. The spore-formers (staphylococci and **β-**haemolytic organisms) were consistently present in most raw milk samples. The relative distribution of various types of organisms in the raw milk was a producer characteristic. Coliform organisms correlated to the greatest extent to penicillin-resistant organisms and total staphylococci. These organisms can be used as a useful index of sanitation level and also public health relation of milk supply. A heat-resistant p-haemolytic bacteria isolated from pasteurised milk was identified as *Staphylococcus albus*. F. C. SUTTON.

Salmonellae and salmonellosis **associated** with milk aod milk products. E. H. Marth *(i. Dairy Sci.*, 1969, 52, 283-315).-The literature on salmonellae and salmonellosis is extensively reviewed, with particular emphasis on occurrence in dairy products and associated food ingredients. (241 ref.) M. O'LEARV.

Comparative study of different methods of counting *E. coli* in foods, using liquid media and the N.P.P. [most probable number] technique. E. Jacqmain, A. Veulemans and R. Lambion *(Revue Fermelll. Illd. alimelll.*, 1969, 24,13-16).--Comparisons showed that results obtained by the method of Mackenzie *et al.*, with a broth containing Brilliant green bile and lactose (with or without glucose), and the rapid (48 h) method of Mundt and Rai using the Hoskins most probable number evaluation technique, were equally reliable. The errors of the methods were generally within ± 30% in relation to the mean (P = 0·95). The presence or absence of glucose in the Mackenzie medium had no effect. P. S. ARUP.

Accumulation of fallout caesium-137 in newborn infants. T. A. linuma, T. Ishihara, S. Yashiro and T. Nagai *(Nature, Lond.*, 1969, 222, 478-480).--Complementary to the studies of Wilson and Spiers *(ibid.*, 1967,215,370), experiments using powdered milk of known 13'Cs content revealed that the 13'Cs concn. in infants between 80 and 170 days of age was higher than in their mothers. These high concn. are explained by a metabolic model of 13'Cs and the intake of powdered milk. The biological half-life of 13'CS in infants, as estimated by fitting the calculated body-burden to experimental data, showed that the internal dose of 13'Cs can be critical in babies up to 180 days old. W. J. BAKER.

3.-8ANITATION, WATER, etc.

Control of hooseDies in Eygpt by chemosterilants. I. Laboratory studies on ***Musca*** *domestica ,icina.* M. Harez, M. F. Osman, S. EI-Ziady, A. A. El-Moursy and M. A. S. Erakey *(i. econ. Ent.*, 1969, 62, 324-329).- When fed to adult flies, the chemosterilants induced sterility in both sexes. This seems to be the most promising form of control as neither larval treatment nor residual application was satisfactory. (19 ref.) C. M. **HARDWICK.**

An apholate-resistant strain of houseflies. I. Resistance to other chemosterilants and to insecticides. R. O. Abasa and E. J. Hansens *(i. econ. Em.*, 1969, 62, 334-338).- Some cross-resistance was shown to metepa but not to methotrexate. After 35 generations of selection with apholate, the strain showed decreased resistance to lindane, diazinon, dimethoate and dimetilan. (15 ref.) C. M. **HARDWICK.**

Biochemical characteristics of tbe microsomal cyclodiene epoxidase system and its inheritance in the hooseDy. M. A. Q. Khan *(i. ecoll. Ent.*, 1969,62,388-392).-(23 ref.) C. M. **HARDWICK.**

Metabolism of 35S-parathion in the [resistant and susceptible] housefly. T. Nakatsugawa, N. M. Rolman and P. A. Dahm *(i. econ. EIII.*, 1969, 62, **408–411).—Two** metabolites were found; sesamex (a pyrethrin synergist) inhibited their production ill *vivo.* (16 ref.) C. M. **HARDWICK.**

Oxidative metabolism of l4C-labelled Baygon by Jiving houseflies and by housefly enzyme preparations. S. P. Shrivastava, M. Tsukamoto and J. E. Casida (I. *eCOll. Ellf.*, 1969, **62, 483–498).—** The susceptibility of various strains to topically applied Baygon was investigated. (43 ref.) C. M. HARDWICK.

Compounds affecting fertility of adult houseflies. R. L. Fye, G. C. LaBrecque, A. B. Borkovec and J. Morgan, jun. *(i. econ. Em.*, 1969, 62, 522-524).-Of 25 compounds screened as chemosterilants, 3,5-bis(dimethylamino)-1,2,4-dithiazolium chloride was the most effective. C. M. **HARDWICK.**

Mosquito control agents derived from petroleum hydrocarbons. **m.** Effect of FLIT MLO on first and second larval instals [of *Culex pipiens quinque/asciatus* and *Aedes aegypti].* D. W. Micks, G. V. Chambers, S. Montalbano and C. Daoud *(i. econ. Em.*, 1969,62, **455–458).—FLIT** MLO was applied to the water surface at 0'**5–4** gal/acre. Growth retardation and mortality for the 2 instars are discussed; development of 2nd instar larvae was much more adversly affected by FLIT MLO than was that of 1st instars. Virtually complete control of 2nd instars was achieved with 2 or 4 gal/acre. C. M. HARDWICK.

Resistance to chlorinated hydrocarbons in *Culex pipiens quinque. fascialus* in Egypt. Z. Toppozada, S. M. Madi and M. E. Eldefrawi *(J. ecoll. Em.,* 1969,62, 440–442).—The degree of resistance in larvae and adults to various insecticides was determined; the strain was collected in 1964 from an area subjected to insecticides since 1950. Both larvae and adults were highly resistant to DDT and lindane, with cross resistance to DDT analogues or cyclodienes. No increased tolerance toward organo-P compounds or carbamates was noticed. (10 ref.) C. M. HARDWICK.

Control of pasture *Aedes* mosquitoes by dripping larvicides into flowing water; residues in a pasture habitat. M. S. Mulla, H. A. Darwazeh, A. F. Geib and W. E. Westlake *(J. ecoll. Em.,* 1969, 62, 365-370).-Emulsifiable concentrates of Dursban (O,O-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate), Abate (0,O-dimethyl phosphorothioate O,O-diester with 4,4-thiodiphenol), fenthion or methyl parathion were dripped into irrigation water to control *A. me/allimoll* and *A. lligramacu/is.* Abate gave complete control at O'17 ppm, Dursban at O'07 ppm, methyl parathion O'14 ppm and fenthion at O'2 ppm. This method cost less than half that of conventional treatment. There was an appreciable decline of residues in grasses after 72 h and residues in the mud soil were also low. C. M. HARDWICK.

Aerial applications of pyrethrum aerosol to control tsetse fly. C. W. Lee, N. S. Irving and J. D. Parker *(Pyrethrum Post,* 1969,9, No.4, 37-40).–A 95% reduction in the population of *G/ossilla pallidipes* was achieved after a third application of O'4% syncrgised pyrethrum in kerosene at 0·016 gal/acre. Further applications failed to reduce the population to a lower level.
E. G. BRICKELL.

DegradabiJity of brewery effluent in the activated sludge process. II. K.-H. Schmidt *(Brauwissellschaft,* 1969, 22, 285-292).–After mechanical pre-clarification, it was found that through-puts of 220 g/m^{3}h produced an output with B.O.D. of < 25 mg/l. In the activation tank, sludge concn. > 2 g/I caused no change in efficiency but between 0·8 and 1·3 gil the efficiency fell by 50%. An 02 level of 1,5-2,0 mg/I is recommended for the activation tank. Absence of mechanical clarification endangers smooth operation because of wild load fluctuation. The activated sludge showed poor sedimenting ability and needed the addition of asbestos, kieselguhr or FeSO,. Full degradative efficiency occurred between pH 6·0 and 9·3 when 90% of the nitrogenous material was degraded. Phosphate in the effluent fluctuated but never reached the **0·05–0·5** mg/I range aimed for. The population in the activated sludge differed from that treating household sludge by containing two strains of *Rotatoria* but they did not adversely affect purification. (51 ref.) J. B. WOOF.

Treatment of wastes from fish meat manufacture. M. Dazai, M. Ogawa and T. Misono *(Rep. Fermem. Res. [list.,* 1968, No. 34, II-18).-In a laboratory investigation, the efficiency of the activated sludge, cultured by other waste, increased considerably when acclimatised with total waste. When B.O.D. value was < 2000 ppm, the purification rate was unrelated to concn. of waste. Max. RO.D. loading of waste was 3·5 kg/m'/day with sludge concn. ~ 5000 ppm at **25°;** under this condition> 96% suspended solids and RO.D. of the waste were removed. Settling of the normal sludge was good and sludge vol. index was **60–70.** Excess sludge produced during the purification was 55% of the total RO.D. of the waste treated. (From English summary.) C. V.

Vegetable canniug process wastes. R. S. Rambo *(Diss. Abstr., B,* 1968,29, 1044).-A study was conducted in two vegetable factories to determine the sources and causes of wastes and excess water usage in the processing of canned peas, maize, beets, potatoes and carrots and to improve processing techniques and reduce pollution loads. The pollution loads expressed as B.O.D. were found to be 12-35 times greater than those of domestic sewage. A high degree of positive correlation between RO.D. and C.O.D. measurements was found for waste flows from the vegetables processed. Mineral analysis of the waste flows indicated that enough nitrogen was present for optimum stabilisation, by microbial waste treatment systems, of the pollution loads from beet, potato and carrot canning wastes. F. C. SUTTON.

Occurrence and significance of pesticide residues in water. H. P. Nicholson(J. *Wash. Acad. Sci.,* 1969, 59, 77-85).- A review covering the principle sources of water contamination by runoff from the land, discharges of industrial waste and accidents, etc. and the significance of pesticide residues in aquatic life and to other water users. (29 ref.) E. G. BRICKELL.

Effect of the association of organic material with clays on parathion and DDT adsorption. W.·C. Wang **(*Diss.*** *Abstr., B,* 1968, 29, 904-905).- Studies were made of the adsorption of parathion and DDT on montmorillonite and kaolinate clays « I μm particle size) in the prcsence of rhodamine B, phenol, methylene blue and organics from a lake, and also of adsorption on a lake sediment and removal of the pesticides from water by copptn. using Fe and Al floes. The results showed that depending upon the type and concn. of adsorbant and pesticide, and the quality of water media, pesticide removal from water solutions by adsorption and/or copptn. may vary markedly. F. C. SUTTON.

Biological concentration of pesticides by algae. B. D. Vance and W. Drummond *(J. Am. Wat. Wks Ass.,* **1969, 61, 360–362).—** The accumulation of commonly used chlorinated pesticides in algal populations and the effects of these pesticides on algal metabolism were studied. *Microcystis aerugillosa* (I), *Anabaena cylindrica, Sce/ledeSllllls qlladricauda* and *Oedogonium* sp. were each grown in the presence of aldrin, dieldrin, endrin andp,p··DDT and were found, wilh the exception of I, to be highly resistant to these pesticides. It was found that algae do accumulate pesticides from the medium in which they are grown and in no case was the concn. of pesticides in the algae < loo-fold under the test conditions. The potentiation and biological transfer of such highly cone. residues to higher members of the food chain, e.g., rotifers and small fish, are discussed. (11 ref.) J. M. JACOBS.

Biological methylation of mercury in aquatic organisms. S. Jensen and A. Jernelov *(Natllre, Land.,* 1969, 223, 753-754).- The evidence reported proves that MeHg+ and HgMe2 are formed in bottom sediments and rotten fish after addition of 0'1-500 ppm of Hg2+ (as **HgCl$_2$**) or MeHg+ and incubation up to 30 days (sediments) or storage for 4 days or more (fish). Concn. of MeHg+ formed in fresh water aquaria sediments were higher than in sediments from a Swedish lake treated with **HgCl$_2$.** The results, which show that living organisms can methylate Hg compounds released in industrial wastes, are discussed briefly in terms of the uptake and distribution of Hg in fish, e.g., pike, and the mobilisation of Hg from bottom sediments into the general aquatic environment.
W. J. BAKER.

Physicochemical behaviour and radioecology in hydrobiological systems of cerium and other lanthanides. R. Bittel *(Serle Bib/phies, Comlliss. Ellerg. atom.,* 1969, No. BIB-138, 88 pp.).- Based on a review of the literature, the physicochemical properties and the behaviour in water and soils are discussed, especially in respect of *wCe,* I44Ce, I55Eu and "'Pm. Detailed consideration is given to complexes of these elements with inorg. and org. compounds present in effluents and contaminated aq. environments, and also to procedures for radiochemical and trace analyses. The radio·ecology of the lanthanides in hydrobiological systems is summarised. (184 ref.) W. J. BAKER.

Schistosomiasis control in water supply sources. J. A. Scott *(J. Am. Wat. Wks Ass.,* 1969, 61, 352-354).-Schistosomiasis (bilharziasis), caused by parasitic worms and transmitted by contact with water in which certain fresh water snails are living, spreads rapidly where new or extended water storage and irrigation projects provide more habitats for the snails. The most hopeful means of checking the disease is through control of the snails. The possible contributions of water engineers to this control, e.g., fluctuations of water level in reservoirs to render the shores un·favourable as snail habitats, changes in the design of irrigation canals to improve flow, lining of canals, weed clearance. etc., are discussed. (14 ref.) J. M. JACOBS.

[Pesticidal] m-amylphenyl, N-chloroacetyl-N-methylcarhamates. Hercules Inc. (B.P. 1,159,559, 30.3.67. U.S., 27.2.67. Addn. to RP. 1.142,170).–Pesticides (especially insecticides with high activity against mosquito larvae) have formula m·C.Hn·C.H,·0'CO'NMe'COCH3_nCln *(n* is 1-3). In an example, MeNCO in toluene is added to a mixture of m-C.Hn·C.H,OH [isomeric mixture of 3 pt. of m·l·methylbutylphenol and I pI. of *m·(I·ethyl·*propyl)phenol)}. toluene and a small amount of Sn octoate, then after 6 h at **70°** toluene is removed at **80°/0·1** mm. Residual Ill-amylphenyl methykarbamate is boiled under N2 with CH2Cl·COCl while keeping at ≯ **130°,** with formation of m-amylphenyl (chloroacetyl)methykarbamate. F. R. BASFORD.

Methylenedioxyphenyl compounds. Shell Internationale Research Mij N.V. (Inventors: J. W. Cornforth and W. Bonthrone) (RP. 1,159,089, 16.5.68).- Compounds useful as synergists for insecticides comprise l-R-2-X-4,5-methylenedioxybenzenes wherein R is CHRI.0([CH2j,0)nRII; R'-RII are alkyl or RI is H; *n* is an integer; and X is halogen or CHRI.0([CH2j,0)nRII. In an example, 4-chloro-I,2-methylenedioxybenzene (prep. described) is added at **60°** to HCI-saturated 40% aq. HCHO and cone. aq.

HCI during 20 min; after 2'5 h at **60–65°** the cooled mixture is poured onto ice and ether. Evaporation of the org. extract affords 2-chloro-1-chloromethyl-4,5-methylenedioxybenzene, m.p. **61–63°** (light petroleum). This in benzene is added during 20 min to reagent prepared by adding a solution of 2-(2-butoxyethoxy)-ethanol in benzene to a suspension of NaOMe in benzene. The mixture is boiled for 12 h, then worked lip to give 1-(2-chloro-4,5-methylenedioxyphenyl)-2,5,8-trioxadodecane, b.p. 156-158'/0'03 mm, which increases the lethal effect of pyrethrin (on flies) 6-fold.
F. R. BASFORD.

4.-APPARATUS AND UNCLASSIFIED

Microbiology. K. L. Burdon and R. P. Williams. 1968, 818 pp. 6th ed., £4-17-6. (New York: Macmillan). S. C. H.

Microbiology of 'pre-gastric' fermentation. R. T. J. Clarke *(Allst.* J. Sci., 1968-69, 31, 141-146).- A review and general discussion. (20 reL) C. V.

Ad,'ances in microbiology of the sea. Ed. M. R. Droop and E. J. F. Wood. 1968, vol. I, 239 pp., 60/-. (London and New York: Academic Press). C. V.

Annual review of microbiology. Ed. C. E. **Clifton,** S. Raffel and M. P. Starr. 1968,596 pp. (Annual Reviews Inc.). C. V.

Analytical serology of micro-organisms. Ed, J. B. G. Kwapinski. 1969, vol. 1,681 pp. (New York, etc.: Interscience Publishers). S. C. H.

Microbial growth. Society for General Microbiology. 1969, 400 pp. (London: Cambridge Univ. Press).- Papers presented at the 19th Symposium. C. V,

Energy exchange between organisms and environment. D. M. Gates *(Allst.* J. *Sci.*, 1968-69, 31, 67-74).- Energy balances in plant and animal systems are discussed. Energy flow and exchange in plants wcre examined in terms of transpiration rates and leaf temp. in relation to various conditions. The energy budget for animals, birds, etc., is discussed and the effect of climatic variables is examined. As a comparative example, the temp. difference between body temp. and radiant surface temp. which the locust, cardinal, jack rabbit and lizard can maintain by virtue of the metabolic ratc, sweating rate, respiratory moisture loss and conductance of body fat, fur or feathers, is clearly shown. C. V.

Design and application of membrane probes for fermentation gases. J. D. Borkowski *(Diss. Abstr., B,* 1968, 29, 468).-Steam-sterilisable membrane probes, for monitoring the dissolved 02 level in liquids or the O, content of gas streams, possess a Ag cathode, a Pb anode, an acetate buffer and a Teflon membrane; those membranes for monitoring the dissolved H, level in liquids or the H, content of gas streams have a Pd anode, a Pb cathode, a Teflon membrane and a Pb $(OAc)_4$ solution as electrolyte. The 0, probes were used to monitor the dissolved 0, concn. in continuous cultures of *Cal/dida mitis* and *Micrococclls rOSCllS* that were O,-limited.
F. C. SunON.

Reducing expenditure and raising profitability of tobacco primary treatment. A. M. Patrikcyev *(Pishch. Teklllllol.,* 1968, NO. 5 (66), IO).- (In Russian.) P. P. R.

Pesticide residue analysis [water and biological material]. D. C. Abbott and J. Thomson *(WId Rev. Pest COl/trol,* 1968,7, **70–83).—** The extraction and clean-up stages are briefly discussed, and are followed by detailed consideration of the end methods of analysis (paper chromatography, t.l.c. and g.l.c.). Tables of R_V values, relative retention times and of pesticide levels in, e.g., birds' eggs, rainwater and human fat are given. (27 ref.) P. P. R.

Thin-layer chromatographic method for the determination of plant pigments in sea-water and cultures. C. Garside and J. P, Riley *(Al/alytica Chilli. Acta,* 1969, 46, 179-191).-Samples are filtered through glass fibre, coated with MgC03, the sediment is extracted with acetone and MeOH, and the dried extract is redissolved in Et,O containing 1% of Et,NH and analysed by I.l.c. The spots are examined with a Chromoscan by reflectance at 430 nm. Chloro·phylls *a, band c,* carotene, many xanthophylls and certain degradation products can be determined. F. C. SAVILLE.

Process for fermentation and recovery of microbial cells. Esso Research and Engng Co. (B.P. 1,144,523,9.1.67. U.S., 17.1.66).- Micro-organisms are recovered from an aq. medium by treating the medium with an immiscible volatile org. liquid (I) at 20-55° and recovering the micro-organisms from the latter. I has sp. gr. > I, a heat of vaporisation less than that of water and is a 1-4C halogenated aliphatic compound, e.g., *CHnX._n(wherel/* isO, 1 or2), CS, or PhMe. In an example, *MicrococcllS cerifical/s* is recovered by extraction with CH2CI, at 70° F for 30 min. The process is designed for recovery of micro-organisms utilising a hydrocarbon feed source. S. S. CHISSICK.

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ABSTRACTS

DECEMBER, 1969

The general arrangement of the abstracts is as follows: I.-AGRICULTURE AND HORTICULTURE. 2.-FoOD; also appropriate Microbiological Processes; Essential Oils. 3.-SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.-ApPARATUS AND UNCLASSIFIED.

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