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THE BACTERIOLOGY AND PROTOZOOLOGY OF RUMINANT DIGESTION *

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Recent successes in the pure culture study of true rumen bacteria responsible for carbohydrate fermentations are outlined. Some account is also given of the rumen ciliate protozoa as fermentative agents. The power of polysaccharide synthesis and storage is very marked in many of these biochemically active rumen micro-organisms, both bacteria and protozoa. On the other hand little is yet known about individual microbial species responsible for extensive nitrogenous transformations in the rumen.

The usefulness of pure culture studies in rumen microbiology

FOR the microbiologist the interesting difference between ruminants and other herbivores is that ruminants pre-ferment their food *before* digestion proper, in that part of the paunch known as the rumen. Hence arises the almost incredible variety of micro-organisms in that very special kind of fermentation vat in which the typical fermentation processes get established under highly reducing conditions when the animal is quite young and the rumen is relatively small. Several factors, which one can hardly hope to reproduce *in vitro*, contribute to the continuity of rumen microbial action during the whole lifetime of the animal. Nevertheless, by a study of the rumen micro-organisms in isolation, firstly the bacteria, then the protozoa, some information about the potentialities of the microcosm has been gleaned. It is important to remember that the rumen microbial association is not built up all at once. In the young calf, at least, the rumen population, before rumination is well established, is quite different from that of the adult rumen, both in numbers and in species.[1, 1a]

By choosing to ferment the most resistant fodder constituent, cellulose, at the start rather than at the end of its digestive tract, the ruminant has necessarily acquired a large number of microbiological problems that would prove difficult to a fermentation chemist. For example, it is not possible to maintain in the rumen a mixed culture devoted almost exclusively to the desired end only, namely cellulose and hemicellulose fermentation to acetic, propionic and butyric acids with concomitant B-vitamin production. In grass itself and especially in root crops like turnips, other fermentable carbohydrates may exceed cellulose in amount and being more soluble and less structurally and mechanically resistant than cellulose, are more readily fermentable by a much wider variety of micro-organisms, not necessary to the same constant ratio of desired products. The marvel is that the microbial association in the rumen can undoubtedly take considerable variations in fodder in its stride, provided the changeover is not too abrupt.

The rumen mixed culture is so complex an association that relatively little is known with certainty about the more important microbial inter-relationships within it.[2] Rumen microbiology is undoubtedly a field in which the great unifying discoveries have yet to be made. Although there are other very fruitful modes of approach, important facts have been gleaned by pure culture studies, which could not have been otherwise obtained. What follows is a brief summary of these facts.

The main problems are to decide whether a given isolate from the rumen is authentic and important, and to isolate the biochemically important rumen micro-organisms. With the larger of these organisms there is little apparent difficulty in identification. Thus the ciliate protozoa for example are so large and usually so numerous that it is only necessary to devise means of separating them from rumen contents in bulk. This is not easy, but in order to study their biochemistry, it is not at first necessary to culture them. Morphology alone is also of great help with large rumen bacteria having a characteristic shape and a clearly defined and recognizable internal structure.[3, 4] It is probable however that mere microscopy, even with all modern

* Read at a joint meeting of the Agriculture and Microbiology Groups, 11 November, 1954; for a report of the Discussion at the meeting see *Chem. & Ind.*, 1954, p. 1579.

refinements of technique, is of limited value for identifying the truly small rumen eubacteria, that is the great majority, owing to their minute size, their liability to extreme pleomorphism and their lack of unambiguous and clearly defined internal structure.

Methods for isolation of true carbohydrate-fermenting bacteria

The biochemical criterion, whether or not the isolated bacterium in pure culture can bring about under conditions resembling those in the rumen a rapid and extensive fermentation of a fodder constituent, or of a plausible intermediate in the breakdown of a fodder constituent, has proved more useful than any other. It seems to be the accepted opinion with regard to bacteria in milk[5] that if a good fermenter is present at a level of 1–10 million or more per gramme, it cannot be ignored in assessing the total fermentative effect. This may well be true for the rumen also. It is now recognized by most workers in this field that, if true anaerobes are sought for, the basal medium should contain a fairly high proportion of sterile clarified rumen liquor[6] and also reducing substances,[7] and a definite bicarbonate ion concentration as in ruminant saliva. Even if the emphasis is on facultative anaerobes, the basal medium should still be essentially rumen liquor with addition, if necessary, of a small amino-acid supplement for aiding bacterial growth, and without added vitamins and growth factors,[8] which are present in adequate proportions in the rumen liquor.

It is useful to divide the added substrates used in such isolation procedures into three groups : cellulose, starch and soluble carbohydrates, and plausible intermediates in carbohydrate breakdown, respectively. Present knowledge seems to indicate that different species of bacteria are involved in each kind of fermentation. Tables I and II show roughly the present position with regard to well authenticated bacterial species concerned with cellulose breakdown on the

Table I

Rumen cellulolytic anaerobes

Name	Morphology	Products	Isolated by
Ruminococcus (? *Streptococcus*) *flavefaciens* and related spp.	Gram-positive coccus	A, L, S Formic ?	Hungate[9]; Sijpesteijn[10]; Bryant & Burkey[11]; Bryant & Doetsch[12]
Bacteroides succinogenes and related spp.	Gram-negative rod	A & S	Hungate[9] / Bryant & Burkey[11]
		A, B & L	Huhtanen & Gall[13]
? (Ferments hemicelluloses)	Gram-negative rod	A, B (from hemicelluloses)	Hungate[14]

A = acetic, L = lactic, S = succinic, B = butyric acids

Table II

Rumen bacteria fermenting starch and soluble sugars

Name	Morphology	Products	Isolated by
Streptococcus bovis (var. *x*, *y*, *z* ?)	Gram-positive coccus; often capsulated; sometimes iodophilic; facultative anaerobe	Lactic acid. Complex capsular polysaccharide. Sometimes amylose, etc., intracellularly	MacPherson[15]; Mann, Masson & Oxford[16]; possibly also by Gall & Huhtanen[17]
Lactobacillus sp. ?	Gram-positive rod	?	Huhtanen & Gall[13]
Selenomonas ruminatium	Motile ovals and crescents (may be two distinct organisms)	Glycogen; ferments glucose, maltose	Woodcock & Lapage[18]; Quin[19]

one hand and starch and soluble sugars on the other. The recent discovery[20] that certain higher volatile fatty acids in rumen liquor serve as growth factors for the cellulolytic *Bacteroides succinogenes* provides a remarkable link between cellulose fermentation and amino-acid breakdown in the rumen. Propionic acid does not seem to be formed directly in any pure culture fermentation so far studied and the products of such fermentations include acids like succinic and lactic, not usually found in the rumen, as well as acetic and butyric. However, as Johns[21] and Sijpesteijn & Elsden[22] have shown, the mixed rumen microflora is able to convert succinic to propionic acid by

decarboxylation with great speed and is also able slowly to ferment lactic acid. Table III lists the pure cultures isolated from the rumen which have these properties. *Veillonella gazogenes* is remarkable in that it will not ferment carbohydrates, but only lactic, succinic and certain related C_4 acids. The point arises here if it is justified to assume that all or most of the propionic acid formed in the rumen arises from succinic acid (see Table IV). A suggested mechanism for the production of succinic acid from cellulose and other hexosan (and perhaps pentosan) carbohydrates is shown in Table IV. Marston's[26] important results for large-scale *in vitro* cellulose fermentation by washed *total* rumen bacteria from a hay-fed sheep are of interest here. These results are not wholly in accordance with the accepted scheme for the production of succinic from pyruvic acid by CO_2 fixation and reduction. A simple calculation shows that there must be

Table III

Rumen anaerobes fermenting lactate and/or succinate to yield propionate

Name	Morphology, etc.	Isolated by
Veillonella gazogenes	Gram-negative coccus (Also in horse colon)	Johns[21]; Gutierrez[23] (Alexander, MacPherson & Oxford)[24]
Coccus LC (unnamed)	Gram-negative	Elsden & Lewis[25] (produces chiefly higher volatile fatty acids)
Propionibacterium sp.	Gram-positive rod (catalase negative)	Johns[21]; Gutierrez[23]
Corynebacterium acnes, renamed *Propionibacterium acnes*	Gram-positive rod (catalase positive)	Gutierrez[23]

Table IV

Mechanism of propionic acid production in rumen

$COOH{\cdot}CH_2{\cdot}CH_2{\cdot}COOH \longrightarrow CH_3{\cdot}CH_2{\cdot}COOH + CO_2$ (Johns, Elsden)

Also possible : $CH_3{\cdot}CH(OH){\cdot}COOH \longrightarrow CH_3{\cdot}CH_2{\cdot}COOH$ (via acrylic acid),
since not all propionic acid-producing bacteria will decarboxylate succinic acid (Johns).

Possible rumen routes to succinic acid (thence propionic acid) from hexose

(*a*) $C_6 \longrightarrow$ 2 pyruvic $\xrightarrow{+\ 2CO_2}$ 2 oxaloacetic $\longrightarrow$ 2 *succinic*
(*V. gazogenes* : *Propionibacterium* sp.)

(*b*) $C_6 \longrightarrow 2C_3 \longrightarrow 2C_2 \longrightarrow C_4 \longrightarrow$ 1 *succinic* ?

[Marston's[26] results for *in vitro* cellulose fermentation by washed total rumen bacteria point to 80% (*a*) + 20% (*b*).]

another mechanism at work beside the accepted one [(*a*) of Table IV] and that this other mechanism can yield only one succinic acid molecule from a hexose unit. The calculation is greatly simplified by virtue of the fact that acetic and propionic acids, CO_2 and methane were really the only products in two out of four experiments. Such *in vitro* fermentations may not, however, give a true picture of the degradation of cellulose in the rumen. Propionic acid is always the chief product *in vitro*, but rumen contents always contain a higher concentration of acetate than propionate.

Nitrogen metabolism

Very little has been discovered yet concerning the finer details of nitrogen metabolism by bacteria bringing about protein, amino-acid and urea breakdown and syntheses in the rumen.[27] Urea is always present because it is a salivary constituent, but it may also be deliberately added as a fodder constituent. Mixed rumen bacteria have a powerful urease action. It is not known which species or even genera of rumen organisms are most active here, nor what their growth requirements are. Obviously it would also be advantageous to be able to distinguish between the bacteria which can convert non-protein nitrogen into cell protein, from those which attack amino-acids and proteins to yield ammonia even in presence of carbohydrate.

Rumen ciliate protozoa as fermentative agents

Recent studies involving the rumen protozoa will now be considered, but bacteria will be mentioned again later in connexion with their chemical composition as distinct from fermentative activities.

Associated with the rumen bacteria in healthy ruminants living together in flocks and herds, there is always an immense population of ciliate protozoa. Their numbers may exceed one million per gramme of rumen contents.[28, 38] These intestinal ciliates, first discovered in 1843, are unusual among animals in being obligate anaerobes. In some of them, biochemical specialization has been carried to the highest possible degree. The rumen ciliates besides providing protein (up to 20% of that leaving the rumen)[28] of high biological value for the nutrition of the host animal, as recently shown by workers at the Hannah and National Institutes for Dairy Research,[29] may also have intense biochemical activity in their own right. Some rumen ciliates may live on, or need, bacteria, but it is now clear that many species among them also compete with bacteria for carbohydrate substrates.

Morphologically the rumen ciliates are organisms propagating always by binary fission. Complications due to encystment, conjugation, and other manifestations of a complex life cycle are not found and the doubts and qualifications which cloud the issue with most parasitic protozoa do not confuse the issue. They can be separated in bulk from rumen contents by methods based largely on their feeding habits. Table V lists the more important rumen ciliates and their feeding habits. Only the holotrichs, that is, two species of *Isotricha* and one of *Dasytricha* (a much smaller ciliate) attack soluble sugars in a bacterium-like way. Table VI lists the soluble sugars attacked by these holotrichs which usually are in a minority among rumen ciliates. Such sugars extend their life in a nitrogen-free bicarbonate buffer medium for the simple reason that the ciliate can convert them in part into insoluble storage material later metabolized. This material consists of tiny grains (2–3 × 1 μ.) of amylopectin,[31] and it is strange that the holotrich cell seems to have little control over the storage which can proceed in a medium too rich in sugar until the cell bursts and the grains are released.[30] The grains themselves resemble yeast cells but have no internal structure. They stain purple with iodine. Up to 70% of the dry weight of the cell can consist of this storage amylopectin-like material.[32] It is clear that the ciliate's own enzymes act on the soluble sugars, since they (the ciliates) are motile and can ferment carbohydrates even in presence of an enormous concentration of streptomycin (1 mg./ml.)

Table V

Genera of ciliate protozoa of sheep's rumen which ingest fodder constituents

Average size: approx. 60 × 30 × 30 μ
Population: up to 10^6/g.

	Genus	Chiefly digest	Occurrence
Holotrichs	*Isotricha* *Dasytricha*	Soluble sugars	Notably on hay and root diet
Oligotrichs	*Metadinium* *Diplodinium*, etc.	Cellulose	Notably on cellulose and soluble protein-rich diet (grass)
	Entodinium (some species only)	Starch	Notably on starch-rich diet (flaked maize, concentrates)

which quickly kills all bacteria in the culture.[33] On the other hand, certain almost insoluble odoriferous plant constituents like skatole and borneol quickly destroy the ciliates, probably without affecting the rumen bacteria very much.[34] *Isotricha* can also digest vegetable starch grains if these are small enough. *Dasytricha* has apparently too small a mouth to be able to swallow any starch grain.[30] The storage carbohydrate laid down from vegetable starch is still amylopectin in the usual granular form. When placed in a buffer without carbohydrate the ciliates proceed to ferment their storage starch to yield acetic, butyric and lactic acids.[35] The same acids are also produced concurrently with amylopectin storage when a soluble sugar is being acted on by the healthy ciliates.[35] All this has been independently confirmed in the U.S.A. in

Table VI

Carbohydrates utilized by rumen holotrich ciliate protozoa[30]

Sugar added (<0·1%)	Length of life of culture in buffer (days)
Glucose	7–8
Fructose	5–6
Galactose	5–6
Sucrose	5
Cellobiose	7–8
Raffinose	5
Inulin	5
Levan	5
Salicin	6
Melibiose	4
No substrate or maltose, xylose, etc.	2–3

Hungate's school quite recently.[36] In addition it has been shown that only the smaller ciliate (*Dasytricha*) attacks cellobiose and β-glucosides.

Another group of rumen ciliates seems to be able to live by digesting cellulose. They include several genera of oligotrichs. Prominent among them is the ciliate known as *Metadinium medium*. It has a large nucleus and also other internal structures known as skeletal plates in which storage material is laid down. It has the power of swallowing pieces of clean sterile cellulose with great rapidity. It then slowly digests the cellulose, at the same time storing amylopectin (or perhaps glycogen) in its skeletal plates and also at its periphery.[37]

The question of course arises, do the ciliates really attack the insoluble cellulose by means of their own enzymes, or do they require cellulolytic bacteria inside themselves to aid in the process? All that can be said at the moment with reference to this point is that a high concentration of streptomycin in the medium greatly slows down cellulose digestion in these ciliates although it does not kill them.[37] It is quite possible that cellulolytic bacteria live in symbiosis with these protozoa.

The power possessed by the rumen holotrich ciliates of ingesting small vegetable starch grains was mentioned above. There are also in the rumen many species of oligotrich ciliates which can ingest vegetable starch grains of medium to fairly large size, in particular species of *Entodinium*. These have a true gastric sac in which the ingested starch grain resides until it is digested.[38] As usual, amylopectin (or glycogen) is laid down as protozoan storage starch this time at the periphery only. Recent Australian work has emphasized the role these ciliates play in starch fermentation in the rumen.[39]

On the other hand, it has been found that starch digestion by *Entodinium* is very susceptible to streptomycin[37] so it would seem that bacteria inside the protozoan gastric sac might play a vital role in starch digestion. Whether these bacteria are similar to the free streptococci mentioned previously has yet to be determined. It is still possible of course that *Entodinium*, like the holotrichs, will prove to have its own amylase.

Rumen bacteria as storage polysaccharide producers

As has been seen, the holotrichs and *Entodinium* do not ferment soluble sugars and starch rapidly to completion—they store a large proportion of the substrate as amylopectin and then ferment the storage material endogenously, at a more or less even rate over a long period of time. Thus the tendency to sudden fermentative bursts, with rapid fall in pH after the entry of fresh fodder into the rumen, is to a certain extent checked. The same provision also exists among certain rumen bacteria. It has long been known that a considerable proportion of rumen bacteria may be iodophilic, and stain blue with iodine. These are particularly in evidence when the ration contains starch.[3] Recently amylolytic *and iodophilic* streptococci in pure culture have been isolated from the calf's rumen[1a] and it is found that they only become iodophilic when maltose or some maltose polymer (e.g. glycogen, dextrin, etc.) is dissolved in the medium. Glucose, fructose, glucose phosphates, cellobiose or sucrose are not converted into intracellular starch by these particular bacteria. It has also become clear recently that capsulation of

bacteria, in other words extracellular bacterial carbohydrate, is abundant in the rumen.[40] This has important serological implications, particularly in the identification of rumen streptococci.[41] Thus, both iodophilic and encapsulated bacteria may be regarded as agents in storing soluble or easily fermented sugars in the form of insoluble and less readily fermentable polysaccharides, in this way tending to retard a rapid rumen fermentation that might well get out of hand.

Unsolved problems concerning rumen protozoa

A large number of problems in rumen microbiology still await solution. Two of these are being studied at the Rowett Institute: (1) It is known that a free-living ciliate called *Tetrahymena pyriformis*, which can be grown indefinitely in a chemically defined medium, requires numerous B-vitamins (in addition to certain amino-acids) to be supplied in the medium.[42] It is surely unlikely that the rumen ciliates are any less exacting than this. They may well be more exacting. It is also well known that rumen bacteria manufacture B-vitamins for the host animal which need have hardly any in its fodder. Is it possible that a large population of ciliates in the rumen may mean that the host animal, the ruminant, is in danger of being deprived of its B-vitamins? In other words: Are any vitamins really destroyed by the living ciliates? (2) Which species of rumen bacteria aid, and which hinder, the continued life *in vitro* of biochemically active rumen ciliates? If this were known, it might be possible to culture the latter indefinitely *in vitro*, for past experience has shown that it is virtually impossible to do so in a sterile chemically defined medium. The whole question of bacterial–protozoan inter-relationships in the rumen in fact lies open to investigation at this moment. That the purely bacteriological problems outstanding are still legion needs no further emphasis.

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For a complete list of references concerning the bacteriology (as distinct from the protozoology) of the bovine rumen up to 1953, consult reference 2 above.

THE MODE OF ACTION OF ANTIBIOTICS IN CHICK NUTRITION. II.*—The Effect of Penicillin in Clean and Stale Premises

By M. E. COATES, M. K. DAVIES, G. F. HARRISON, S. K. KON and J. W. G. PORTER

Premises where chick growth had been suboptimal without penicillin in the diet were thoroughly cleaned and disinfected. The first few batches of chicks reared there after the disinfection grew well without penicillin, but growth deteriorated in subsequent batches and finally became indistinguishable from that in ' stale ' premises.

In field units that had previously housed other chicks, growth of new chicks was depressed in the absence of penicillin. In new field units on fresh ground chicks grew as well without as with the antibiotic, but two further batches of chicks showed a small increase in weight with penicillin even though the units had been disinfected and moved to fresh land.

Introduction

Several hypotheses have been put forward to explain the ability of antibiotics given by mouth to stimulate the growth of young animals. Many workers have reported a sparing action by antibiotics on certain vitamins and other nutrients but, as our own experiments and those of others have shown, antibiotics can exert a beneficial effect on chicks even when added to diets rich in all known essential nutrients and some further effect must therefore be involved. We have suggested previously[1] the existence among most chicks of a growth-depressing ' infection ', hitherto unrecognized, which prevents the chick from growing at its optimal rate and which can be counteracted by antibiotics. The ' infection ' is otherwise symptomless and can be transmitted by direct contact between chicks. It may possibly be air-borne. It is apparently associated with old poultry houses that have been in continual use, whereas it is initially absent from premises not previously occupied by poultry. Our work has now been confirmed by other workers, including Hill *et al.*[2] and Lillie, Sizemore & Bird,[3] and something similar with turkeys in more practical conditions has been reported by Wilson.[4] King & White[5] do not record consistent responses to penicillin even in old premises, but in our opinion it is doubtful if the number of birds they kept in their room at any one time would be sufficient to maintain the condition of ' infection '.

This report is a record of our further experiences with the feeding of penicillin to chicks in ' infected ' and ' uninfected ' environments.

Experimental

By our definition the ' infection ' is a transmissible condition which prevents optimal growth of chicks on an adequate diet unless that diet is supplemented with an antibiotic. In experiments on the nature of this ' infection ' a good growing diet is therefore essential, and throughout the work described here a mash which in our experience supports excellent growth in young chicks was used. It had the following composition in parts by weight : ground maize 35, ground wheat 30, wheat offals 8½, fish meal 10, dried skim milk 7½, dried grass 3, yeast 3, limestone 1½, salt mixture ½, arachis oil 1. Each g. of arachis oil had dissolved in it 680 i.u. of vitamin A and 64 i.u. of vitamin D_3. Procaine penicillin was added where required at the rate of 2·5 mg. of penicillin/100 g. of diet.

Experiments in the chick rooms

The premises in which we first detected the presence of the growth-depressing ' infection ' were our usual chick rooms, referred to as Shinfield 1, where some 1500 chicks up to the age of 4 weeks are regularly kept at any one time in Hearson electric battery-brooders. It seemed desirable to eliminate the condition if possible from these rooms while maintaining the ' infection ' for experimental purposes in another smaller room, referred to as Shinfield 2, some distance removed from the first. Shinfield 2 was ' infected ' by transferring to it chicks and equipment from Shinfield 1 and was then maintained as an isolated unit attended by a separate staff.

* The paper published in *J. Sci. Fd Agric.*, 1952, **3**, 43 is regarded as Part I of this series.

Shinfield 2 contained two brooders each housing 120–180 birds. Every two weeks the chicks in one or other of the brooders were replaced, so that the total population in the room remained approximately constant throughout the experimental period. Meanwhile Shinfield 1 was emptied of birds and the floors, walls, ceilings and all equipment were scrubbed several times in strong Lysol solution. The rooms were fumigated twice with formaldehyde and left empty for three weeks. When day-old chicks were re-introduced into the room, growth was much improved and responses to penicillin were negligible, whereas, in the newly-infected Shinfield 2 room, growth was significantly depressed in the absence of the antibiotic. However, as more and more chicks passed through Shinfield 1, growth began to deteriorate unless penicillin was included in the diet, and in spite of liberal use of disinfectants including aerosols, repeated fumigation and the exclusion of everyone but the necessary attendants, the condition of ' infection ' could not be completely eliminated so long as the rooms were filled to capacity. Table I gives some typical responses to penicillin during a period of about 10 months in both premises.

Table I

Mean body weights in g. at four weeks of age of groups of fifteen chicks in two different environments. Standard error in parentheses

Time after setting up of premises (weeks)	Shinfield 1 (after disinfection)			Shinfield 2 (after deliberate ' infection ')		
	Without penicillin	With penicillin	Increase %	Without penicillin	With penicillin	Increase %
2	317 (13·3)	325 (12·3)	2·5	249 (10·2)	311 (14·4)	24·9
4	311 (12·5)	339 (13·7)	9·0	273 (11·8)	302 (14·2)	10·6
20	330 (11·3)	360 (23·6)	9·1	276 (19·7)	334 (11·9)	21·0
40	273 (18·8)	333 (15·2)	22·0	277 (14·5)	306 (6·5)	10·5

Field experiments

It seemed of interest to determine whether the ' infection ' was peculiar to intensive methods of rearing or whether a similar condition is established in chicks reared out of doors. For these experiments, which were carried out during the summer months, the chicks were brooded in fold units on grass runs. Heat was provided inside the sleeping compartment by electrically heated ' Raydown ' hovers. At the beginning of this series of experiments all the equipment was new and the land had not previously been used for poultry. Two of the folds were deliberately ' infected ' by housing in them a group of four-weeks-old ' infected ' chicks from the chick rooms, for several days immediately before the first experiment ; these two units were not cleaned or moved on to fresh ground during the first experiment. The other two folds were set up at some distance from the ' infected ' pair and were managed by different attendants to minimize the risk of cross-infection. Between experiments the two ' uninfected ' folds were moved on to fresh ground after having been scrubbed with disinfectant, and all detachable parts as well as food and water containers were steamed. The ' infected ' folds were only brushed out and moved over a limited area so as to include within the run some ' infected ' ground.

Groups of about 50 day-old chicks were placed in the folds immediately on arrival from the hatchery. One group in each pair of folds received the diet alone and the other was given the diet supplemented with penicillin. The chicks were weighed at three weeks of age ; the first and third experiments were continued for a further week. In all, three experiments were carried out with intervals of a fortnight between each. The mean body weights of the groups at the end of each experiment are given in Table II.

Table II

Response to penicillin under field conditions of groups of fifty ' infected ' or ' uninfected ' chicks. Mean weight in g. with standard error in parentheses

Experiment No.	Age of chicks (weeks)	' Uninfected ' chicks			' Infected ' chicks		
		Without penicillin	With penicillin	Increase %	Without penicillin	With penicillin	Increase %
1	4	318 (8·2)	336 (10·4)	5·7	296 (9·1)	343 (7·6)	15·9
[2	3	211 (3·5)	226 (4·1)	7·1	179 (4·4)	196 (3·8)	9·5
3	4	292 (5·9)	316 (6·7)	8·2	260 (7·6)	301 (7·3)	15·8

The response to penicillin of the ' uninfected ' group in the first experiment was small and not significant ($P > 0.1$). At the same time the difference between the two groups on the ' infected ' land was highly significant ($P < 0.001$). In the second and third experiments a trend similar to that in the disinfected chick room became apparent, in that a relatively small but significant response to penicillin occurred in the ' uninfected ' folds, although the weights attained there were greater than those in the ' infected ' folds.

Discussion

These results confirm our previous finding that in thoroughly clean fresh premises chicks show little or no improvement in growth when penicillin is added to an adequate diet. However, in the conditions prevailing in our chick room, where about 1500 chicks are continuously kept in a total area of 750 sq. ft., it is apparent that the ' infection ' is readily established. Throughout the time these experiments were being carried out exceptional care was paid to cleanliness and hygiene. The risk of ' infection ' being brought in from outside was minimized by excluding all but a few attendants who wore clean overalls and disinfected boots while working in the chick rooms. It seems likely therefore that the development of the ' infection ' depends very much on the density of the chick population in a building. Possibly environmental factors such as ventilation also affect the ease with which it is established and some such difference in external conditions might explain why the findings of King & White[5] are at variance with our own.

It is evident that the ' infection ' is not confined to buildings but can also occur in field conditions. It was to be expected that land and equipment once used for ' infected ' birds would transmit ' infection ' to new chicks. It is perhaps more surprising that ' infection ' should appear in equipment that had been thoroughly cleaned and ' rested ' for a short period, particularly equipment in which only ' uninfected ' chicks had been kept. Since in both the field units and the disinfected rooms extremely careful precautions were taken to prevent the ' infection ' from being accidentally brought in from outside, the rapidity with which it became established suggests that the causative agent is widespread and is very likely airborne. It also emphasizes the difficulty of investigating this problem under ordinary conditions.

It was noticeable that both in the rooms and in the field, the weights of the ' infected ' groups were in general lower than those of the ' uninfected ' groups. In our original observations[1] penicillin restored the weights of chicks in the ' infected ' room to the level of those in the ' uninfected ' premises, but possibly the degree of ' infection ' then was less than in the present series of experiments. Throughout the period now under discussion the penicillin treatment, although it improved growth, rarely resulted in chicks as heavy as the best of the ' uninfected ' groups. As successive batches of birds passed through the originally ' uninfected ' premises the general level of weights attained became nearer to that in the ' infected ' premises. This finding suggests that penicillin cannot completely reverse the effects of a heavy ' infection ', even at the very high level used in these experiments. It seems likely therefore that in more practical conditions administration of penicillin may partly mitigate the effects of ' staleness ' of buildings or equipment but not entirely counteract them.

Acknowledgments

We should like to thank Mr. J. P. Fordham and Mr. J. B. Kennedy for care of the chicks.

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THE MODE OF ACTION OF ANTIBIOTICS IN CHICK NUTRITION. III.*—The Nature of the 'Infection' Counteracted by Penicillin

By M. E. COATES and J. W. G. PORTER

Isolation units were designed for the study with chicks of the growth-depressing 'infection' counteracted by penicillin. Preliminary results indicated that the 'infection' could be transmitted by feeding gut contents from 'infected' chicks to newly-hatched chicks. It was not transmitted by similar gut contents after autoclaving or by gut contents from 'uninfected' chicks.

Introduction

It was apparent from the results quoted in the previous paper[1] that no very detailed investigation of the nature of the 'infection' counteracted by penicillin could be made under the conditions prevailing in the chick rooms or in the field. Isolation units were therefore designed for the closer study of its identity.

Experimental

Isolation units

The design of a unit is illustrated in Fig. 1. They were constructed of ¼-in. Perspex and measured 2 ft. 4 in. in length, 1 ft. 10 in. in depth and 1 ft. 2 in. in height. All joints were sealed with Perspex cement and the structure reinforced with brass pins. The dimensions allowed for the accommodation of two wire cages each large enough to contain five chicks. The cages were fitted with ¼-in. wire-mesh screen floors standing over a tray of sawdust. Asbestos heaters (200 watts), measuring 1 ft. 6 in. × 8 in., were fitted at the back of each pair of cages. They were connected through a variable resistance to the electrical supply and by this means the temperature in the boxes was maintained at 95° F for the first day and gradually reduced throughout the time of experiment to about 85° F.

The chief technical difficulty we encountered was the maintenance of an adequate supply of air throughout the system. A workable, though not entirely satisfactory, arrangement was reached by connecting a 1-in.-diameter rubber tube from the front of each unit to an electrically driven vacuum pump. A similar air inlet at the back of each box was connected to a 2-in. rubber hose leading in through a window. Thus clean fresh air was drawn through the system and as the inlets were situated next the heaters the air was warmed before reaching the chicks. The used air was expelled at a long distance from the intake. If the units were made entirely of Perspex, humidity within them became very high, but when the front was replaced by a sheet of plasticized Cellophane 0·003 in. thick, sufficient transpiration occurred to reduce the humidity considerably. There was considerable loss of efficiency throughout the system, but sufficient air change occurred in the boxes to keep the chicks to about 14 days of age without apparent distress. An emergency tube led from each box to a water pump in case of a power failure or mechanical breakdown of the main pump.

The diet was that given in the previous paper.[1] It was supplied in troughs 12 in. long × 4 in. wide with a patent non-scatter device. On most occasions the units had to be opened once during the experiment in order to replenish the diet. Each cage was fitted with a small glass drinking bowl with a constant-level arrangement connected through glass and rubber tubing to an external reservoir.

In all, eight separate units were constructed, thus allowing for four different treatments in duplicate at any one time. The units were arranged in rows along a metal rack so that external conditions such as illumination and ventilation were as uniform as possible. Between each experiment all the equipment inside the units were sterilized by autoclaving at 15 lb. pressure for 30 min. The Perspex boxes were washed in strong Lysol solution and then soaked for 1 h. in a solution of sodium hypochlorite containing 250 p.p.m. of Cl.

* Part II: *J. Sci. Fd Agric.*, 1955, **6**, 419

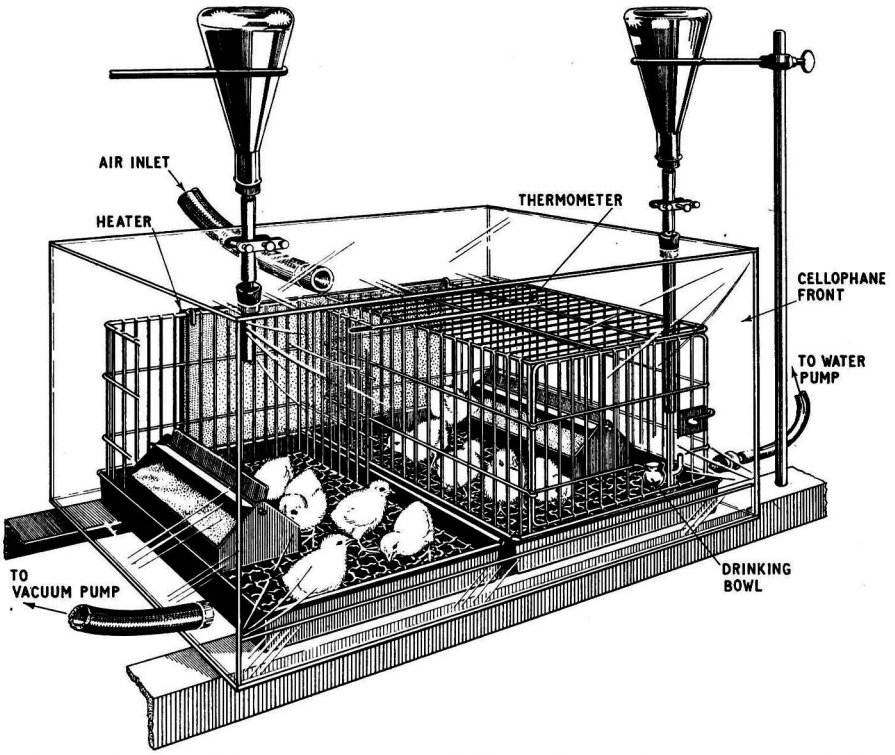

FIG. 1.—*Perspex isolation unit for young chicks. (Part of the left hand cage has been cut away to show detail of the interior)*

Immediately on arrival from the hatchery, day-old chicks were sorted into equal weight groups and allotted to the cages inside the units. The food was distributed so that one cage in each unit had unsupplemented diet and one the diet with penicillin. The two units chosen for controls were sealed with Cellophane before any material suspected of being ' infectious ' was brought near. The remaining groups were then treated and sealed in ascending order of expected ' infectivity '. When it became necessary to replenish the food during an experiment the units were opened in the same order. It was not practicable to carry the experiments beyond the 12th or 14th day as the difficulties of disposal of droppings and of supply of food could not be overcome without frequent opening of the units. To minimize experimental error due to the inevitably small weight differences and the small number of birds each experiment was repeated several times. Occasionally results for one box had to be excluded when during an experiment it became obvious by the condensation inside that some blockage of the air tubes had occurred.

Transmission of ' infection ' by means of gut contents

As antibiotics exert their maximal growth effect when given by mouth it seemed likely that the ' infection ' described by us might be present in the gut and transmissible by means of gut contents. Gut contents rather than droppings were chosen so that any bacteriological investigation arising from these studies might not be complicated by the results of microbial activity occurring in droppings if not collected immediately after voiding. Guts were removed from freshly-killed birds and the entire contents from the crop down to and including the caeca were squeezed out and stirred thoroughly. They were mixed with a small quantity of diet and scattered on top of the food at the rate of about 25 g. of gut contents per cage of five birds. Table I shows the results of several experiments when gut contents from ' infected ' chicks were administered as described above.

Table I

Effect of penicillin on chicks in isolation boxes given gut contents from 'infected' birds. Mean weights in g. at 12 days of age of groups of five chicks

No. of groups per treatment	Control chicks		Chicks given whole gut contents	
	Without penicillin	With penicillin	Without penicillin	With penicillin
1	100	97	77	95
2	—	—	89	114
2	107	109	—	—
2	107	112	92	101
2	109	118	85	101
3	106	109	79	84
2	99	106	80	88
Mean	105	110	84	97

The effect of feeding gut contents from chicks showing or failing to show signs of the infection was also investigated with the results shown in Table II.

Table II

Effect of penicillin on chicks in isolation boxes given gut contents from 'infected' and 'uninfected' birds. Mean weights in g. at 12 days of age of groups of five chicks

No. of groups per treatment	Control chicks		Chicks given whole gut contents			
			From 'uninfected' birds		From 'infected' birds	
	Without penicillin	With penicillin	Without penicillin	With penicillin	Without penicillin	With penicillin
1	99	115	117	109	103	129
1	—	—	97	98	77	88
2	112	115	101	101	79	88
1	113	119	110	108	—	—
Mean	108	116	106	104	85	102

From the foregoing experiments there appeared to be a growth-depressing factor in the gut contents of 'infected' birds which was absent from the gut contents of chicks not showing signs of 'infection'. The effect of autoclaving gut contents was next tried. In order to minimize the possibility of any part of the material escaping treatment it was packed in ½-in. layers in 4-in. diameter stainless steel bowls and a pressure of at least 30 lb./sq. in. was maintained in the autoclave for 1 h. On two occasions the sample was tested bacteriologically after autoclaving and found to be sterile. Table III shows the effect of feeding the autoclaved gut contents to chicks in the isolation units.

Table III

Effect of penicillin on chicks in isolation boxes given autoclaved gut contents from 'infected' chicks. Mean weights in g. at 12 days of age of groups of five chicks

No. of groups per treatment	Control chicks		Chicks given whole gut contents from 'infected' chicks			
			Autoclaved		Not autoclaved	
	Without penicillin	With penicillin	Without penicillin	With penicillin	Without penicillin	With penicillin
2	104	101	114	107	—	—
2	114	104	120	123	—	—
2	109	119	116	122	85	101
3	106	109	103	105	79	84
2	99	106	96	99	80	88
Mean	106	108	110	111	81	91

Discussion

The work reported here has been in progress for over 2 years and it has become apparent that the full solution of this problem is likely to take a very long time. The ease with which the 'infection' is evidently established has resulted in considerable technical difficulties which we are

attempting to resolve. In the meantime this account is presented only as a preliminary report indicating the lines on which we are tackling the problem of the nature of the ' infection ' counteracted by penicillin. No statistical analysis of the data has been attempted and any conclusions drawn must necessarily be very tentative.

The results so far support our suggestion that the growth-promoting action of penicillin added to a diet complete in all known essential nutrients may be due to the suppression of an unidentified ' infection '. It appears that the agent responsible for the depression of growth counteracted by penicillin is present in gut contents of ' infected ' chicks and can be transmitted orally to new chicks not given penicillin. The infective power is destroyed by autoclaving and is absent from the gut contents of ' uninfected ' chicks. On some occasions the growth depression caused by feeding gut contents was not completely reversed by penicillin. This phenomenon is similar to that in heavily ' infected ' chick rooms or fold units[1] and may have occurred because the quantity of ' infective ' material given was too large. Before proceeding further with this investigation it has become necessary to improve the design of the units since experiments have frequently been invalidated by chance contamination, probably because of the close proximity of other chicks. The ease with which ' infection ' of the units can occur, coupled with the fact that the infective power of the gut contents is destroyed by autoclaving, suggests that the responsible agent is a living organism or group of organisms. The possibility that it is a heat-labile toxic material seems unlikely because of the ready transmissibility of the condition. Work is in hand on improving the technique, and further discussion of this problem must be reserved until more results are available.

Acknowledgments

We should like to record our gratitude to our colleague Mr. S. F. Suffolk and other members of the Engineering Department of this Institute for the invaluable help they have given us in the construction and maintenance of the isolation units, to Mr. C. Machin for preparation of the figure, and to Dr. S. K. Kon for his help and encouragement throughout the work.

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MOISTURE EQUILIBRIUM OF PALM KERNELS

By B. SOMADE

Determinations have been made of the moisture contents of whole palm kernels kept for times up to 35 days in desiccators over saturated salt solutions for which the relative humidities at 30° are known. The temperature fluctuated between 24° and 31°.

Introduction

The moisture content of palm kernels has an important bearing on their keeping qualities. If the kernels have a high moisture content they become mouldy rapidly and also heat up with attendant risk of spontaneous ignition, several occurrences of which have been recorded :[1] if too dry some oil exudes, especially at a low relative humidity and at temperatures above 24°.

Information was required concerning the hygroscopic equilibrium and the rate of its attainment under normal storage temperatures encountered in the tropics. In the tests made, no attempt was made to keep the temperature constant during the experiment. Maximum and minimum daily readings of the temperature during the period were taken, however, and these ranged from 76° F to 88° F.

A preliminary investigation to determine the moisture content of whole palm kernels was carried out. Whole kernels and sliced kernels were heated in an air oven at 102° ± 1° for 2, 3, 5, 7, 12 and 18 hours. It was found that the weight remained constant at ± 0·001 g. after 5 hours for sliced kernels, while for whole kernels constant weight was obtained after 18 hours; the difference between the moisture content of whole kernels and sliced kernels being less than 0·005%. It was therefore decided to determine the moisture content during the experiment by heating the sliced nuts for 5 hours at 102°.

Experimental

Portions of the kernels with previously determined moisture content of 8·9% were put in Petri dishes which were stored in large desiccators containing zinc gauze placed over mixtures of saturated salt solutions and excess of salts. At regular intervals, samples of the kernels were taken out, sliced, placed in closed moisture dishes and weighed. The moisture content was then determined. When three successive samples indicated not more than 0·05% change in weight, the kernels were considered to be at equilibrium and the moisture contents were calculated.

To determine the rate at which equilibrium was attained from a low moisture content, the kernels were first heated in an air oven for 3 hours at a temperature of 102°. The moisture content of the sample had then been reduced to 1·3%. The experiment described above was then repeated using this sample.

The salts used and the relative humidities maintained by their saturated solutions at 30° are given in Table II. The values are taken from 'Handbook of Chemistry and Physics', 32nd edn., and 'Handbook of Chemistry' by Lange.

Results

Table I

Moisture content of palm kernels at different relative humidities

R.H.	22·5%		30%		43·7%		53·7%		68·6%		75·4%		81·1%		92·5%	
Days	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
0	8·9	1·3	8·9	1·3	8·9	1·3	8·9	1·3	8·9	1·3	8·9	1·3	8·9	1·3	8·9	1·3
7	3·4	1·7	3·8	1·8	5	2·1	5·3	2·7	6·4	3·8	7·9	4	8·5	4·4	11·8	6·7
14	3·1	1·9	3·4	2·0	4·6	2·3	4·9	3·1	6·0	4·1	7·5	5·1	8·2	6·0	12·2	9·8
21	2·8	2·0	3·3	2·2	4·6	2·8	4·9	3·3	5·8	4·6	6·7	5·6	8·1	6·5	12·4	10·5
28	2·2	2·0	3·3	2·6	4·3	3·3	4·8	3·9	5·6	4·9	6·5	5·9	7·9	6·9	12·5	11·8
35	2·0	2·0	3·2	2·5	4·3	3·3	4·7	3·9	5·6	4·9	6·5	5·9	7·9	6·9	12·6	11·9

H = Samples with initial moisture content of 8·9%. L = Samples with initial moisture content of 1·3%.

Table II

Relative humidity of air above saturated salt solution at 30°

Relative humidity, %	Solid phase
92·5	$NH_4H_2PO_4$
81·1	$(NH_4)_2SO_4$
75·4	NaCl
68·6	NH_4Cl and KNO_3
64·4	$NaNO_2$
53·7	$Na_2Cr_2O_7$
43·7	K_2CO_3
30·0	$CaCl_2,6H_2O$
22·5	Potassium acetate

Discussion

It was observed that after 6 days, kernels having a moisture content of 8·9% became mouldy at relative humidity of 92·5%. At relative humidities of 64·4% and below, oil exuded from the kernels in increasing amount as the relative humidity decreased.

Whole kernels having an initial moisture content of 8·9% attained equilibrium in 28 days, while those having the lower initial moisture content of 1·3% took 21 days. The rate of absorption and dehydration depends on the difference between the actual moisture content and the moisture content at which equilibrium is finally attained.

Samples having an initial moisture content of 8·9% maintained at 92·5% R.H. did not attain equilibrium on account of mould growth. The average relative humidity of the palm kernel area in Nigeria is approximately 82% for the 4 months June–September and approximately 76% for October to May, corresponding roughly to the wet and dry seasons, respectively. The Department of Marketing and Exports (Produce Section) stipulates a moisture content of not greater than 9% for commercial palm kernels which is close to the hygroscopic equilibrium for palm kernels at the average diurnal temperature and relative humidity at which they are stored.

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THE VACUUM CONTACT-PLATE DEHYDRATION OF FOODSTUFFS. I.*—A First Appraisal

By E. G. B. GOODING and E. J. ROLFE

Factors which must be considered in the drying of foodstuffs are discussed. The conventional hot-air drying and the vacuum contact-plate drying methods are described and compared. The latter yields better dried products than the former, both in appearance and palatability of the reconstituted, cooked material, and there is some evidence that the storage properties are also improved. Relevant factors are the absence of oxygen and the milder heat treatment of the foodstuff during vacuum contact-plate dehydration. This technique is versatile and may be used to dry a wide range of commodities, many of which cannot be dried satisfactorily by conventional methods.

Introduction

An ideal dehydrated food would have the appearance, palatability and nutritional quality of the freshly prepared food as usually cooked and served, and would reconstitute rapidly when water is added ; it would also have a long storage life under a wide range of climatic conditions, a high packaging density, low processing losses and economy in manufacture.

During and since the Second World War there have been substantial developments in food dehydration, and food preserved in this way can be made into attractive and highly acceptable dishes, but it has not yet been possible to combine all the properties of the ideal in a single dehydrated foodstuff. Both pre-treatment and drying methods are important in achieving these ends, and in this paper some considerations will be given to drying methods and their effects on the ideals listed above.

Hot-air drying requires the material undergoing dehydration to be in a relatively finely divided form—purées, minces, shreds, or strips or dice of small dimensions. For example, contracts for the Services stipulate that root vegetables shall be cut into strips of cross-section dimensions $\frac{3}{16}$ in. × $\frac{5}{16}$ in., which is the largest size that will dry and reconstitute satisfactorily.

* Read at a Joint Conference of the Agriculture and Food Groups and Aberdeen Section, 30 September–2 October, 1954.

This size factor severely limits the application of air drying and means that the product will not necessarily conform with the ideal of being closely similar in appearance to the original material as usually eaten. Larger particles of tissue cannot be dried satisfactorily because of the reduction of evaporative surface per unit weight and the lengthening of the path for water from the centre of the material to the evaporative surface. Both factors greatly prolong the drying time.

Experiment has shown that, generally speaking, when portions of larger cross-section are dried by hot air, a hard product is obtained which is only very slowly penetrated by water during reconstitution, and usually, even after very prolonged soaking, does not reconstitute adequately.

In the particular case of meat, further factors have to be considered. Not only is drying limited to minced tissues, but it is necessary to cook the material, since raw meat case-hardens during drying and this involves a considerable extension of the drying time, while, moreover, the product from raw meat does not reconstitute well and is tough and fibrous when cooked. At high fat contents the rate of drying of cooked mince is retarded, while dehydrated meat of low fat content is dry in texture. The best balance between these opposing factors is obtained with a product containing 30% fat, calling for skilled trimming of the raw meat. Higher fat contents are best achieved by incorporating rendered fats after drying. Pork fat is less stable than that of beef, and pork mince dried in hot air was very often rancid, although recent work has shown that the careful feeding of the pigs to be used for dehydration can overcome this difficulty. Oxidation during air drying has presented problems in the dehydration of vegetables as well as in that of meats. For example, there is evidence that oxidative deterioration of carotenoid pigments takes place in tomato during air drying, and the ascorbic acid loss suffered by vegetables during air drying is also probably due to oxidation.

Clearly then, in connexion with appearance, palatability and nutritional quality, much can be said for a drying process which avoids the passage of heated air over the foodstuff. Vacuum drying seemed the obvious approach, but unfortunately it had always proved impracticable for material other than thin films of liquid or purées, because of the very slow transfer of heat into other types of material undergoing dehydration. It was a marked advance, therefore, when vacuum dehydration between movable pairs of heated plates was devised—especially as it was claimed that this technique allowed the dehydration of large portions of foodstuffs. Experience during the past few years has shown that this method does, in fact, justify many of the hopes which have been associated with the idea of vacuum dehydration, and the following paragraphs give a brief account of some of the observations and comparisons made on experimentally prepared hot-air-dried and vacuum contact-plate-dried dehydrated foods.

Experimental

In the Ministry of Food Experimental Factory at Aberdeen there are hot-air dryers working on the conventional principle and also a new system referred to as the vacuum contact-plate process.

For the drying of vegetables, the plant consists of three drying cabinets forming in effect a three-stage system. The first cabinet holds one pair of trolleys, each with 56 trays of 32 in. × 16 in. ($3\frac{1}{2}$ sq. ft.). Two pairs of trolleys form one batch of material. The second and third cabinets each hold four pairs of trolleys (four batches). The usual loading is about 5 lb. of potato strips per tray, equivalent to a batch weight of about 560 lb. of scalded vegetable. Air flow is across the trays and in the first cabinet is about 850 ft./min., and about 800 ft./min. in the other two. Each batch is dried in succession in each cabinet. A typical drying cycle for potato would be 35 minutes in Cabinet I at 99–100° dry-bulb and approximately 57° wet-bulb, 2 h. 20 min. in Cabinet II at 74° dry-bulb and 50° wet-bulb, and 2 h. 20 min. in Cabinet III at 63° dry-bulb and 30° wet-bulb.

The hot-air dryer used for meat is also a cross-flow type, but has a smaller capacity than the vegetable dryers. It has an output of 100 lb. of product per batch.

The minced cooked meat is loosely spread on to wire mesh trays at the rate of 2 lb./sq. ft. and loaded into the trolley. The trolley is wheeled into the dryer and the doors closed. Hot air is circulated over the trays of meat by means of a fan, using baffles to ensure that the flow of air is uniform over the trays. The air is heated by thermostatically controlled steam coils, and the humidity of the air is regulated by bleeding out air from the system and taking in fresh air from

the outside. As drying is more rapid at the leading edge of the trays, it is necessary to remove the trolley and turn through 180° every hour. Suitable drying conditions are 80° F dry-bulb and 52° F wet-bulb temperatures, reducing after the first hour to 70° F dry-bulb temperature.

The vacuum contact-plate dryer is of novel design and is fully described in another paper.[1] The foodstuff is spread on aluminium trays, each of which is then covered by an aluminium lid, and then sandwiched between movable pairs of heated plates (4 ft. × 8 ft.) contained in a vacuum cabinet. The essential feature is that the plates can be moved together at the will of the operator, thus maintaining thermal contact with both sides of the foodstuff undergoing dehydration as it shrinks during drying.

The operation of the plant is basically similar for all materials. The trays are loaded with up to 100 lb. of the prepared product, the vacuum is applied and dehydration is started with the plates pressing only very lightly on the material and the plate temperature rising quickly from 60° to 100°. Thermocouples in the material undergoing dehydration show the temperatures actually attained in the foodstuff. Shrinkage may occur very rapidly during the early stages of dehydration and the plates are closed at a rate which has been established by experience in each case. The essential points in plate closure are to close rapidly enough for good heat-transfer to be maintained, but not so rapidly as to squash the material and damage its structure or to press the portions of material so closely together that evaporation will be retarded by blocking passages through or between the pieces of the foodstuff. Experience, and an examination of the temperature curves of the material and the vacuum gauges on the cabinet, will indicate whether evaporation is proceeding rapidly or whether additional plate closure is indicated. The maximum temperature which the material can be allowed to reach compatible with good quality is in all cases 60–65° and after a period of drying the plate temperature has to be gradually lowered to this level. The material is removed when all recorded temperatures have been together for about 1 h., when the final moisture contents will be in the range of 3–5%.

Before dehydration, the foodstuffs are given some form of pre-treatment and this operation is of paramount importance if a product of good quality is to be obtained.

Results

An extended series of runs on the various dryers described above has yielded a considerable amount of data, leading to the general indications outlined below.

(*a*) *Appearance, palatability and nutritional quality*

As was expected, the fact that air is not drawn over the material reduces or eliminates oxidation, and in the case of pork the stability of fat is not so critical as for hot-air drying. Similarly, vegetables containing carotenoids (e.g. tomato) can be dried with relatively little oxidative destruction of their pigments, and ascorbic acid retention in cabbage is improved by vacuum dehydration.

In air drying the rate and extent of dehydration is markedly influenced by the distance through which water must travel to an evaporative surface, and though it would be expected that a similar effect would appear in vacuum dehydration, in practice large portions can readily be dehydrated by the latter method—e.g. fish in the form of whole fillets, meat as steaks or chops or cubes, root vegetables in slices or thick strips, and fruit whole or in halves or rings, according to the kind being handled.

The good thermal contact, maintained as the material shrinks during drying, is fundamental in bringing this about, but the mechanism by which water escapes from the interior of large portions of tissue to the evaporative surface is not clear and may be different for different tissues. For example, in potato it seems that steam forming within the tissues under the low atmospheric pressures during dehydration, produces a system of cavities which may be the channels by which the water vapour escapes. These cavities enable vacuum-dried potato to reconstitute much more rapidly than air-dried potato. A similar effect can be produced in fish, meat and carrot depending upon the manipulation of the drying equipment.

Case hardening, which was a disadvantage of hot-air-dried raw meat, does not appear in the product obtained by the vacuum contact-plate dehydration process and there is no difficulty in reconstituting raw meat dried by the latter method. Also the fat content appears to have little

influence on the rate of drying, and dehydrated cooked minces with up to 60% fat have been prepared without difficulty.

It has been found by the use of thermocouples that the temperatures in the foodstuffs are lower during most of the vacuum dehydration period than during hot-air drying: in the early stages of the process the temperature of the tissue may remain as low as 15–20° for a considerable time, and only a short time before the end of the drying period does the temperature rise to that of the adjacent heating plates. This is in marked contrast to conditions during hot-air drying, when the temperature of the material starts at the wet-bulb temperature of the circulating air, 50–60°, and then rises to the dry-bulb temperature, remaining there for the last 3 or 4 hours of a 5- or 6-hour drying period (Figs. 1–3). The consequence is that heat damage during vacuum

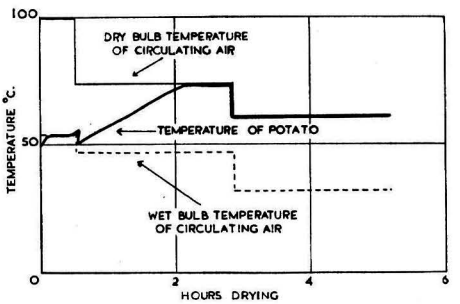

FIG. 1.—*Temperature of potato during drying. Hot-air drying: three-stage, cross-flow dryers. (Strips* $\frac{3}{16} \times \frac{5}{16}$ *in. cross-section)*

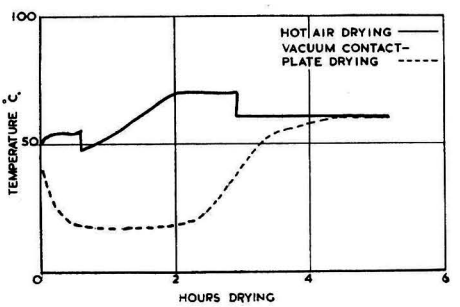

FIG. 2.—*Temperature of potato during drying. Vacuum contact-plate process. (Strips* $\frac{3}{16} \times \frac{5}{16}$ *in. cross-section)*

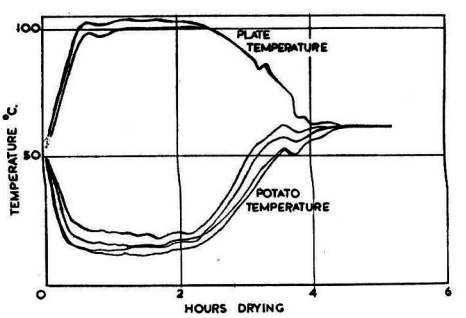

FIG. 3.—*Temperature of potato during drying. Hot-air drying in three-stage cross-flow dryers and vacuum contact-plate. (Strips* $\frac{3}{16} \times \frac{5}{16}$ *in. cross-section)*

contact-plate dehydration is considerably less. In both vegetable and animal tissues heat damage is usually shown by the development of the ' browning ' reaction. In nearly all cases the vacuum-dried product is superior in colour to the corresponding air-dried material. For example, in cabbage the whites remain clear and bright and do not develop the slight dullness characteristic of even the best air-dried cabbage, while the retention of the green colour is better. Vacuum-dehydrated raw meat is obtained as a red material and there is none of the discoloration or dulling associated with cooking, and during reconstitution the water becomes red with blood pigments.

(*b*) *Storage life*

The most serious cause of deterioration in dehydrated foods when stored under tropical temperatures is the non-enzymic browning initiated by a Maillard-type reaction between amino-groups and reducing sugars. At low moisture contents this reaction is greatly retarded.

The drying process influences tropical storage life by the possibility of actually initiating this type of browning, and by its effect on the final moisture content of the product. In hot-air drying the main factor limiting the temperatures which can be used is the danger of causing browning, and even with great cautions in hot-air drying a small amount of browning frequently occurs. There is abundant evidence that material which has suffered browning during drying has a shorter tropical storage life than material in which no browning has appeared.

In vacuum contact-plate dehydration, as already mentioned, the temperatures throughout most of the drying period are lower than during hot-air drying, and browning is rare. This may be expected to have its effect on tropical storage life and accelerated tests on cabbage and on potato have suggested that this effect exists ; further long-term tests are in progress.

As mentioned above, low moisture content is one of the most important factors leading to long storage life of dehydrated foods at elevated temperatures, and the question at once arises of the relative merits of air- and vacuum-drying as methods of attaining low moisture contents. For drying to take place at all the partial pressure of water vapour in the surrounding atmosphere must be less than that exerted by the water in the foodstuff. The concept of relative humidity (R.H. $= p/p_s$ where p is the partial pressure of water vapour in the atmosphere and p_s the saturation pressure of water vapour at the same temperature) has been applied to the problem, and Gane has published tables[2, 3] showing the relative humidity of the atmosphere in equilibrium with a wide range of foods at different moisture contents and temperatures.

The usual finishing temperatures for air drying are 60–65° dry-bulb and 30–33° wet-bulb, equivalent to a relative humidity of about 0·1 : it is evident, therefore, that unless the drying air itself be artificially desiccated, the lowest level of moisture content theoretically attainable with air drying will be that in equilibrium with air of R.H. 0·1.

In the case of vacuum drying, consideration must be given to the value of p/p_s. The absolute pressure, p, is determined by the nature and mechanical efficiency of the vacuum equipment ; p_s is the saturation pressure of water vapour at a given temperature, and can be found from published tables. For 60° (the approximate finishing temperature generally used in vacuum contact-plate dehydration), p_s is approximately 150 mm. Hg.

As stated above, the relationship between R.H. and partial pressures of water vapour is R.H. $= p/p_s$. Hence, for a vacuum dryer to be theoretically equivalent in this connexion to an air dryer working under the conditions discussed above, p/p_s must equal 0·1, or, $p = p_s \times 0{\cdot}1$. Substituting for p_s we have $p = 150 \times 0{\cdot}1 = 15$ mm. Hg. If, therefore, an absolute pressure of 15 mm. Hg can be obtained in the vacuum cabinet towards the end of the drying period, the machine should theoretically be able to dry to moisture contents similar to those attainable with an air dryer. If lower absolute pressures can be obtained, lower moisture contents should be possible.

The above relationship may be used to calculate the absolute pressure theoretically necessary to bring a foodstuff to any required moisture content (provided that sufficient is known of the water relations of the foodstuff), or, if the lowest absolute pressure attainable in the cabinet is known, to calculate the lowest moisture content that could possibly be attained in the foodstuff dried in that cabinet. For example, it is desired to bring potato to a moisture content of 2·0% at a finishing temperature of 60°. From Gane's figures it appears that the equilibrium R.H. is

about 0·04. As mentioned above, the saturation vapour pressure of water at 60° is 150 mm. Hg. Applying the formula R.H. $= p/p_s$ and substituting we have $p = 0{\cdot}04 \times 150 = 6{\cdot}0$ mm. Hg: viz. in order to obtain dehydrated potato of 2% moisture content, an absolute pressure of 6·0 mm. Hg or below in the vacuum cabinet would be necessary.

A few other examples are tabulated below:

Foodstuff	Temp. of finishing °C	Moisture content required %	Maximum absolute pressure, mm. Hg.
Pork	60	7·9*	120
Pork	60	2·5*	15
Beef	60	8·4*	75
Beef	60	2·3*	15
Potato	60	6·7	45
Potato	60	4·0	15
Carrot	60	4·4	45
Carrot	60	1·2	15

*The figures for meat refer to pre-cooked, air-dried material, and moisture contents are expressed as g. of water per 100 g. of fat-free dry weight.

In the large-scale vacuum contact-plate dehydration cabinet, absolute pressures of 2·5–3·0 mm. Hg are easily attainable, and when it is remembered that an absolute pressure of 15 mm. Hg is equivalent to the relative humidity of 0·1, which is approximately that normally attainable by hot-air drying, the theoretical superiority of the vacuum contact-plate process is evident. Further, the above discussion applies to a plant which is free of leaks. If there are leaks (and in practice there usually are), the value of p (water-vapour partial pressure) will be lower than the vacuum gauge reading, and the moisture contents theoretically attainable will be lower than suggested by the gauge reading.

In practice, small-scale experiments on a pilot dryer with plates 2 ft. × 1 ft. holding 5–8 lb. of foodstuff, and attaining a final absolute pressure of 15 mm. Hg, have produced dehydrated vegetables with moisture contents near the theoretical limit—e.g. potato 3·8%, and cabbage 1·9%.† Only two experiments have so far been carried out on the larger plant in which attempts were made to approach the lower theoretical level of moisture, and these have not been successful; the final moisture content of the product (potato) lay between 3·7% and 3·9%, instead of below 2% as might have been expected. The reasons for this failure are not clear and further study, with plant especially constructed for the collection of experimental data, is to be carried on.

(*c*) *Packaging density*

Although only light pressures are applied to the foodstuff by the pairs of heating plates, many substances are produced in a more compact form than by air drying: vegetables, for example, come off as sheets instead of loose shreds or strips, and the sheets have a packing density about twice as high as the loose material. While this is of no advantage in foodstuffs which can be compressed, it will be valuable for those materials for which no satisfactory compression technique can be developed—and there may be such foodstuffs: e.g., it probably will be a very long time before the compression of dehydrated potato is mastered.

(*d*) *Processing losses*

There is little difference in the losses occurring during vacuum contact-plate dehydration and hot-air drying.

(*e*) *Economy*

The labour required for loading and unloading the vacuum contact-plate dryer is less than that for conventional cabinet or tunnel-type air-dryers, but the steam consumption (mainly due

† It is not possible to deduce with any accuracy a figure for cabbage at 60° from Gane's figures, but 1·9 seems to be a fair approximation.

to the steam vacuum augmentor) is higher per lb. of water evaporated and the capital cost of the equipment is greater. Full costing figures are not yet available.

Conclusions

Enough has been said above to show that the vacuum contact-plate dehydration process possesses certain very substantial advantages over air-drying methods when considered in relation to the 'ideal' dehydrated foods.

There is a considerable amount of developmental work yet to be done, but even with present knowledge it is possible to produce a much wider and superior range of dehydrated products than could be prepared by ordinary methods of hot-air drying. The main limitation is the considerable cost of the equipment which must be reflected in the price of the product, although its effect may be small when handling expensive raw materials such as meat and would be minimized by using the plant throughout the year to dehydrate any commodity in season. This is undoubtedly a process of considerable technical interest which, by reason of its versatility, as well as the quality of the product, may well prove to be a commercially feasible proposition.

Ministry of Food Experimental Factory
Aberdeen

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THE VACUUM CONTACT-PLATE DEHYDRATION OF FOODSTUFFS. II.*†—Equipment

By J. M. HAY

On account of the lower temperatures of vaporization at reduced pressures, vacuum evaporators and vacuum dryers have for long been in use for the drying of thermally unstable materials. During the Second World War attempts were made to dehydrate foodstuffs using vacuum-drying equipment of the types then available but, except in the case of finely divided materials, the results were unsatisfactory. The equipment described in this article comprises a large steel vacuum cabinet accommodating 21 trays enabling about a ton of material to be dried per batch. The movable heating plates, arranged in such a manner as to maintain good thermal contact with foodstuff undergoing dehydration, are responsible, more than any other feature of the plant, for the successful drying of meat steaks, fish fillets, sliced root vegetables and certain whole fruits.

Introduction

The dehydration of foodstuffs by the vacuum contact-plate process has been described in the previous paper (see Part I); in the present article further consideration is given to the drying process and the equipment is described in some detail.

Water may be held by any one material in different ways—in interstices between fibrous or

* Part I: *J. Sci. Fd Agric.*, 1955, **6**, 427
† Read at a Joint Conference of the Agriculture and Food Groups and Aberdeen Section, 30 September–2 October, 1954

cellular formations, enclosed within the walls of the cells themselves or in combination with some of the constituents as water of crystallization or hydration. The water may reach the surface at which evaporation is taking place by capillary action or by diffusion. In the drying of most foods the movement of the water to the surface is controlled mainly by diffusion. The whole problem is complicated by the fact that the water present contains sugars, salts and other solids in solution. As the water moves to the surface during drying the solutions in the outer cells become more concentrated. At the same time shrinkage of the material occurs, so that while the moisture gradient falls and the force driving the water to the surface decreases, the resistance in its path continues to increase. Thus drying at low moisture contents becomes progressively more difficult. Very dry material is hygroscopic and will reabsorb moisture, so that there is a limit to the degree of drying that may be effected depending on the temperature and water-vapour pressure of the surrounding atmosphere. This factor is of great importance when attempting to dry down to very low moisture contents.

In the food industry, equipment employing the principle of direct or hot-air drying is widely used and appears in many forms such as rotary dryers of various types, pneumatic dryers for finely divided solids, as well as spray dryers for liquids like milk and eggs. For the dehydration of vegetables and minced cooked meat or fish cross-draught tray dryers of the tunnel and cabinet types and through-draught dryers of the batch and conveyor types are amongst the most commonly used.

Most biological materials, to an extent dependent on their nature, suffer some degree of deterioration when heated above a certain temperature level ; the longer the time of exposure to such elevated temperatures, the greater being the severity of the damage. Fortunately for the success of hot-air drying, the temperature of the material does not greatly exceed the wet-bulb temperature of the air until about half the moisture originally present is removed. During this stage the rate of drying is roughly proportional to the wet-bulb depression of the air. For this reason, provided the relative humidity of the heated air is low, fairly high dry-bulb temperatures may be used in the initial stage of drying without appreciable degradation of the material. As drying progresses, however, the temperature of the material approaches that of the dry bulb and it is then necessary to avoid overheating the material by employing lower circulating air temperatures.

The earlier stage of drying, when moisture is reaching the surface of the material fairly freely, is generally referred to as the constant-rate period, and this is succeeded by the falling-rate period when water finds its way to the surface only with considerable difficulty. The moisture content at the rather indefinite point of transition between the two phases of drying is known as the critical moisture content. There is some doubt as to whether or not the water ultimately may find its way to the surface in the vapour state when low moisture contents are reached : if this is so, then we must assume three stages of drying.

In order to ensure reasonably rapid drying rates for foods by the hot-air method, dry-bulb air temperatures in the order of 100° to 120° or more may be used in the first stage, but, to avoid scorching of the material it is seldom advisable to exceed 65° in the final stage. Even these temperatures, applied as they are for several hours during the process, are sufficient to denature some of the more heat-sensitive substances in the material and such prolonged heating in the presence of oxygen may induce rancidity of certain fatty constituents and initiate other longer term changes affecting storage life.

Vacuum drying

On account of the lower temperatures of vaporization of water, as well as because of the diminished oxygen concentration of the ambient atmosphere at reduced pressures, and to avoid some of the inherent drawbacks of the hot-air process, vacuum dryers and vacuum evaporators of various types have for long been in use for the drying of thermally unstable materials, including a number of foodstuffs. Rotary, pan and plate vacuum dryers, designed in most cases primarily for other purposes, were used experimentally during the war for the dehydration of cooked minced or diced meat and other foods with some success, but efforts to dry larger portions of raw material such as meat or vegetable slices or fish fillets were not so encouraging.

In recent years the processing of such labile substances as penicillin, streptomycin and blood

plasma has given an impetus to drying by sublimation of ice in the frozen material under high-vacuum conditions, care being taken to supply just sufficient latent heat for the process to avoid melting the ice. This method has since been employed in the dehydration of meat steaks and other foods, giving a very porous, and, consequently, rapidly reconstituting product which retains to a marked degree the colour, appearance, texture and nutritive qualities of the original material. The volume undergoes little change during this process of drying.

Vacuum contact-plate dehydration plant

The type of vacuum drying equipment described in this paper gives in less time a denser product, which otherwise approximates to that obtained by freeze drying, but without the attendant complications and costs of refrigeration and extremely low pressures. Indeed, by modern standards in vacuum engineering, it might be more accurate to refer to the process as drying at reduced pressure. During dehydration the absolute pressure in the drying cabinet falls gradually from around 13 mm. Hg at the commencement of the process to about 2 mm. Hg as the moisture content decreases and the rate of evaporation slows down. Throughout the greater part of the drying period the temperature of the material is normally between 30° and 40° rising to about 60° only at the end of the process. These temperatures, which are measured by thermocouples inserted at several points in the material and automatically recorded, are easily controlled, either by altering the steam supply to the heater or by manipulating the plate pressure, and may, if so desired, be kept much lower than the values stated above, but at the expense of a longer drying period.

For the investigations in vacuum drying now in progress at the Experimental Factory in Aberdeen, the equipment* comprises a robust cabinet of fabricated steel construction (Fig. 1), roughly rectangular in form, and measuring internally 8 ft. 9 in. in length, 5 ft. 6 in. in width and

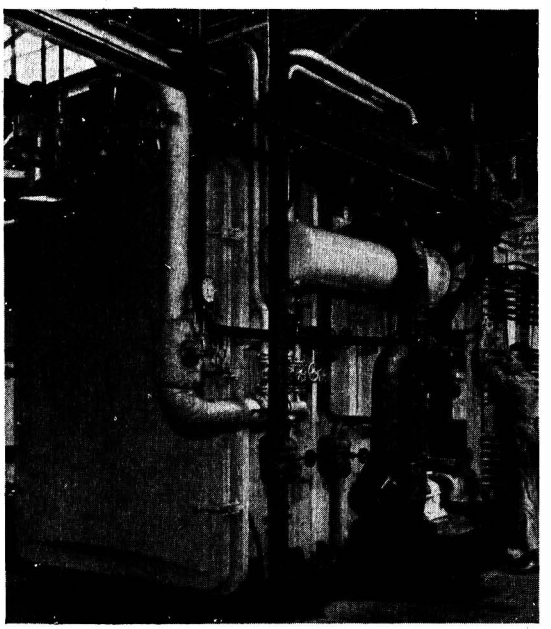

FIG. 1.—*Heater side of vacuum dehydration cabinet*

The heater at top of picture is painted a dark colour: the light coloured cylindrical vessel below it is the cooler. The four-way cock for reversing flow through heating plates is on the right: behind it, attendant is operating lever for the two three-way cocks used for changing ever to cooler

* This system is covered by a patent held by A/S Atlas Maskinfabrik, Copenhagen.

8 ft. 4 in. in height. The ends are closed by large doors rendered vacuum tight by rubber seals engaging on the flanged faces of the cabinet. As these doors are each over a ton in weight they are suspended from trolleys running on overhead rails to facilitate opening and closing when cleaning or charging the plant. The shell of the cabinet is substantially ribbed externally, and the doors internally, to withstand the pressure of the atmosphere with a minimum of distortion, the load on each side being of the order of 66 tons when the plant is in operation.

Heating system

Probably the most outstanding feature of the equipment is the arrangement of the 24 horizontal mild steel heating plates, which are grouped in three banks, one above the other, with eight plates per bank, each bank thus accommodating seven trays of material (Fig. 2). These plates measure, as do the aluminium alloy trays and lids, 8 ft. in length by 4 ft. in width. The plate spacing is such that the layer of foodstuff on the trays may be initially up to 1½ in. in thickness: this allows batches of as much as 1 ton of raw material to be processed per run. The plates are suspended by side links, through short connecting rods, from hydraulically actuated double-armed levers—two on either side—mounted in extension boxes on top of the cabinet. Large vacuum-tight glands are provided at the four points where the lever shafts enter the extension boxes. In each bank, the four lower plates and the four upper plates are connected on opposite sides of the levers, and at distances from the fulcra so proportioned that the plates more remote from the horizontal central plane of the bank move towards each other faster than the others, in such a manner that the spacing between adjacent plates diminishes by the same amount for a given angular movement of the levers. Thus, as shrinkage occurs during drying, satisfactory

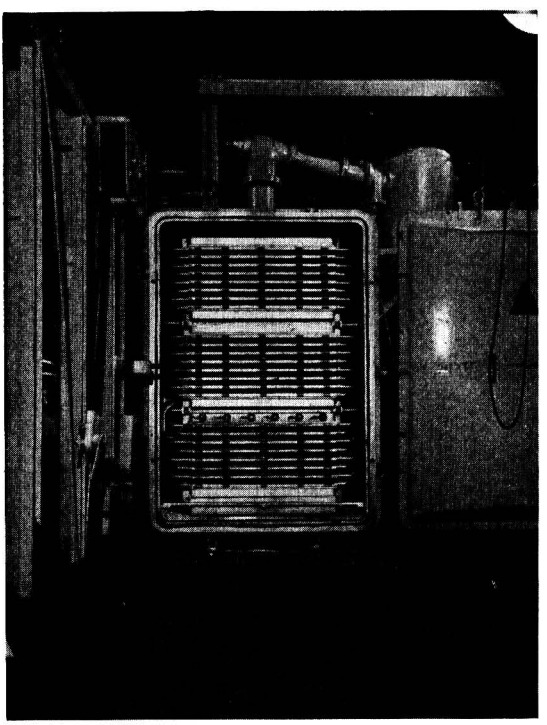

FIG. 2.—*Loading end of cabinet*

Door has been moved to right to show arrangement of heating plates. The rack-car is on the left. On top of the cabinet the smaller of the two steam-jet vacuum augmentors can be seen with its discharge pipe leading to the condenser at the top right-hand corner

heat transfer by conduction from plates to material is ensured throughout the process. This feature is of prime importance in vacuum drying when the heating medium is at only moderate temperatures, because very little heat is imparted to the material by radiation and none by convection. The pressure on the material may be gradually increased from zero at the beginning to about 1¾ lb./sq. in. at the end of the dehydration period. In the case of many foods, however, it is desirable only to move the plates together sufficiently to follow up the shrinkage without applying appreciable pressure on the material : this gives a product of open texture, thus reducing the time required for reconstitution.

The flat, hollow, mild steel heating plates through which water is circulated from a shell-and-tube steam heater, are about 1¼ in. thick. They are ribbed internally with dividing plates forming a 12-path labyrinth in each to give good temperature distribution over the entire upper and lower surfaces. These ribs also impart to the plates the requisite strength and rigidity to withstand the loads imposed in normal usage. It would be impracticable, however, to make the plates strong enough to withstand pressures on one side only without some buckling. In practice, therefore, it is necessary to operate the plant with any one or more of the banks completely filled, that is at ⅓, ⅔ or full load. The top and bottom heating plates in each group are backed by heavily ribbed reinforcing plates to provide the necessary support.

The inlet and outlet water connexions to the plates are made by flexible metallic tubing from semicircular-section ducts welded on either side of the cabinet. The temperature drop of the circulating water between inlet and outlet is about 20° during the warming-up stage, falling to only a degree or so at the end. In order, therefore, to obviate uneven drying, which this temperature difference would otherwise cause, the direction of flow through the plates is reversed periodically by changing over a four-way cock in the external piping. Heating-water temperatures up to 140°, corresponding to a gauge pressure in the system of 38 lb./sq. in., may be used. Any water temperature above 100° of course implies a pressure above that of the atmosphere and for this reason the hot-water system is closed, an expansion tank, normally about half full of water, and fitted with a safety valve, being placed at a short distance above the level of the cabinet to allow for changing volume at different temperatures. Water is forced through the system by a centrifugal pump driven by a 3-h.p. motor.

Mention need now be made of only one other component of the circulating water system, namely, the cooler which is of similar construction to the heater : it is supplied with cold water from one of the ejector pumps. At the end of the process the circulating water flow and return are changed over from heater to cooler by two three-way cocks operated simultaneously by one lever. In routine operation, emergency use of the cooler should never be necessary, but when experimenting with new types of material, and there happens to be an unexpected tendency for the temperature of the substance to rise to an undesirable level, the condition can be controlled, and damage to the material averted, by changing over quickly to the cooler.

Heat transfer from the circulating water to the material is adversely affected by the presence of rust or scale on the heating plates and it is necessary to have the plate surfaces scraped at frequent intervals to ensure constancy of operating conditions. Internal rusting of the plates has been successfully controlled by the addition of sodium nitrite to the water as a corrosion inhibitor to form a 0·3% solution. The volume of water in the system is about 450 gallons and the water is changed as infrequently as possible. One other factor influencing the uniformity of heat transfer is the degree of contact between plates and trays. Care must be taken to see that the trays are kept as flat as possible. Slight irregularities of contact are discounted to some extent by the high heat conductivity of the aluminium used in the construction of the trays.

The vacuum system

The vacuum in the cabinet is produced by two multi-jet, single-stage steam vacuum augmentors working in conjunction with two water ejectors. The water ejectors are of the same size and type, each being supplied with 160 gallons of water per minute at 60 lb./sq. in. gauge pressure by a centrifugal pump absorbing about 15 h.p. Working alone, the water ejectors can reduce the pressure in the cabinet to about 27 mm. Hg absolute, but in practice it is usual to bring one or other of the augmentors into operation after the ejectors have been working for 8 to 10 minutes, by which time the absolute pressure in the system will have been reduced to between

40 and 50 mm. Hg. The ejectors do not evacuate directly from the cabinet but, through non-return valves, from the augmentor condenser, at which point they receive the advantage of the compression afforded by the steam jets of the augmentor. The main object of the ejectors is, of course, to remove the air from the system.

Unlike the ejectors, the augmentors are not equal in size, the one being about twice the capacity of the other. The ideal method of operating them would be to use both augmentors at the beginning of the process when evaporation is rapid, leaving the larger one alone to maintain the vacuum throughout the greater part of the run, then finally bringing the smaller again into operation and shutting down the larger during the remaining stage. The normal procedure, however, adopted in the Factory is to use only the larger augmentor throughout the run for full load and the smaller one for light load operations. It is unfortunate that the large augmentor uses steam at the rate of about 1200 lb. per hour, and the small one about half that amount, but it is difficult to suggest any equally convenient alternative means of achieving a vacuum in the cabinet of the order of 12 down to 2 mm. Hg now given by these extremely simple devices. A valve is provided at one side of the cabinet to break the vacuum at the end of a run and a safety valve is fitted to prevent any excessive rise of pressure that might result from a fracture at some part of the internal heating system.

Condensation of the augmentor steam and vapour from the material is effected by a spray condenser at the side of the cabinet. The mixture of condensate and cooling water is drawn from the bottom of the condenser vessel by a centrifugal pump driven by a 10-h.p. motor and discharged to a tun dish outside the building, whence it is piped to a still-pond overflowing into the harbour at a temperature little above that of the atmosphere. It should be explained that the water used for condensing the steam, operating the ejectors and supplying the cooler is pumped from the harbour to two large tanks outside the factory and adjacent to the plant. The water for the spray is sucked up by the vacuum in the condenser from one of the tanks, while the water for the ejectors is recirculated through the other tank, only enough additional water being supplied to ensure that at no time does the temperature rise to 20°. To prevent corrosion the pipes used in the salt water circuits are bitumen lined, while the inlets are fitted with wire mesh strainers to avoid choking of the spray or damage to pumps.

Auxiliary equipment

Working at intervals with individual batches of diverse materials, as must of necessity be the case in an experimental factory, it is difficult to assess the manufacturing costs of any product: one example, however, may be cited. In the case of the production of ' vac-packed ' meat bars, it has been estimated that in a small factory, comprising a single vacuum dehydration cabinet capable of turning out 200 tons of the product per annum, the overall manufacturing cost, including rents, depreciation, management, supervision, labour, services, scientific control and packaging, would be less than one-third of the total cost, the raw material accounting for the remaining two-thirds. This cost could be considerably reduced for any material if a battery of, say, from three to six cabinets were in continuous use.

In battery operation a tray-lift and rack-car would be required to reduce labour costs in charging and discharging the cabinets and, while not absolutely essential for a single cabinet plant, such adjuncts are useful in reducing the effort required when inserting and withdrawing the trays. This rack car runs on rails laid in front of the drying cabinet (or cabinets) and is provided with 21 racks fitted with skid bars on to which the loaded trays are automatically slid, one at a time, from the electrically operated tray-lift: each rack is on the same level as the corresponding space between the plates in the cabinet. The trays are then slid simultaneously into the cabinet by a vertical push-bar, which is suspended from an overhead rail on the car. At the end remote from the cabinet this rail is carried round so that when trays are being slid on to the racks from the tray-lift the push-bar can be moved to the side, and brought back to a central position behind the trays when they are to be pushed into the cabinet. The push-bar engages with chains above and below the racks, the chains being operated manually through suitable gearing from cranked handles on either side of the car. For the withdrawal of the trays from the cabinet the push-bar is fitted with a clamping device which can be made to lock on lugs welded to the front edges of the trays. The trays are withdrawn individually from the rack-car on to the

hoist platform of the tray-lift by an electrically operated chain and pawl mechanism and finally returned to the loading table which is used prior to dehydration for spreading the raw material on the trays.

The plant at the Experimental Factory was used in the first instance mainly on the drying of fish fillets, and it was not long before it was confirmed that variations in the thickness of the layers caused wide discrepancies in the final moisture content at different points in the material, the thinner portions drying more slowly. Trouble due to this cause has been largely overcome by the use of a machine (Fig. 3) developed to cut oversize fillets down to a uniform maximum thickness. This flat cutting machine as it has been called, comprises a serrated rubber belt on which the fillets are placed : the fillets are carried by the belt under a band knife and are held in position against the drag of the knife at the point of cutting by sharp-edged rollers which can rise and fall individually in conformity with the contours of the fish. The thin slices from the tops of the fillets can be used to make up the thickness of the layers at the tail portions.

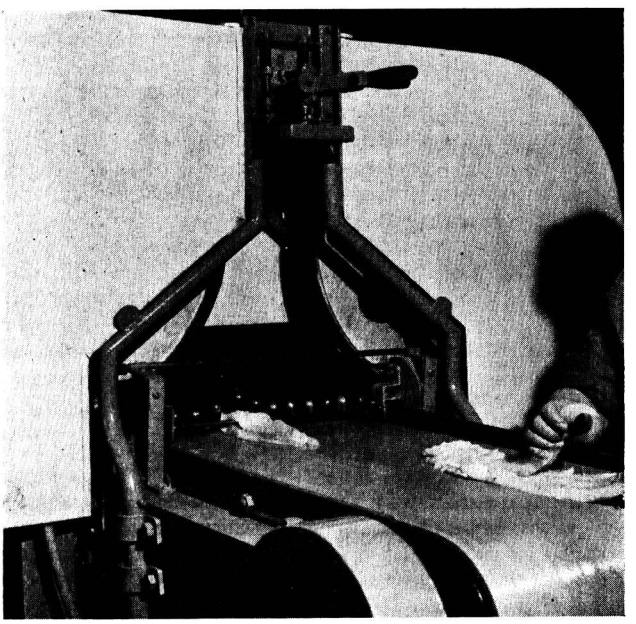

FIG. 3.—*Flat cutting machine*

Part of the guard over the band knife has been removed to show rollers which hold the fillet against knife. The operative at the discharge end of the belt is lifting a top slice which represents excess thickness of fillet

In the production of, so called, pressfish blocks drying is done in two stages. The fillets are first dried down to about 15% moisture content. The layer of material is then removed from each tray as a coherent mat and cut by a circular saw into portions measuring 16 in. × 15 in., these portions being afterwards left for two or three days to equilibrate in moisture content in a homogenizing room set aside for this purpose. At 15% moisture content the material is sufficiently plastic to withstand compression without undue fragmentation. The hydraulic compression plant (Fig. 4) comprises three presses : at one end an up-stroke press exerting 15 tons is used for dealing with trimmings from the edges of the mats : the central press is really a combination of two presses, a 5-ton down-stroking press and a 30-ton up-stroking press, the former being used in charging the mould and the latter in ejecting the compressed material : the main pressing operation is carried out by the 800-ton up-stroking press at the other end of the plant. In charging the press six layers of fillets are placed in the bottom of the mould, then a chromium-plated, separating plate is inserted, then another six portions of fillet mat, a separating plate, and

so on until, after intermittent pressing by the quick-acting charging press, the mould is fully loaded, when it is pushed along guide rails on its bogey wheels to the main press where the full pressure of 800 tons is applied for a dwell period of 10 minutes. The 1-in. thick, 16-in. × 15-in. slabs of compressed fillets ejected after this treatment are then cut into blocks of a size suitable for packaging and these are once more put in the vacuum dryer to bring the moisture content below 5%. Unfortunately, although these blocks present the nutritive constituents of fish in the most concentrated form obtainable, they take rather long to reconstitute and further investigation will be required to overcome this drawback.

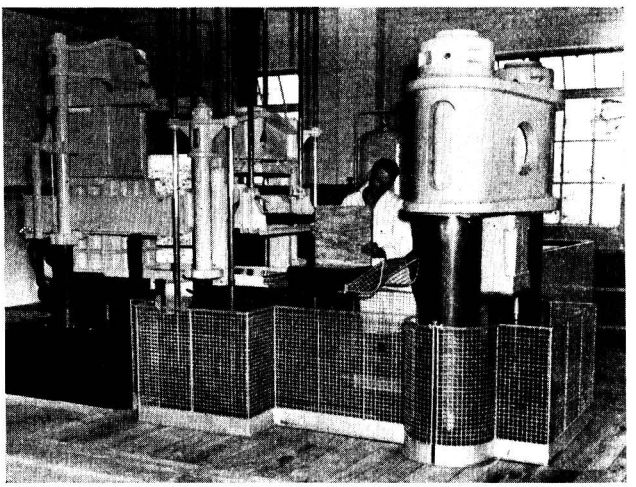

FIG. 4.—*Hydraulic fillet compression plant*

The press for trimmings is on the left. The mould on its bogey wheels is in position at the centre press for charging or ejecting. On the right the 800-ton main press

Pilot equipment

Much useful work of an exploratory nature has been done at the Experimental Factory using a small pilot vacuum contact drying cabinet in which one tray between two heating plates, measuring 28 in. × 14 in., is used to dry up to about 10 lb. of raw material. Difficulties have been encountered, however, in applying to the commercial-sized plant procedures that have been developed from the small-scale plant. The uncertain effects of the considerable degree of friction in the sealing glands and other parts of the plate operating mechanism, together with the large difference in the mean lengths of vapour paths in the two cases, give rise to somewhat intractable discrepancies in the scale effect. For these reasons, as well as to obtain precise information on the vacuum dehydration process, a new pilot cabinet has been designed and is at present in course of construction in the Factory workshop. There will be two reinforced heating plates to take one tray measuring 4 ft. × 4 ft. and provision is being made for the direct measurement of the principal variables involved in the process.

Many problems in vacuum contact dehydration of foodstuffs still remain to be solved, and it is hoped that this new pilot cabinet will enable much more information to be gained on heat transfer and the effects of changes in the time–temperature–pressure cycles than is possible with either the main plant or the existing small-scale cabinet. Even at the present stage of development, however, products can be obtained with the vacuum contact-plate dehydration process that are markedly superior to those given by normal conventional methods.

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COLORIMETRIC ANALYSIS OF *p*-CHLOROBENZYL *p*-CHLOROPHENYL SULPHIDE (CHLORBENSIDE) RESIDUES IN PLANT AND ANIMAL TISSUE

By D. J. HIGGONS and D. W. KILBEY

A method is described for the colorimetric analysis of residues of *p*-chlorobenzyl *p*-chlorophenyl sulphide on leaf and fruit samples and in animal tissue. The method is based on pre-oxidation of the spray residues followed by intensive nitration of the resulting *p*-chlorobenzyl *p*-chlorophenyl sulphone, and the formation of a purple colour on interaction of the nitrated products in benzene with sodium methylate.

The method is sensitive to 0·05 mg. and is specific in the presence of all likely contaminants except DDT.

Introduction

In a previous publication[1] announcement was made of the discovery of outstanding biological properties in *p*-chlorobenzyl *p*-chlorophenyl sulphide (recommended common name—Chlorbenside) for the control of Tetranychid mites and this was followed[2] by a brief résumé of potential colorimetric reactions for this substance. As outlined in the latter paper, Chlorbenside itself is a rather inert substance and it has not yet been possible to find any satisfactory reaction capable of development into a practicable microanalytical technique. Work on the sulphone of Chlorbenside, however, has brought to light two potential colorimetric reactions: (*a*) intensive nitration to form the trinitro derivative followed by reaction with sodium methylate in benzene; (*b*) condensations on the active methylene group.

As the trinitro derivative of Chlorbenside sulphone has properties similar to the tetranitro derivative of DDT reported by Schechter *et al.*,[3] this reaction presented the most rapid approach to the development of a colorimetric procedure.

The method presented in this paper depends on the pre-oxidation of spray deposits of Chlorbenside to its corresponding sulphone with hydrogen peroxide in glacial acetic acid. This reaction is quantitative as long as reaction conditions are not too severe. The resulting residue of Chlorbenside sulphone can then readily be nitrated in the presence of extraneous plant extract to the corresponding trinitro derivative. The isolation and development of the colour of this derivative then proceeds along lines very similar to those reported by Schechter *et al.*[3] for DDT.

As briefly reported previously,[1] there is some evidence that spray residues of Chlorbenside may be slowly oxidized on leaf surfaces to either the sulphoxide or even sulphone. The analytical method developed will, of course, report residues as a total of Chlorbenside, the sulphoxide and the sulphone. However, the biological and toxicological properties of the two oxidation products are very similar to those of Chlorbenside, so for practical purposes the total residue is the figure required.

Separation techniques for Chlorbenside and its sulphoxide and sulphone are being worked out to make it possible to adapt the colorimetric procedure to a study of the kinetics of oxidation on the leaf surface: this work will form the basis of a separate communication.

Experimental

Standard Chlorbenside solutions

0·1 g. of recrystallized Chlorbenside is dissolved in acetone and diluted in 100 ml.; 5 ml. of this solution are diluted in 100 ml. with acetone. 1 ml. of dilute solution contains 0·05 mg. of Chlorbenside.

Preparation of standard curve

Aliquots of the standard solution containing 0·05–0·5 mg. of Chlorbenside are pipetted into 150-ml. beakers. Two ml. of a 2·5% solution of oleic acid in benzene are added and the volume is reduced to 5 ml. on a hot-plate. Five ml. of glacial acetic acid are added followed by 3 drops of 100-vol. hydrogen peroxide. After setting aside for 5 minutes, the beaker is returned to the hot-plate and the evaporation allowed to proceed slowly until the volume is reduced to 1–2 ml.

A further 2 ml. of glacial acetic acid are added followed by 2 drops of 100-vol. hydrogen peroxide and the evaporation continued to dryness.

The residue in the beaker is transferred to a boiling tube with small amounts of acetone, a small glass bead is added, and the acetone is evaporated in a boiling water-bath. Any residue left on the side of the tube is washed down with a small volume of acetone and the evaporation is completed, last traces of acetone being removed in an air current. Five ml. of nitrating acid (equal volumes of fuming HNO_3 and concentrated H_2SO_4) are added and the tube is returned to the boiling water-bath for exactly 1 hour. After cooling, 15 ml. of distilled water are added to stop the reaction.

The contents of the tube are transferred quantitatively through a small funnel into a 150-ml. separating funnel with three 15-ml. portions of distilled water followed by three 15-ml. portions of ether. The funnel is shaken well and, after separating, the lower layer is run off and discarded. The upper ether layer is washed with 10-ml. portions of 2% sodium hydroxide solution until the washings are alkaline, and finally with two 10-ml. portions of saturated sodium chloride solution. It is then filtered through a plug of cotton wool in a Gooch crucible funnel into a 150-ml. spoutless beaker using three 10-ml. aliquots of ether for washing. The ether is evaporated on a hot-plate and the last traces removed with an air current.

Five ml. of dry benzene are added, the beaker covered with a watch-glass and gently swirled until the residue has dissolved. Two ml. of this solution are then pipetted into a Biochem Absorptiometer test tube, 4 ml. of sodium methylate reagent* are added and, after mixing, the tube placed in the instrument which has been previously set to zero with water using a Chance glass OG1 filter. The peak reading obtained after 1–2 minutes is recorded.

Treatment of leaf samples

50–200 leaves are collected and one disc is taken from each leaf with a No. 12 cork borer. The discs are placed in a 500-ml. Q.F. conical flask, 100 ml. benzene are added and the flask is shaken periodically over half an hour. The benzene extract is filtered through a No. 1 Whatman filter paper and a 50-ml. aliquot is transferred to a 150-ml. beaker, 1 ml. of oleic acid solution (2·5% pure acid in benzene) is added and the volume reduced to 5 ml. as above.

Treatment of fruit

A suitable quantity of fruit (about 500 g. of apples or pears) is weighed into a 7-lb. jar, 150 ml. of benzene are added and the cap is screwed on tightly. The jar is shaken for 5 minutes in an end-over-end shaker and the benzene extract then filtered and a 50-ml. aliquot treated as above.

With fruit samples, rather large quantities of wax may be carried through to the nitration causing a violent reaction on addition of the nitrating acid. In these cases, the tube should be warmed gently for the initial reaction to subside before being placed in the boiling water-bath.

Treatment of animal tissue

(*a*) *Faeces.*—10 g. of rabbit faeces (2 g. for rats) are macerated completely with 100 ml. of benzene in an Ato-Mix. The solid material is allowed to settle and 50 ml. of the filtered supernatant extract is taken for the analysis.

(*b*) *Liver, kidney and muscle.*—10–20 g. of these organs are weighed and macerated in the mechanical macerator with 100 ml. of benzene. The solid particles are allowed to settle and a 50-ml. filtered aliquot taken as before. Results on this type of tissue are not very accurate and recoveries decrease as the quantity of fat increases.

The method cannot yet be applied to urine, blood, milk and fatty tissue.

* *Sodium methylate reagent.*—10·0% ± 0·1% w/v of sodium methylate in dry redistilled methyl alcohol. The methyl alcohol is dried over anhydrous sodium sulphate for 48 hours and the clear supernatant liquid is decanted and distilled. Slightly more than the theoretical amount of clean sodium is carefully dissolved with cooling in the dried methyl alcohol under nitrogen. The solution is standardized against hydrochloric acid and the concentration adjusted to 10·0% ± 0·1% by the addition of dry methyl alcohol. Any sediment is allowed to settle and the clear supernatant reagent decanted for use.

Results

The study of reaction conditions rapidly showed that from the stage of nitration, optimum conditions were very similar to those worked out by Schechter and his co-workers[3] for DDT. For completeness these are briefly outlined below.

Analytical procedure

(*a*) *Development of colour.*—Chlorbenside sulphone nitrates to a trinitro derivative which can be isolated practically pure by adding water to the nitrating mixture. This derivative has been characterized having a melting point of 222° (found C 35·3, H 1·6, N 9·6. Trinitro derivative requires C 35·8, H 1·6, N 9·6%) and its isolation simplifies studies on colour development. It can readily be reduced, diazotized and coupled to give coloured products, but these methods offer no advantage over that chosen.

The trinitro derivative reacts in benzene with sodium methylate in methyl alcohol to give an unstable purple colour reaching a peak of intensity 1–2 minutes after development (Fig. 1).

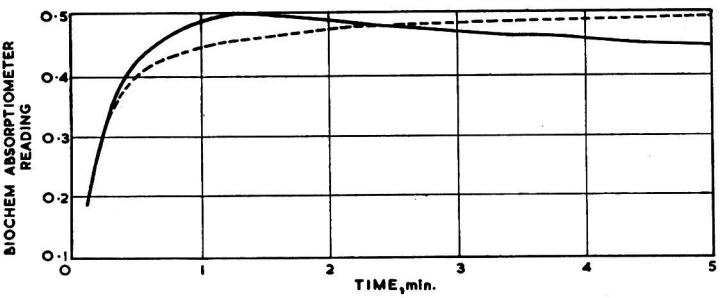

FIG. 1.—*Rate of development of colour : 0·25 mg. of Chlorbenside (continuous line) ; 0·10 mg. of DDT (broken line)*

The exact form of the curve varies with temperature, but the peak value is exactly reproducible and it is possible to obtain good replication of results by the recording of this peak in a direct reading colorimeter such as the Biochem Absorptiometer. All results given below are of the peak value unless otherwise stated.

The spectrophotometric curve for the colour is given in Fig. 2 and has absorption peaks at 356 mμ and 575 mμ.

Variations in the 1 : 2 ratio of benzene to sodium methylate solution have no advantage. Lowering the ratio of benzene increases sensitivity, but the peak is reached too rapidly for accurate

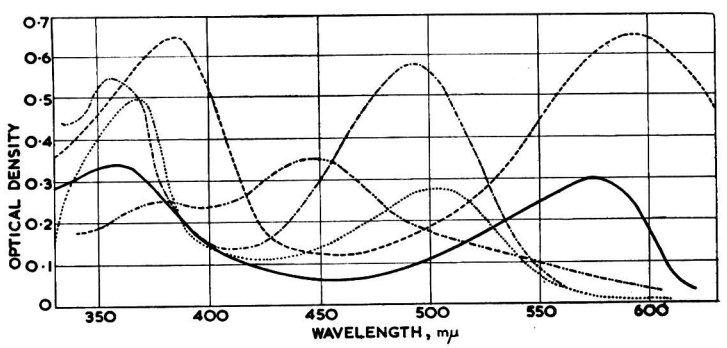

FIG. 2.—*Spectrophotometric curves of colours derived, without pre-oxidation, from*

(i) 0·01 mg. of Chlorbenside sulphone
(ii) 0·05 mg. of Chlorbenside
(iii) 0·01 mg. of DDT
(iv) 0·05 mg. of *op*-Chlorbenside sulphone
(v) 0·05 mg. of *mp*-Chlorbenside sulphone

recording. The 1 : 2 ratio gives a peak reading 1–2 minutes after development and allows easy mixing of reagents without undue loss of sensitivity.

The strength of the sodium methylate reagent is very critical. Maximum sensitivity is reached at a concentration of 9·7% and falls fairly steeply above and below this concentration. It is therefore necessary to standardize the strength of this reagent to an accuracy of ± 0·1%.

Traces of water decrease sensitivity so both benzene and methyl alcohol must be dried before use.

A typical calibration curve for the reaction is given in Fig. 3.

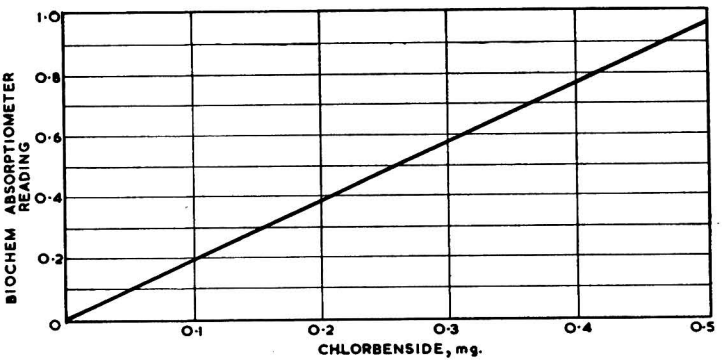

FIG. 3.—*Typical calibration curve*

(*b*) *Conditions of nitration.*—Preliminary experiments showed that conditions for quantitative nitration of Chlorbenside sulphone are similar to those for DDT.[3] A 1 : 1 mixture of fuming nitric and concentrated sulphuric acid gives quantitative nitration after about 1 hour at 100°. Nitrations in the cold or with concentrated nitric acid are incomplete.

Extraction of the nitro derivative follows along the lines of those used for DDT,[3] except that direct extraction from the nitrating mixture by benzene or other solvents[6] is unreliable because of the interference by traces of water.

(*c*) *Oxidation of spray residues.*—As mentioned above, spray residues may consist of either Chlorbenside or its sulphoxide or sulphone, and to obtain complete recovery it is necessary to pre-oxidize all residues to sulphone before nitration. Hydrogen peroxide in glacial acetic acid proved to be the most satisfactory reagent. Typical recoveries on this oxidation procedure are shown in Table I.

Table I

Recovery of 0·1 mg. of compounds in oxidation procedure

Compound	Treatment	% recovery
Chlorbenside	Pre-oxidized with 5 ml. of acetic acid + 0·5 ml. of H_2O_2 as in ' Method '	92
Chlorbenside sulphoxide		99
Chlorbenside sulphone		97
Chlorbenside sulphone	No pre-oxidation	100

Residues of Chlorbenside sulphoxide and sulphone are quite easy to handle, but with Chlorbenside itself poor recoveries are obtained unless the oxidation is carried out very carefully under low heat. The addition of 0·05 g. of oleic acid under these conditions prevents loss of Chlorbenside by volatilization and does not interfere in the final colorimetric reaction as it is mainly removed in the alkali wash.

Results from a considerable number of analyses show that the whole method has a standard deviation of 6%.

Specificity

Chlorbenside and its sulphoxide will not normally appear in the analysis because of the pre-oxidation technique outlined above, but both can nitrate to form derivatives[2] which it has not

yet been possible to isolate or characterize. These react in benzene with the sodium methylate reagent to give a pale orange colour. The spectrophotometric curve for this colour reaction is given in Fig. 2 and shows absorption peaks at 365 mμ and 505 mμ. The colours obtained are not sufficiently sensitive, however, to warrant development as analytical techniques for the present purpose.

A typical series of results obtained by analysis of Chlorbenside and the sulphoxide and sulphone, omitting the pre-oxidation stage, are given in Table II.

Table II

Absorptiometer readings for 0·1 mg. of Chlorbenside compounds

	1½ min. after reaction	½ hr. after reaction	3 hr. after reaction	Colour of solution
Chlorbenside	0·025	0·045	0·042	Orange-purple
Chlorbenside sulphoxide	0·042	0·055	0·050	Orange-purple
Chlorbenside sulphone	0·198	0·140	0·063	Purple

Technical Chlorbenside is controlled to have a content of 90% or over of the active isomer *p*-chlorobenzyl *p*-chlorophenyl sulphide, and the majority of possible impurities have now been characterized. Their reaction under the conditions of test is shown in Table III.

Table III

Reactions of impurities in commercial Chlorbenside

Substance	Chemical composition	Max. % in technical Chlorbenside	Colour produced	Biochem reading (OG1 Filter) for 0·1 mg. of substance	% sensitivity by weight in relation to Chlorbenside
Chlorbenside	*p*-chlorobenzyl *p*-chlorophenyl sulphide	—	Purple	0·198	—
op-Chlorbenside	*o*-chlorobenzyl *p*-chlorophenyl sulphide	5%	Orange	0·077	39
mp-Chlorbenside	*m*-chlorobenzyl *p*-chlorophenyl sulphide	2·5%	Yellow	0·075	38
2 : 4-dichloro-Chlorbenside	2 : 4-dichlorobenzyl *p*-chlorophenyl sulphide	Trace	Yellow	0·035	18
—	*p*-chlorobenzaldehyde	2%	None	0·002	1
—	bis-*p*-chlorophenyl disulphide	1%	None	0·005	2
Dimercaptal	*p*-chlorobenzaldehyde di-(*p*-chlorophenyl) mercaptal	10%	None	0·005	2
—	*p*-chlorobenzylidene chloride	1%	None	0·005	2

It is clear that none of the impurities occurring in technical Chlorbenside will interfere in the method in the quantities they are likely to occur. The spectrophotometric curves for the *op*- and *mp*-sulphones are given in Fig. 2 for the sake of completeness.

These results are confirmed by the analyses shown in Table IV on a series of batches of Chlorbenside of varying purity. Results refer to the *pp*-isomer content.

There is no interference to the method by any of the normally used fungicides or insecticides except DDT. 1 mg. aliquots of Dieldrin, C.P.B.S., parathion, ferbam, Aldrin, toxaphene,

Table IV

Analyses of different samples of Chlorbenside

Batch	Analysis by colorimetric procedure, %		Chlorbenside content by gravimetric procedure, %[4]	
1.	100	100	100·5	99·7
2.	100	99·4	97·6	98·2
3.	94	95·5	94·5	94·3
4.	80·2	83·1	84·3	84·1
5.	79	78	76·6	77·2

Malathion, γ-BHC, nicotine, and phenyl mercuric salts give no coloration whatever under the conditions of test. C.P.C.B.S. and Captan give a very slight colour but are not likely to be encountered in practice in such large quantities.

As would be expected, however, DDT interferes seriously with the method and no means has been found of removing this interference. The method is only applicable therefore in the absence of DDT. The details of this interference are dealt with in the following section.

Interference by DDT

The colour derived from DDT is very similar to that from Chlorbenside sulphone having absorption peaks at 595 mμ and 385 mμ in contrast to Chlorbenside sulphone which has absorption peaks at 575 mμ and 356 mμ (Fig. 2). It is not possible therefore to eliminate interference by the use of filters. The colour is also about twice as sensitive, weight for weight of substance, when measured on the Biochem Absorptiometer and its rate of formation is different (Fig. 1) creating a very difficult interference problem.

Two lines of approach were made in attempts to eliminate interference:

(*a*) *Separation techniques.*—Attempts at selective hydrolysis or oxidation were not successful as the degradation products of DDT give interfering colours, while Chlorbenside is not removed. The introduction of the two oxygen atoms into Chlorbenside to form the sulphone appears to activate the methylene group sufficiently to form loose sodium derivatives. Reaction conditions are however too critical to adapt this to routine separation.

(*b*) *Properties of the nitro derivatives.*—The general properties of the trinitro derivative of Chlorbenside sulphone are almost identical with those of the tetranitro derivative of DDT. It gives a red coloured product with aniline in the same way as DDT and also reacts in acetone solution with alkali to give a light orange colour.[3] After reduction of the nitro-compounds and diazotization, coupling with a variety of agents gives a series of colours. The sensitivity of these colours for both compounds is again too near to form any basis of a separation (see Table V).

Table V

Absorptiometer readings of colours from reduced nitro-compounds from 1·0 mg. of Chlorbenside sulphone and of DDT after diazotization and coupling with various reagents

No.	Coupling agent	Colour	Filter	Chlor-benside	DDT	Blank setting
1.	1-naphthylamine-4 : 6 : 8-trisulphonic acid	—	OG1	0·162	0·115	0
			OB2	0·228	0·171	0
2.	1-naphthol-2-sulphonic acid	No colour	—	—	—	—
3.	1-amino-8-naphthol-3 : 6-disulphonic acid	—	OB2	0·68	0·595	—
4.	2-naphthol-3 : 6-disulphonic acid	—	OB1	0·86	0·88	0·3
5.	2-amino-5-naphthol-7-sulphonic acid	—	OB2	0·64	0·55	0
6.	*m*-toluidine ethyl hydrogen sulphate	—	OB2	0·175	0·335	0
7.	2-naphthylamine-6-sulphonic acid	Turbid orange	—	—	—	—
8.	2-naphthol-6-sulphonic acid	—	OG1	0·17	0·19	0

Reduction was carried out with zinc in acidic alcohol followed by diazotization and condensation as described by Holleman for tetrachloroaniline.[5]

With no separation available it must be accepted that a 10% contamination of DDT significantly affects the results for Chlorbenside. Similarly a 10% contamination of Chlorbenside interferes with the results for DDT by the Schechter–Haller method.[3]

Treatment of extract

Both Chlorbenside and its oxidation products are reasonably soluble in benzene and, as this solvent is easy to handle, it was selected for routine extraction purposes. As relatively large volumes are involved in the extraction, it is necessary to evaporate these down to 5 ml. or less before oxidation, to keep the volume of acetic acid used as mutual solvent as low as possible. No Chlorbenside is lost during this evaporation provided a certain amount of plant waxes are present. The inclusion of oleic acid is considered desirable in all analyses, however, as the quantities of natural plant waxes may not be sufficient with some samples to hold the Chlorbenside

against volatilization. The presence in the extract of oxidizable matter, either of plant or animal origin, weakens the oxidizing power of the hydrogen peroxide, but a total of 5 drops (0·2 ml. of 100 vol. reagent) is sufficient in all cases.

Typical recovery figures for a variety of plants are given in Table VI.

Table VI

Recovery of Chlorbenside added to plant materials

Variety and type of sample	Chlorbenside added, mg.	Chlorbenside found, mg.	% Recovery
Bramley leaves	0·1	0·102	102
Cox leaves	0·1	0·103	103
Worcester leaves	0·1	0·105	105
Gooseberry leaves	0·1	0·086	86
Gooseberry leaves	0·15	0·161	107
Lanes Prince Albert fruit	0·2	0·202	101
Rev. Wilkes fruit	0·2	0·224	112
Newton Wonder fruit	0·2	0·225	112

With animal tissue also, benzene is used, extraction being carried out in a high-speed macerator. With faeces the method is satisfactory, but with fatty tissue recoveries are poor. Reasonable results can be obtained with kidney, liver and muscle if all adhering fat is removed before maceration. The method is not applicable in its present form for analysis of fat, milk, blood or urine.

Typical recovery figures are given in Table VII.

Table VII

Recovery of Chlorbenside added to biological materials

	Wt. of sample, g.	Chlorbenside added, mg.	Chlorbenside found, mg.	% Recovery
Rabbit faeces	5	0·1	0·102	102
Rabbit faeces	5	0·1	0·098	98
Rat liver	5	0·1	0·062	62
Rat muscle	4	0·1	0·099	99
Rabbit kidney	7·5	0·1	0·098	98
Rabbit liver	25	0·1	0·041	41

Discussion

The method of analysis of Chlorbenside in this paper is based on the fact that the trinitro derivative of Chlorbenside sulphone forms a purple colour in benzene with sodium methylate which, although not stable, reaches a reproducible peak 1–2 minutes after development. Under similar conditions the nitro-derivatives of the sulphide or sulphoxide give a pale orange colour which forms slowly and is stable. By a pre-oxidation technique, all spray residues of either the sulphide or sulphoxide are converted to the sulphone and the method gives an additive result for all three substances. This is desirable as the oxidation products of Chlorbenside, which it is believed can be formed under some conditions on plant surfaces, have biological and toxicological properties similar to those of Chlorbenside.

The method is specific for the active *pp*-isomer both in the presence of the normal impurities in the commercial material and to all other spray materials except DDT. The last-named insecticide interferes seriously, as its tetranitro derivative forms a stable blue colour under the same conditions, with absorption peaks too close for separation by colour filters. Attempts to remove this interference from DDT have failed and the method as it stands is only valid in the absence of this substance. For work on fruit crops in this country this is not a great disadvantage as DDT is rarely used post-blossom because of its destructive action on predatory insects. In other countries and on other crops in this country it constitutes a serious disadvantage, however, and work is continuing to devise completely specific techniques for the analysis of Chlorbenside.

Very little work has been carried out on the mechanism of the colour reaction apart from

establishing the empirical formula of the trinitro sulphone. The rapid fading of the colour would appear to be connected, however, with the splitting off of a nitro-group under the strongly alkaline conditions.

Acknowledgments

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CHANGES IN THE OXIDATION–REDUCTION POTENTIAL OF THE *STERNO-CEPHALICUS* MUSCLE OF THE HORSE AFTER DEATH IN RELATION TO THE DEVELOPMENT OF BACTERIA

By ELLA M. BARNES and M. INGRAM

The oxidation–reduction potential of the *sterno-cephalicus* muscle of the horse falls after death from $E_H > +250$ mv to about -130 mv. The greatest change takes place immediately after death and is probably due to the withdrawal of the last traces of oxygen from the tissues. After *rigor mortis* the potential remains steady, provided that bacterial growth is inhibited, which can be effected either by holding the muscle at 5° or incorporating 1–2 p.p.m. of aureomycin. If bacterial growth occurs the potential falls to about E_H -250 mv or lower.

It has often been observed that changes take place in the microbial flora of large muscles during storage, particularly when slow cooling allows the multiplication of internal mesophilic organisms. For example it was shown that, in the whale, facultative anaerobes such as the faecal streptococci were slowly replaced by clostridia (Robinson, Ingram, Case & Benstead[1]). Although the relationship of bacterial growth to the oxidation–reduction potential of the medium has frequently been studied in pure culture (Hewitt[2]), no previous attempts have been made to apply such studies to *post mortem* changes in muscle tissue. As a preliminary to such an investigation it was necessary to determine the nature of the changes in the oxidation–reduction potential of the muscle itself.

A search of the literature revealed surprisingly few observations on the oxidation–reduction potential changes in muscle and, in fact, none which followed the changes for several hours immediately after death. Okuyama[3] found that the E_H of rat muscle (*m. quadriceps*) was + 150 mv ; ligature of the blood vessel, or killing the animal, resulted in a shift of the potential towards reduction and 1 hour after death it had fallen to + 50 mv. Cater & Phillips[4] found a potential of about + 250 mv in rat muscle. This fell after death but was considerably influenced by the method of killing the animal. When an intravenous injection of cyanide was used the ' electrode potential ' remained practically unchanged for 10 min. and then fell slowly. Death from ether gave a similar slow fall. A very rapid fall in potential occurred after death from ' coal gas '. Kovalsky & Glesina[5] inserted electrodes into the legs of rabbits and found that the normal E_H varied from + 292 mv to + 473 mv : they also found that the potential of the pectoral muscle of the pigeon varied from + 49 to + 130 mv and of isolated frog muscles from + 172 to + 190 mv. Uchimura[6] recorded the oxidation–reduction potential of normal frog muscle as about + 230 mv. Experiments with minced muscle extracts have been reported by Chagovetz[7] and by Pincussen & Seitz[8] but, when the muscle structure is destroyed in this way, the changes associated with *rigor mortis* are disturbed.

In the experiments described below, changes in the oxidation–reduction potential of intact horse muscle, kept under anaerobic conditions, have been followed more or less continuously for 1 to 2 days *post mortem*. The *rigor mortis* changes have been followed concurrently by means of pH determinations combined with a visual assessment of the state of parallel samples of muscle.

Experimental

In this kind of investigation there are two serious difficulties : (i) There is evidence that the oxidation–reduction potential of muscle *post rigor* depends on whether it contains or does not contain dissolved oxygen (Brooks[9]). Hence it is necessary, when working with small pieces, to exclude air from the muscle as far as possible and to arrange the insertion of the electrodes etc. so that the minimum amount of oxygen is admitted deep into the tissue. Although the muscle itself consumes oxygen, and so is capable of reducing the potential, again, this process is inconveniently slow ; (ii) the electrodes are liable to be poisoned, for reasons which are unknown. This is minimized by the use of polished electrodes with a large surface area. Nevertheless, several electrodes must be used simultaneously, so that any electrode which gives erroneous readings can be detected. Wire electrodes were found to be too small and spade electrodes could not easily be inserted, so conical spear electrodes were used (see below).

Treatment of horse muscle

As soon as the horse had been killed, one or both of the *sterno-cephalicus* muscles were removed with as little contamination as possible. The fascia and fatty tissue were stripped off and the muscle then divided into two parts—one for E_H and one for pH measurements. When aureomycin was used to delay bacterial growth, a concentrated solution was injected at several points in the muscle, immediately after removal of the sheath and withdrawal of a sample for bacterial count. The concentration of aureomycin used was such as to give 1–2 p.p.m. if distributed evenly throughout the tissue.

Bacteriological analysis

Samples for bacteriological analysis were withdrawn aseptically from the centre of the piece of muscle, in some experiments by a difficult procedure using a metal borer inserted through one of the holes in the Perspex disc, which would normally carry an electrode (Fig. 1).

Viable counts of aerobic bacteria were made using tryptic digest agar plates. The plates were incubated at 37° and 22° for 2 and 4 days respectively.

Presumptive clostridial counts were made by incubating serial dilutions in Robertson's cooked meat medium at 37°. Positive tubes showing growth and gas formation were checked by microscopical examination.

When aureomycin had been injected into the muscle the plates and meat broths were incubated for a longer period (up to a week), to see if any aureomycin was carried over and delayed growth. There was, however, no evidence of inhibitory action by residual aureomycin

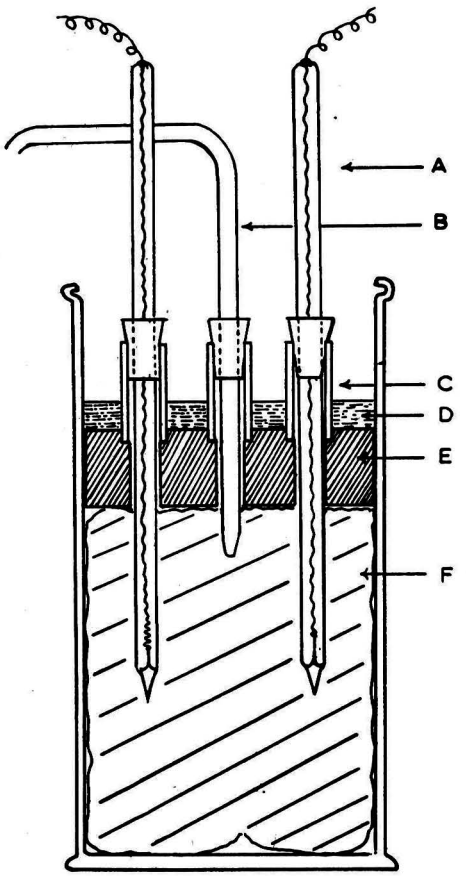

FIG. 1.—*Apparatus for measuring the oxidation–reduction potential of muscle*

A. Platinum electrode
B. Agar/KCl bridge
C. Perspex collar
D. Paraffin wax seal
E. Perspex disc
F. Muscle

even in the plates and tubes corresponding to the lowest dilutions of the meat. If bacteriostatic concentrations of aureomycin had been present, those agar plates containing the highest concentrations would have given relatively lower bacterial counts than the higher dilution plates; this did not occur.

pH measurements

The section of muscle for pH determinations was kept at 37° in a screw-capped bottle under nitrogen. This was opened at hourly intervals, a sample removed, and chopped up in a few ml. of 0·005M-sodium iodoacetate to inactivate the enzymes involved in the anaerobic breakdown of glycogen (Lundsgaard[10]). The pH was measured with a glass electrode after allowing the mixture to stand for 30 min.

Oxidation–reduction potential measurements
Whole muscle

The muscle, which was about 12 cm. long and 4 cm. diameter, was folded over so that its longitudinal surface was uppermost, and squeezed into a straight-sided glass jar which had previously been filled with nitrogen. A Perspex disc (5·8 cm. diameter) containing five holes, each with a 2-cm. high Perspex collar, was pressed down on the meat and sealed in place with paraffin wax (Fig. 1). The only part of the muscle now exposed to the air was that part immediately beneath the holes. Nitrogen was blown down each tube continually while a small but deep incision was made in the muscle with a sharpened metal probe and platinum spear electrodes mounted in rubber bungs were sunk to about 2 cm. below the surface of the muscle, the bungs fitting tightly into the collars. An agar bridge saturated with potassium chloride was inserted through the central hole. This bridge connected through a saturated KCl reservoir with the standard calomel half-cell. Alternatively the calomel electrode was seated in a tap funnel, the stem of which contained the saturated agar bridge.

Details of the care and use of platinum electrodes and calomel cells are given by Hewitt[2] together with the method for calculating E_H values, so that only a few points will be mentioned below.

(*a*) *Electrodes.*—The platinum electrodes were made to the following specification—Platinum tips 15 mm. long × 2 mm. diameter tapered to a spear point at one end. At the other end, a 2-cm. wire 0·5 mm. diameter connected to a central copper wire. The electrode was sealed into a glass tube.

The electrodes were tested in quinhydrone buffer solutions of known E_H and found to agree to within 1 mv.

In the experiments described below the average reading obtained from three or more electrodes is always given. Immediately after insertion of the electrodes the readings differed by as much as 100 mv, but these variations rapidly diminished, and after the first 3 hours the differences between the electrodes was usually not greater than 30 mv and generally much less.

The object of inserting the electrodes through raised tubes in the disc was to enable one to be withdrawn in case of trouble (e.g. poisoning of the electrode) without disturbing the others and the paraffin wax seal.

To sterilize the electrodes (which should not be heated) they were left in 70% alcohol (v/v) overnight, washed with sterile distilled water and dried carefully with a sterile cloth or cotton wool.

(*b*) *Nitrogen.*—The last traces of oxygen were removed from the nitrogen by bubbling through two pyrogallol reservoirs and then over heated copper gauze.

Homogenized muscle

The muscle was mixed with an equal quantity of distilled water or buffer at the required pH and pulped in a rotating-knife homogenizer. About 30 ml. were then put into 50-ml. tall beakers each fitted with a bung carrying the nitrogen inlet tube, the agar bridge, the platinum electrode, the gas outlet tube (connected to a pyrogallol reservoir) and an inoculating or sampling cup. Nitrogen was blown through the sample in a steady stream ; tests in which air was admitted to homogenates of *post rigor* muscle of known E_H showed that it was 2–3 h. before the effect of the incorporated air on the potential of the homogenate was eliminated.

Results

Most of the experiments were carried out under anaerobic conditions at 37° (see above). The pH fell continuously until a constant level was reached and this point was taken as an indication of the end of the rigor changes. The time taken for these changes was remarkably constant, and in Table I the initial and final pH values are shown together with the time after death of the

Table I

Initial and final ('ultimate') pH values of the sterno-cephalicus *muscle from a number of horses*

Sample	pH measurements		Time *post mortem* to reach the final pH, h.
	Initial*	Final	
1	6·86	5·76	8
2	6·92	5·76	8
3	7·1	5·8	9
4	6·98	5·76	8¼
5	6·81	5·72	8
6	6·72	5·74	9½
7†	7·04	5·74	9
8†	7·02	5·88	8¼

* At least 1 h. *post mortem*
† Aureomycin-treated muscle

end of the pH change. The recorded changes, associated with *rigor*, were (1) the loss of the power of contraction on stimulation, (2) the temporary toughening, (3) the darkening in colour, (4) the production of moisture on the surface.

It was found that there was initially a rapid fall in oxidation–reduction potential probably due to the removal of the last traces of residual oxygen from the tissue followed by a period of slower fall and finally, a further fall to a very low E_H after the pH had reached its ultimate level (Fig. 2). As no effort had been made to control bacterial growth it was thought probable that the continued fall in E_H was due to an increase in the bacterial population. Initial and periodical counts were made and the relationship of bacterial count to pH and E_H is shown in Table II. These results supported the view that the final low E_H might result from the metabolic activity of the large numbers of bacteria which had developed by that time.

In order to separate the changes in potential due to bacterial growth from those which were a function of the *post mortem* changes *per se*, it was necessary to repeat the experiments under conditions where bacterial growth would be delayed or inhibited. As it was not possible to

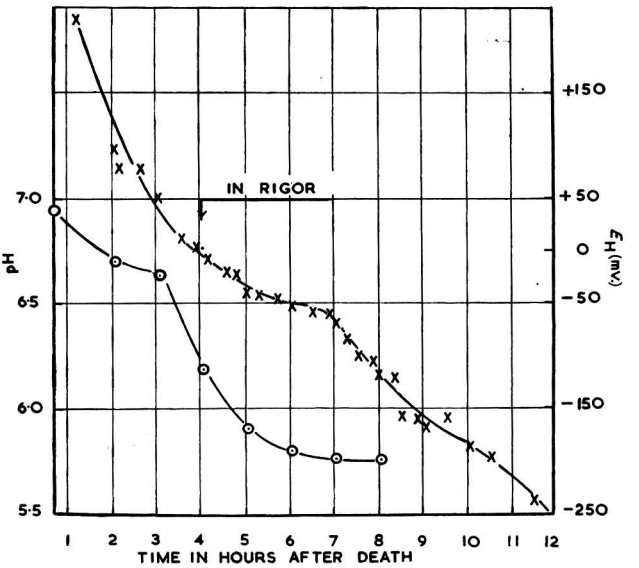

FIG. 2.—*The oxidation–reduction potential and pH of the* sterno-cephalicus *muscle of the horse* (post mortem) *kept anaerobically at* 37°
×—× E_H ⊙—⊙ pH

Table II

The relationship of bacterial count to E_H and pH

Time *post mortem*, h.	pH of muscle	E_H, mv	Bacteriological condition	
			Total aerobic count at 37°	Presumptive clostridial count
0·75	6·93	> +.230		
2	6·53	+ 98	$2·9 \times 10^4$	$3·9 \times 10^2$
5·75	5·8	− 44	$1·6 \times 10^5$	$1·6 \times 10^2$
8·5	5·76	− 121	$7·6 \times 10^5$	2×10^4
11·25	5·76	− 229	$2·1 \times 10^6$	$9·7 \times 10^5$
13·75	5·76	− 300	$2·5 \times 10^6$	$2·6 \times 10^6$

obtain sterile horse muscle two methods were tried : (i) holding the muscle at 5° instead of 37° which, although delaying the *rigor* changes, delayed bacterial growth even more ; (ii) incorporating 1–2 p.p.m. of aureomycin and then carrying out the experiment at 37° as previously.

(i) The changes in potential at 5° are shown in Fig. 3. After death the potential fell rapidly but, as would be expected, not so rapidly as at 37°. When the experiment was carried out on the left and right muscles of the same horse, that at 5° took $8\frac{3}{4}$ h. to reach an E_H of 0 mv, whilst that at 37° took only 4 h. to reach the same value. After the ultimate pH was reached (about 20–24 h.) the potential remained fairly steady for the next 24 h. between E_H — 110 and — 140 mv. The total aerobic count at 22° and 37° showed no change during this time whilst there was a slight decrease in the clostridial count.

(ii) The incorporation of aureomycin did not have any obvious effect on rigor changes and it was found to delay bacterial growth for 24–48 h. Results of two experiments are given in Table III. In the first experiment there was no increase in the number of bacteria present even after 72 h. and the potential *post rigor* remained steady for the whole of this time. In the second experiment the count had increased after 48 h. The changes in oxidation–reduction potential of the muscle in the presence of aureomycin are shown in Fig. 4. The E_H of the living muscle is

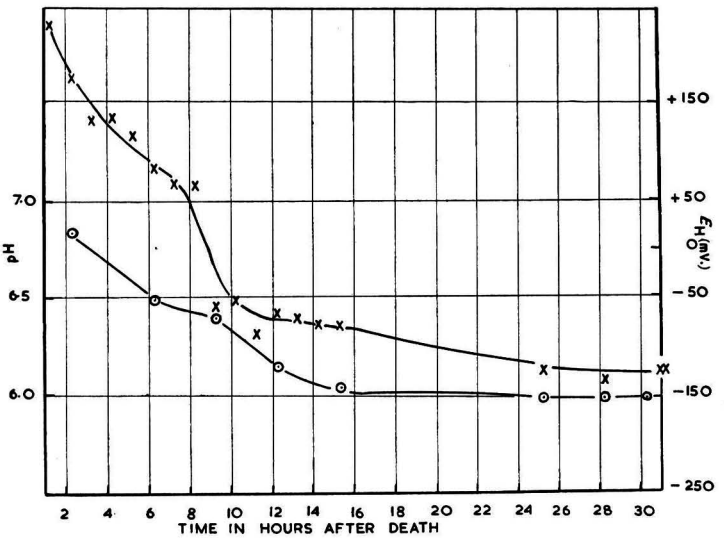

FIG. 3.—*The oxidation–reduction potential and pH of horse muscle* (post mortem) *kept anaerobically at* 5°
×—× E_H ⊙—⊙ pH

Table III

Bacteriological condition of horse muscle treated with aureomycin and held at 37°

Experiment No.	Time *post mortem*, h.	Total aerobic count at 37°	Presumptive clostridial count
1	1	$2{\cdot}03 \times 10^4$/g. (micrococci and sporing rods)	< 10/g.
	72	$4{\cdot}54 \times 10^3$ (micrococci and sporing rods)	< 10/g.
2	1	$4{\cdot}3 \times 10^4$/g. (sarcina and micrococci)	< 10/g.
	48	$8{\cdot}52 \times 10^5$/g. (pseudomonas)	$1{\cdot}56 \times 10^4$/g.

not known, but the earliest *post mortem* determinations showed it to be greater than + 250 mv. Within the first 4 h. there was a rapid fall in E_H to about − 70 mv, by which time *rigor* had commenced. The potential then fluctuated and dropped only slightly after the final pH had been reached. It remained steady for a further 16 h. at about − 130 mv. After a further 24 h. it had dropped to about − 250 mv, and the bacterial count had increased as shown in Table III, experiment 2.

Both these methods agree in showing that under conditions where the *rigor* changes proceed whilst bacterial growth is prevented, there is no continued fall of oxidation–reduction potential in the period after the ultimate pH is established. The low values attained under normal conditions (about − 250 mv) must therefore be a consequence of the metabolic activity of bacteria. The ultimate potential of the muscle itself would, it seems, be about E_H − 130 mv.

Homogenized muscle

It was of interest to see whether a similar potential would be reached if the muscle structure were destroyed by homogenizing. The duplicate neck muscle to that used in the experiment (with aureomycin) shown in Fig. 4 was also treated with aureomycin and homogenized with an equal quantity of distilled water. About 30 ml. of homogenate were then put into 50-ml. beakers as described above. The rest was stored under nitrogen for pH determination. The pH of the homogenized sample had fallen to 6·1 and the oxidation–reduction potential rapidly fell to

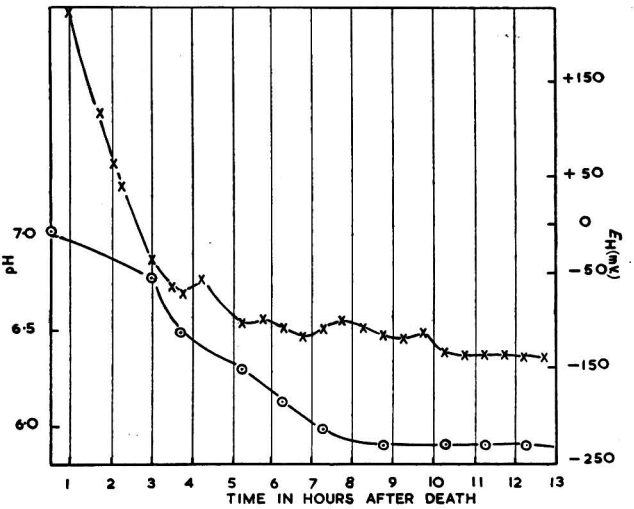

FIG. 4.—*The oxidation-reduction potential and pH of aureomycin-treated horse muscle* (post mortem) *kept anaerobically at* 37°

×—× E_H ⊙—⊙ pH

— 112 mv (3½ h. *post mortem*) as the oxygen was eliminated from the apparatus. For the next 9 h. the potential fluctuated between — 158 and — 197 mv. Throughout the experiment, there was a consistently lower potential in the homogenized sample and hence this is not considered to be a valid method for determining the *post mortem* changes in oxidation–reduction potential. Thus homogenization reduced the pH more rapidly and this was accompanied by a much more rapid fall in E_H.

Discussion

The overall change in the oxidation–reduction potential of the *sterno-cephalicus* muscle of the horse *post mortem* is of the order of 400 mv, i.e. from an $E_H > + 250$ to about $E_H - 130$ mv.

The major part of this change in potential takes place within the first 3–4 h. at 37°, and the end of the rapid fall in potential appears to coincide with the onset of *rigor* as judged by pH change. Most of the oxygen in the muscle must be consumed within the first 10 min. after death as the anaerobic production of lactic acid begins almost immediately, but it seems reasonable to interpret the continued fall in potential during the first few hours as being due to the consumption of the last traces of oxygen in the system. The interpretation of the initial fall in potential, as consequent on the consumption of residual oxygen by the respiration of the muscle, receives support from the observations of Cater & Phillips,[4] cited above. When they killed rats with coal gas, the carbon monoxide from which would displace the residual oxygen from the muscle, the potential fell rapidly ; whereas when, on the other hand, they poisoned the animal with cyanide, which largely inhibits uptake of oxygen by muscle, the potential remained higher.

During the *rigor* changes there is a further fall in potential. Subsequently the potential remains steady unless bacterial growth occurs.

The above conclusions are based on the average of the potentials obtained from several electrodes simultaneously. These potentials sometimes differed considerably as already indicated above. So far, there is insufficient evidence to say whether these different potentials represent real differences between different sites in the muscle. The greatest variation was found immediately after insertion of the electrode into the muscle.

The number of organisms required to produce a shift in potential was less than 10^7/g. (Table II). Both facultative anaerobes and clostridia had multiplied by the end of the pH

changes in the muscle, but when the potential reached a low level there was evidence of preferential growth of clostridia compared with that of other organisms. The inter-relationships of these different groups of organisms are being further investigated.

The addition of aureomycin to the horse muscle enabled the oxidation–reduction changes to be determined successfully without the complicating factor of bacterial growth. Incidentally, it also demonstrated a possible limitation of the proposed use (Weiser, Kunkle & Deatherage[11]) of such antibiotics in preserving meat: viz. the selection of resistant organisms leading to spoilage by unusual types of micro-organisms. In the experiment quoted above (Table III, experiment 2), it is clear that the pseudomonads, which were present at first in very small numbers, grew whilst micrococci were inhibited. In this case the anaerobes present had also developed though slowly. The action of the antibiotic depends entirely on the types of bacteria present initially.

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PLANT PROTEINS. IV.*—Amino-acids Present in Alcoholic Extracts of Grass and Legume Hays and Fenugreek Seeds

By J. KOLOUŠEK† and C. B. COULSON‡

The amino-acids of alcoholic extracts of lucerne hay cut in different stages of growth and mature grass and legume hays are estimated quantitatively by a rapid approximate paper chromatographic method. It is found that the amino-acids of the lucerne cut in varying stages of growth decline in quantity with increasing age, although some of the amino-acids form a fairly constant percentage of the total nitrogen at each stage. Differences in asparagine–aspartic acid content of lucerne hay from two different sources, as well as those of glycine, serine, alanine and proline, caused by manuring, are noted. Certain family amino-acid differences are observed among the mature hays. The alcoholic extracts of fenugreek seeds are found to contain few free amino-acids; other acids exist in peptide combination.

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Introduction

Some years ago Clarke[1] investigated the influence of growth stage and cutting on *Medicago media* (Ontario variegated lucerne) hay. He suggested that the leaf/stem ratio change accounted for the nitrogen change and found that the amino-acids and peptides were at a minimum (calculated as a fraction of total nitrogen) in the bud stage. Recently Bathurst[2] found that fertilizers changed the amino-acid pattern of the free amino-acids and peptides of grasses and that most of the free amino-acids were accounted for by aspartic acid and glutamic acid (and their amides) as well as alanine and serine. Rabideu[3] examined the free and bound amino-acids of four range grass seedlings during growth and found a general decrease in amino-acid content throughout growth. Synge[4] has examined the non-protein nitrogenous constituents of *Lolium perenne*. A general examination of total amino-acid content of grasses, legumes and other herbage was carried out by Armstrong[5] who suggested that there was a greater variation in amino-acid composition than was supposed by other authors, but this variation is presumably caused by the free amino-acids and peptides and not by the protein fraction. A recent note[6] has recorded the free and bound amino-acid composition (quantitative) of various plants. Variation in free amino-acids and peptides of *Pisum sativum* during growth development have been noted by Hyde.[7] The free amino-acids of the developing maize endosperm[8] and oats[9] have been examined.

A semi-quantitative evaluation of the free amino-acids of the hays examined in the present report, together with other hays, has been published.[10]

Experimental

Materials.—The materials used were lucerne (*Medicago sativa* L.) hay cut at five different stages of growth (from the State Agricultural Research Station, Liběchov near Mělnik) and a sample cut at maturity (from the State Agricultural Research Station, Měšic near Tábor) as well as other mature samples of red clover (*Trifolium pratense* L.), timothy (*Phleum pratense* L.) and orchard grass (*Dactylis glomerata* L.) (all from Tábor).

The lucerne cut in varying stages of growth (Liběchov) were from land unmanured before use ; the mature grasses and legumes were from land treated with sulphate of potash and basic slag before use. The hays were those used previously.[11]

'Amide' nitrogen.—This is the arithmetical difference between total nitrogen (Kjeldahl) and the Barnstein (copper precipitable) nitrogen.[11, 12] These have been previously determined.[12]

Extraction.—The milled hay (lucerne cut in varying stages of growth : 0·5 g. ; mature grasses and legumes : 1·0 g.) was extracted several times with 75% ethanol (10 ml. × 5), the residue removed by filtration through a fine sintered glass filter several times and the extract allowed to evaporate in a vacuum desiccator over calcium chloride.

Hydrolysis.—The resultant residue (after evaporation) was taken up in 6N-hydrochloric acid (2 ml.) and hydrolysed in a sealed tube (105°, 24 hours). The residue was removed by filtration through a sintered glass funnel and the hydrolysate evaporated in a vacuum desiccator over sodium hydroxide and calcium chloride.

Paper chromatography.—The dried unhydrolysed extracts of the mature hays (Tábor) were dissolved in 75% ethanol (2 ml.) and small quantities (80μl.–140μl.) used for two-dimensional chromatograms. The dried hydrolysed extracts of the various lucerne samples were dissolved in distilled water (0·5 ml.) and smaller quantities of these were used for quantitative paper chromatography.

The free amino-acids in the unhydrolysed extracts of the mature hays and those of the hydrolysed extracts of the various cuts of lucerne were estimated by a method involving two-dimensional paper chromatography.[13]

The hydrolysed ethanolic extracts of the mature hays and fenugreek (*Trigonella foenum graecum* L.) were evaluated semi-quantitatively.

Results

The quantitative results for samples of lucerne cut in varying stages of growth (Liběchov) are given in Tables I and II and the quantitative results for the unhydrolysed free amino-acid extracts of the mature hays (Tábor) are given in Tables III and IV. The results are expressed

in two forms, firstly as the amount of amino-acid (mg.) per 100 g. of dry hay (Tables I and III), and secondly, amino-acid nitrogen as a percentage of total nitrogen (Kjeldahl) of the sample (Tables II and IV).

Table I

Amino-acid composition of acid-hydrolysed 75% ethanolic extracts of lucerne (Liběchov) cut in varying stages of growth (mg. of amino-acid/100 g. of dry hay)

	Growth stage				
	1. Very young	2. Young	3. Beginning of bloom	4. Full bloom	5. Beginning of seed formation
Height of plant	12 cm.	14 cm.	46 cm.	55 cm.	70 cm.
Aspartic acid	690	480	400	360	410
Glutamic acid	580	400	250	190	170
Serine	150	480	230	200	90
Glycine	60	20	10	5	5
Threonine	420	390	140	60	40
Alanine	290	290	220	160	160
Tyrosine	160	*	*	*	*
Proline	190	410	400	120	1230
Valine + methionine	540	1000	500	300	210
Phenylalanine	110	210	120	60	50
Leucine(s)	890	520	560	210	170
Histidine	220	*	*	*	*
Lysine	520	151	90	40	40
Arginine	360	110	60	160	210

* Present in trace only

Table II

Amino-acid composition of acid-hydrolysed 75% ethanolic extracts of lucerne cut in varying growth stages (amino-acid N as % total N). 'Amide' N† as a percentage of total nitrogen

	Growth stage				
	1.	2.	3.	4.	5.
Aspartic acid	1·5	1·3	1·2	1·5	1·7
Glutamic acid	1·2	1·0	0·7	0·7	0·7
Serine	0·4	1·7	0·7	1·1	0·5
Glycine	0·2	0·1	0·1	0·05	0·05
Threonine	1·0	1·2	0·5	0·3	0·2
Alanine	1·0	1·2	1·0	1·0	1·0
Tyrosine	0·3	*	*	*	*
Proline	0·5	1·3	1·5	0·6	6·1
Valine + methionine	1·1	2·6	1·3	1·2	0·8
Phenylalanine	0·2	0·5	0·3	0·2	0·2
Leucine(s)	2·0	1·4	1·7	0·8	0·7
Histidine	0·4	*	*	*	*
Lysine	1·8	0·6	0·4	0·3	0·3
Arginine	2·0	0·7	0·7	1·7	2·3
Total	13·6	12·6	10·1	9·45	14·5
Amide' N†	36·8	38·5	21·6	30·6	31·6

* Present in trace only

† Difference between total nitrogen and Barnstein (copper precipitable) nitrogen calculated as a percentage of total nitrogen

A semi-quantitative evaluation of the amino-acids present in the hydrolysed alcoholic extracts of the mature hays and fenugreek seeds is given in Table V. An unknown ninhydrin-reacting spot (A) was noted in the chromatograms of the hydrolysates of lucerne ethanolic extracts. Substance A gave the following values:

R_F phenol–water/NH_3 0·83 (range: 0·80–0·87);

R_F butanol–acetic acid–water 0·33 (range: 0·3–0·37). For comparison the values for γ-aminobutyric acid are

R_F phenol–water/NH_3 0·77 (range: 0·74–0·81)

R_F butanol–acetic acid–water 0·37 (range: 0·33–0·41)

Table III

Amino-acid composition of unhydrolysed 75% ethanolic extracts of mature hays (mg. of amino-acid/100 g. of dry hay)

	Red clover	Lucerne	Timothy	Orchard grass
Aspartic acid	40	50	60	40
Glutamic acid	80	110	200	130
Serine	230	420	370	310
Glycine	300	410	320	—
Threonine	110	100	210	300
Alanine	470	410	750	980
Tyrosine	0	0	50	90
Proline	530	400	140	210
Valine + methionine	490	230	270	590
Phenylalanine	90	30	130	30
Leucine(s)	280	90	180	140
Histidine	0	0	0	0
Lysine	0	0	0	0
Arginine	0	0	0	0
Asparagine	1080	1100	1320	790
Glutamine	110	50	140	230

Table IV

Amino-acid composition of unhydrolysed 75% ethanolic extracts of mature hays (amino-acid N as % total N) 'Amide' N as a percentage of total nitrogen*

	Red clover	Lucerne	Timothy	Orchard grass
Aspartic acid	0·2	0·3	0·4	0·2
Glutamic acid	0·3	0·6	1·1	0·7
Serine	1·2	3·3	2·8	2·5
Glycine	2·3	4·6	3·4	—
Threonine	0·5	0·7	1·4	2·1
Alanine	3·0	3·8	6·7	9·4
Tyrosine	0·0	0·0	0·2	0·4
Proline	2·6	2·9	1·0	1·6
Valine + methionine	2·0	1·3	1·5	3·5
Phenylalanine	0·3	0·1	0·6	0·9
Leucine(s)	1·2	0·6	1·1	1·0
Histidine	0·0	0·0	0·0	0·0
Lysine	0·0	0·0	0·0	0·0
Arginine	0·0	0·0	0·0	0·0
Asparagine	9·3	14·0	15·9	10·2
Glutamine	0·8	0·6	1·5	2·7
Total	23·7	32·8	37·6	35·2
'Amide' N*	16·7	25·0	29·6	34·8

* Difference between total nitrogen and Barnstein (copper-precipitable) nitrogen calculated as a percentage of total nitrogen

Spots corresponding to β-alanine, pipecolinic acid, methionine sulphone, and γ-aminobutyric acid were observed on the two-dimensional chromatograms of the extract hydrolysates of the hays. (Fenugreek extract hydrolysates showed only the last of these amino-acids and also substance A.)

A spot corresponding to what may be 3 : 4-dihydroxyphenylalanine[10, 13] was observed on the two-dimensional chromatograms of the hay extract hydrolysates.

A semi-quantitative evaluation of the amino-acids in the hydrolysed alcoholic extracts of mature hay and fenugreek is given in Table V.

Table V

Semi-quantitative evaluation of the hydrolysates of 75% ethanolic extracts of mature hays (Tábor) and fenugreek seeds

	Red clover	Lucerne	Timothy	Orchard grass	Fenugreek seeds
Cysteic acid	2	2	2	2	2
Aspartic acid	5	5	5	4	5
Glutamic acid	5	5	5	5	5
Serine	5	5	4	3	5
Glycine	5	5	3	3	4
Threonine	4	4	4	3	4
Alanine	5	5	5	5	5
Tyrosine	—	—	1	1	1
Proline	5	5	5	4	1
Valine + methionine	3	4	3	3	3
Phenylalanine	2	2	2	2	4
Leucine(s)	3	3	3	3	5
Histidine	—	—	1	1	4
Lysine	2	2	2	2	4
Arginine	2	2	2	2	5
γ-aminobutyric acid	3	3	3	3	3

1. Very weak
2. Weak
3. Medium
4. Strong
5. Very strong

Discussion

The non-protein-nitrogen fraction is more variable in its composition as a result of season cutting and use of fertilizers than is the protein fraction.[2, 13] The non-protein-nitrogen fraction undergoes alteration during wilting[14] (in the main as a result of proteolysis).

The amino-acid content of the lucerne at various stages of growth (expressed as % of total N) (Table II) is at a minimum at the third growth stage (which approximates to late budding) and the fourth (which is full flower). The ' amide ' nitrogen is also at a minimum during the third stage. Clarke[1] showed that a minimum is reached in his ' sub-peptone ' fraction (as a fraction of the total nitrogen) at the budding stage in Ontario variegated lucerne (*Medicago media*) hay. The ' sub-peptone ' corresponds to the amino-acids and peptides present. It is suggested[1] that the increase in this fraction (and the total soluble nitrogen) following budding is due to protein degradation facilitating translocation of simpler nitrogenous materials required for the process of flowering. This minimum amino-acid-N/total N is achieved at the third growth stage (Table II) when extractable protein shows an increase on the second growth stage.[11] This is presumably a result of conversion of free and peptide amino-acids to bud proteins (and flower proteins). The extractable protein of the lucerne (Liběchov) is at a maximum in the first stage,[11] as is also the amount of alcohol-soluble amino-acids (Table I).

Table I shows that the majority of the amino-acids decline quantitatively with age presumably due to the general fall-off in metabolism (a fall in the ratio of actively growing points to general plant structure), which is related to the leaf/stem ratio (in reality, dilution by carbohydrates). This general decline in amino-acid content is also recorded by Rabideu[3] for range grass seedlings. The rough pattern of these changes is given in the semi-quantitative evaluation of the free amino-acids of the growth stages and mature grasses.[23] A rough correspondence is to be noted between the contents of ' amides ' and the amino-acids (Tables II and IV). This correspondence was previously noted.[23]

A distinct difference is shown between the unmanured lucerne cut in varying stages of growth and the mature lucerne hay together with the other mature hays which were obtained from fertilizer treated plots, in the contents of aspartic acid–asparagine as well as of glycine, serine, alanine and proline (Tables I and III). The asparagine is estimated as aspartic acid in the case of the lucerne cut in varying stages of growth. Bathurst[2] showed that the manuring of the plots results in a changed free amino-acid pattern. The difference between the lucerne cut in varying stages of growth on the one hand and the mature hays on the other might therefore be caused by manuring. The relatively high levels of aspartic acid, glutamic acid and their amides in both

groups may arise from the wilting process. The general conditions present in haymaking are somewhat similar to those obtaining with plant leaves starving in the dark following excision. It has been shown that excised plant leaves undergoing starvation in the dark synthesize appreciable amounts of glutamine and especially asparagine[15] (the predominance of one or the other amide depends on the particular species), and this may be a factor contributing to the high levels found of these and related compounds.

The major free amino-acids present in fresh grass samples are : aspartic acid, glutamic acid (and their amides), serine and alanine.[2] The free amino-acids present in the mature grass and lucerne hays (Table III) show aspartic acid and asparagine (but not glutamic acid and glutamine) and also alanine, serine and glycine to be the predominant ones.

Serine, valine + methionine, and phenylalanine show a maximum in the second growth stage of lucerne. Most of the others show a maximum in the first growth stage (Table I). Red clover and lucerne (Tábor) exhibit higher quantities of proline than timothy and orchard grass. Red clover and orchard grass show higher amounts of valine + methionine than lucerne and timothy (Table III).

An examination of amino-acid nitrogen as a percentage of total nitrogen (Tables II and IV) reveals that aspartic acid, alanine and phenylalanine in the various lucerne samples (Liběchov), and aspartic acid and leucine(s) in the mature hays (Tábor), vary little in their contribution to the total nitrogen present. In lucerne (Table II), glutamic acid, glycine, threonine and lysine show a decline in their contribution with increasing age. Some differences seem to exist between the free amino-acid composition of the mature legume hays as a percentage of total nitrogen and that of the mature grasses (Table IV). The legumes (Tábor) have lower contents of threonine, phenylalanine, alanine and glutamine, but higher proline contents than timothy and orchard grass, while tyrosine and histidine are found to be absent (Table IV). Distinctly anomalous results are obtained for proline (Table II). An appreciable increase in proline is found to take place in wilting grass.[14] Thus the variation found in the lucerne sample may arise from the effects of wilting.

Other amino-acids and ninhydrin-reacting substances were also detected and have already been mentioned. The unidentified ninhydrin-reacting substance (A) found in the lucerne extract hydrolysates has a yellowish brown colour. Steward & Thompson[16] record further unknown ninhydrin-reacting substances in the alcohol-insoluble residues of lucerne. The occurrence of ninhydrin-reactive substances giving brown spots has been examined in detail.[17]

The alcoholic extract of the defatted fenugreek seeds contained in the main aspartic acid, glutamic acid, alanine, valine + methionine, leucine and γ-aminobutyric acid (serine, glycine and threonine were present in mere traces only). On hydrolysis the following amino-acids were detected : cysteic acid, serine, glycine, threonine, tyrosine, phenylalanine, arginine, hystidine, lysine and proline (and substance A), which are presumably originally present in peptide combination. It is interesting to note that in this respect the fenugreek seeds resemble marine algae.[18] The free amino-acids present are, in the main, those directly involved in metabolism.[19]

Although the nitrogen content of feeding stuffs is important, the form of the nitrogen is not so critical for ruminants as for non-ruminants because ammonia forms an important nitrogen source in the rumen[20] and the carbohydrates, which show significant growth stage changes in grass,[21] are a factor influencing the value of protein and non-protein nitrogen because they are being simultaneously fermented by the bacteria in the rumen.[22]

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THE SEED OILS OF *BOMBAX SESSILE* AND OF *LUPINUS TERMIS*

By D. N. GRINDLEY and A. A. AKOUR

The seed oils of the species *Bombax sessile* and *Lupinus termis* have been examined in detail and their fatty acid composition determined. The former has a composition closely resembling that of palm oil, while the latter resembles other members of the Papilionaceae in that it contains minor quantities of linolenic acid and also of higher saturated fatty acids.

Bombax sessile (Benth.) Bakh.

Until recently, this plant was known in the Sudan as *Pachira aquatica* Aubl, and the species described under this name by E. L. Adriaens in his book 'Les Oleagineux du Congo Belge' appears to be the same as the Sudan species. Typical specimens recently sent to Kew from the Yambio Experimental Farm were identified as *Bombax sessile* (Benth.) Bakh., of the family Bombacaceae. This tree is a native of tropical America, and is grown for its oil in Brazil. It has also been cultivated in the Belgian Congo, and has been recently introduced into the Sudan as an experimental oil crop for the Southern Provinces. It is a small tree, with flowers up to 14 cm. long with linear pinkish or purplish petals. The fruit is an ovoid woody capsule up to 20 cm. long × 10 cm. on average, dehiscent along the locules by five valves. The number of seeds varies from 10 to 20. The ripe seeds are bedded in a soft silky fibre resembling kapok, and the seed itself is attractively marked with concentric white rings. The average size of the seed is $1\frac{1}{2} \times 2$ cm. The fresh seed germinates readily and, given the proper conditions, germination is almost 100%, but germination capacity falls off with time. The seed oil is characterized by the presence of palmitic, oleic and linoleic acids as major constituents, thus resembling the oils of the seeds of other families of the Malvales.

The oil is described by K. A. Williams[1] as being soft and greasy at ordinary temperatures, tending to separate into solid and liquid portions. The fat, in common with the kernels, possesses a pleasant smell reminiscent of a mixture of liquorice and fenugreek and would be an excellent soap fat. It has been suggested that the non-fatty residue might prove attractive as a cattle-food on account of its pleasant odour. Samples of seeds grown in the Congo have been examined by Pieraerts *et al.*,[2] who state that the cake is free from glucosides, and that the oil consists of glycerides of palmitic, oleic and linoleic acids. These workers were unable to detect stearic, arachidic or myristic acids.

Reference has been made[3] to the use of the wood of *Pachira* species as a paper-making material, which gave 46% of unbleached pulp (43% bleached) similar in character to common soda wood pulp.

It appeared that this species was worth further investigation, particularly from the standpoint of the edible oil which should be suitable after refining and deodorization, to replace palm oil in all its uses. It has the additional advantage that, being a seed fat and not a pericarp fat, it is free from the presence of lipoclastic enzymes which cause such rapid development of acidity and deterioration in palm oil, and it would not only keep better, but the need for immediate extraction of the oil after harvesting is not so vital. Furthermore, the oil-palm is a crop requiring a fairly heavy rainfall, and it is not well suited to the climate of the southern Sudan, whereas the *Pachira* species grows extremely well under rather poor conditions. Most of the country's requirements of palm oil have to be imported, and it is recommended that the species under consideration be cultivated to provide a replacement.

Analysis

The analysis of the kernels, which weigh about 1¾ g. each, is given in Table I. The high oil content (44%) is worthy of note. The kernels were extracted with light petroleum, when a pale yellow oil having a sweetish odour was obtained. The residue after extraction of the oil was shown to be free from alkaloids. The oil gave a very strong red colour with the Halphen reagent, in common with many other oils from the families Bombacaceae, Malvaceae, Sterculiaceae and Tiliaceae, which together make up the natural order Malvales. This property appears to be a characteristic of the entire order, and is even more pronounced in this species than in either cottonseed or kapok oils.

Table I

Composition of seeds

Species Family	*Bombax sessile* Bombacaceae	*Lupinus termis* Papilionaceae
Wt. of 100 seeds	175·7 g.	40·77 g.
Kernel, %	77·05	—
Analysis of kernel		
Oil, %	43·64	9·13
Protein, %	16·67	34·79
Moisture, %	4·76	7·51
Ash, %	3·19	2·88
Crude fibre, %	0·37	3·06
Carbohydrate (by difference), %	31·37	42·63
	100·00	100·00
Alkaloids	Absent	Present

The oil had a low acidity and low content of unsaponifiable matter. After removal of the latter, the fatty acids were recovered and their iodine and thiocyanogen values were determined, thus enabling the composition of the total fatty acids to be calculated, using the empirical value of 96 for the thiocyanogen value of pure linoleic acid (cf. Hilditch & Murti[4]). The fatty acids were next brominated in ethereal solution at 0°, but no precipitate of hexabromides was obtained, indicating the absence of linolenic acid. On removal of the ether and recrystallizing the residue from light petroleum a small yield of crystals was obtained, presumably tetrabromides derived from the linoleic acid present in the oil. These were insufficient to purify for identification purposes, so the mixed fatty acids were subjected to a controlled oxidation to identify the unsaturated acids originally present in the oil, using the conditions recommended by Lapworth & Mottram.[5] 5 g. fatty acids and 5 g. sodium hydroxide were dissolved in 2 litres of water and 700 g. of ice was added to the solution. Potassium permanganate (7·6 g.) was dissolved in ice-water (360 ml.) and this solution was added to the soap solution over a period of 3 minutes, with shaking. The mixture was set aside for a further 12 minutes, when sulphur dioxide was passed in to decolorize the permanganate, and concentrated hydrochloric acid (100 ml.) was added. The mixture was set aside overnight to flocculate, and the oxidized acids were filtered off at the pump and sucked as dry as possible. They were then boiled with light petroleum to remove any unoxidized fatty acids and saturated acids originally present, and the insoluble residue was boiled with 1500 ml. of water, filtered hot through a Büchner funnel and thoroughly washed with a further 2 litres of boiling water to remove any tetrahydroxystearic acids which might have been formed from linoleic acid originally present. The combined water washings were concentrated

to about 500 ml. and cooled overnight, when tetrahydroxy-acids crystallized out and were filtered off. The yield was too small for separation of the two isomers, as was done for the acids derived from *Lupinus termis* (*vide infra*), but the material was shown to have m.p. 154° and mol. wt. 346. It is therefore considered that this material is a mixture of the two isomeric tetrahydroxystearic acids arising from the mild oxidation of linoleic acid, whose presence in the original oil is thus proved. The residue left on the Büchner funnel after the hot-water treatment was recrystallized from ethyl acetate several times, when a pure white product, having m.p. 130·5°, mol. wt. 315, was obtained. This was the 9 : 10-dihydroxystearic acid corresponding to oleic acid which is thus shown to be the monoethenoid acid of this oil.

The saturated fatty acids were determined by Bertram's oxidation method,[6] and the results agreed closely with the figure derived thiocyanometrically. After the oxidation mixture had been extracted with light petroleum to remove the saturated fatty acids, the aqueous layer, after concentration, was further extracted with diethyl ether. Large yields of a dibasic acid were obtained which after recrystallisation from water had melting point and equivalent weight corresponding closely with azelaic acid. It is thus evident that in this species there is no unsaturated acid present having a double-bond nearer to the carboxyl group than in the 9 : 10 position, as in oleic acid.

The saturated acids obtained in the Bertram process had m.p. 61·5° (sharp) and mol. wt. 260·0, and appeared to consist of almost pure palmitic acid. To make certain of the absence of appreciable quantities of higher saturated fatty acids, the oil was subjected to Bellier's turbidimetric test (Evers' modification[7]). Turbidity was obtained at 26·5°. A synthetic mixture of pure palmitic acid and pure oleic acid containing the same percentage of saturated acids as found in the oil was similarly tested, and also gave a turbidity at 26·5°. It is therefore concluded that higher saturated acids are absent from this oil, the saturated acids of which consist almost entirely of palmitic acid. The constants and fatty acid compositions are given in Table II.

Lupinus termis Forsk.

The seeds of this plant, which is a member of the family Papilionaceae, form an important article of diet of the people of the Sudan and neighbouring countries where they provide a valuable source of protein. The composition of the seeds is included in Table I. However, it is necessary that the seeds be thoroughly soaked in water prior to being eaten, as they contain poisonous bitter principles which have been identified by Clemo & Leitch[8] as lupanine and related alkaloids. Symptoms of poisoning have been recorded which have been attributed to the use of these seeds when they have been eaten without proper washing. The detection of these alkaloids in viscera has been the subject of a recent communication from these laboratories,[9] sparteine having been shown to be a constituent in addition to the other lupin alkaloids. The alkaloids are readily soluble in water and also to a certain extent in light petroleum, with the result that some alkaloid passes into the oil when it is prepared by extraction with light petroleum. However, the quantity and character of the oil are such that its separation would not be warranted as an economic proposition, and it is the whole seed that is of value, after washing.

The plant is a herb or sub-shrub, and a native of the Levant. The flowers are white, with a bluish tint, and the pod is $2\frac{1}{4}$ to 4 in. long by $\frac{1}{2} \times \frac{3}{4}$ in. broad, being stout beaked and hairy. The seeds are whitish, nearly orbicular, compressed and about $\frac{1}{3}$ in. across. The species is very close to *Lupinus albus*, from which it differs in the presence of deciduous bracts to the calyx, and in the colour of the flowers. The plant is cultivated in the extreme north of the Sudan extensively as both a food and a fodder.

The oil was obtained from the seeds by extraction with light petroleum, and had a dark brown colour. The unsaponifiable matter was extracted by the usual method, and contained all the alkaloids which had been extracted with the oil. It was intensely orange-coloured and had the peculiar violet-like odour of oils rich in carotene, of which these seeds are probably a useful source.

Analysis

The treatment the oil was given was similar to that described for *Bombax sessile*, the fatty

acids being recovered after the removal of the unsaponifiable matter. On bromination of these in ethereal solution at 0° and keeping overnight, a small deposit of hexabromostearic acid was obtained, which melted at 181°, thus proving the presence of $\Delta^{9:12:15}$-octadecatrienoic acid (linolenic acid) in the original oil. After filtering off the hexabromides, the ether was evaporated from the filtrate and the residue was recrystallized from light petroleum, when a small crop of crystals was obtained, again presumably tetrabromostearic acid, but the yield was too small to identify.

The mixed fatty acids were subjected to the mild alkaline permanganate oxidation process described above for *B. sessile*, and the presence of oleic acid in the original oil was proved by the isolation of a pure specimen of dihydroxystearic acid by crystallization of the fraction of the oxidation products which was insoluble in both light petroleum and hot water. This had m.p. 130·5° and mol. wt. 317. On concentrating the hot water washings of the oxidation product and cooling, a good crop of crystals was obtained, which were filtered off at the pump. By treatment with boiling ethyl acetate these could be separated into two fractions, one sparingly soluble, having mol. wt. 349 and m.p. 156° and another insoluble, having mol. wt. 348 and m.p. 171°. These are therefore the well-known pair of isomeric tetrahydroxystearic acids resulting from the mild oxidation of *cis-cis*-$\Delta^{9:12}$-octadecadienoic acid (linoleic acid), the presence of which in the original oil is thus established.

The saturated acids were determined as before by Bertram's method and their high mean molecular weight suggested the presence of higher fatty acids. These were determined by Evers' modification of Bellier's process, when a turbidity was produced at 37·75°, corresponding to 5·1% higher acids. The presence of these was confirmed using the modification of Renard's method recently described by us,[10] in which the total saturated acids obtained in the Bertram process were employed. This gave 5·9% higher acids, of m.p. 72° and mol. wt. 336·5. This oil

Table II

Properties of the oils

Species	*Bombax sessile*	*Lupinus termis*
Colour	Pale yellow	Dark brown
Odour	Sweetish	—
Free fatty acids (as oleic acid), %	0·80	0·74
Acid value (mg. KOH/g.)	1·59	1·48
Saponification value (mg. KOH/g.)	196·7	179·3
Iodine value	49·89	102·78
Thiocyanogen value	42·90	77·49
Refractive index at 40°	1·4604	1·4686
Unsaponifiable matter, %	0·82	3·48
Total fatty acids, %	94·75	92·47
Mol. wt. of total fatty acids	270·2	286·2
M.p. of total fatty acids (slip-point)	52·0°	32·5°
Iodine value of fatty acids	52·22	107·73
Thiocyanogen value of fatty acids	44·92	80·88
Saturated acids (Bertram), %	50·66	17·21
Mol. wt. of saturated acids	260·0	298·8
M.p. of saturated acids	61·5°	58·5°
Bellier's test (turbidity)	26·5°	37·75°
Higher saturated acids (Bellier–Evers), %	—	5·1
Higher saturated acids (Renard), %	—	5·88
M.p. of higher acids	—	72°
Mol. wt. of higher acids	—	336·5
Hexabromides, %	Nil	3·18
M.p. of hexabromides	—	181°
Tetrabromides	Present	Present
Halphen's test	Strongly positive	Negative
Composition of fatty acids		
Linolenic acid, %	—	6·80
Linoleic acid, %	8·54	23·40
Oleic acid, %	40·80	52·59
Saturated C_{16}–C_{18}, %	50·66	11·33
Higher saturated C_{20}–C_{24}, %	—	5·88
	100·00	100·00

therefore resembles those from other species of the three sub-families of the Leguminoseae, all of which are characterized by the presence of these higher saturated fatty acids, and some of them also by minor amounts of linolenic acid.

The final fatty acid composition of the oil was determined by the thiocyanogen method, making use of the figure for the saturated acids determined above, and assuming the empirical values of 96 and 163 for the thiocyanogen values of linoleic and linolenic acids respectively.[4] The amount of linoleic acid found is in agreement with that expected from the yield of hexabromides, though the precipitation of the latter is by no means quantitative. Re-extraction with diethyl ether of the aqueous solution from the Bertram oxidation after removal of the saturated fatty acids gave a good yield of azelaic acid, again proving the absence in this oil of fatty acids having a double-bond nearer to the carboxyl group than the 9 : 10-position. The constants and fatty acid composition of the two oils are given in Table II.

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A MODIFIED 'PYRETHRIN II' ASSAY

By WM. MITCHELL and F. H. TRESADERN

The 'pyrethrin II' determination by the A.O.A.C. (or Seil) method is modified by separating chrysanthemumdicarboxylic acid from accompanying water-insoluble acidic matter, before titration. Lower and more accurate results are thereby obtained.

The determination of 'pyrethrin II' by the A.O.A.C. method,[1, 2] and also by the Seil method,[3] is non-specific. In each case, after the chrysanthemic acid has been removed, the residual aqueous liquid, having been concentrated, is saturated with sodium chloride, acidified with hydrochloric acid, and the liberated acidic matter is extracted with ether. After removal of the ether, the residue is titrated with alkali, and the 'pyrethrin II' content is calculated on the assumption that the total acidic matter represents chrysanthemumdicarboxylic acid derived from the hydrolysis of true pyrethrin II and cinerin II originally present in the sample.

Chrysanthemumdicarboxylic acid is readily soluble in boiling water, whereas it has been

observed that the material titrated, normally yellowish-brown in colour and consisting of crystals mixed with resinous matter, is only partly soluble. This fact suggested the following additional procedure as likely to give more accurate results:

> Boil the residue, obtained after evaporation of the ether, with distilled water (75 ml.), and filter the hot mixture. Wash the residues in the dish and filter paper with boiling distilled water (5 or more portions, each of 20 ml.) until the filtrates are neutral to litmus. Titrate the combined filtrates with 0·02N-sodium hydroxide (phenolphthalein).

Typical results, obtained by application of this additional procedure to samples of Kenya pyrethrum flowers, are given in Table I, in comparison with the results obtained by direct titration of the ether residue obtained by the usual A.O.A.C. method,[1] as modified[2] (using hydrochloric acid to liberate the chrysanthemic acid). Also included are the apparent figures for 'pyrethrin II' obtained by dissolving the water-insoluble residues in 95% v/v ethanol (25 ml.), diluting with water (50 ml.), and titrating with 0·02N-sodium hydroxide (phenolphthalein). Table II gives results similarly obtained on commercial extracts made from Kenya pyrethrum flowers.

Table I

Determination of 'pyrethrin II' in Kenya pyrethrum flowers by the A.O.A.C. method (a) *as usual,* (b) *after separating the water-insoluble acidic matter, and* (c) *based on the water-insoluble acidic matter*

Sample reference	'Pyrethrin II' (%)			
	a	*b*	*c*	Ratio *b*/*a*
23B	0·53	0·49	0·03	0·92
24A	0·61	0·56	0·05	0·92
25	0·60	0·56	0·04	0·93
4/3068/L	0·86	0·80	0·06	0·93
4/3068/D	0·78	0·73	0·04	0·94
HT 4	1·11	1·00	0·13	0·90
HT 5	1·24	1·01	0·19	0·81

Table II

Determinations of 'pyrethrin II' in commercial extracts, made from Kenya pyrethrum flowers, by the A.O.A.C. method (a) *as usual,* (b) *after separating the water-insoluble acidic matter, and* (c) *based on the water-insoluble acidic matter*

Sample reference	'Pyrethrin II' (%)			
	a	*b*	*c*	Ratio *b*/*a*
1881	10·7	9·3	1·5	0·87
2369	15·3	13·7	1·4	0·90
C12/54	8·8	8·0	0·9	0·91
Aerosol grade M28/54	12·9	12·4	0·6	0·96

From these results it is evident that a substantial amount of the 'pyrethrin II' recorded in the A.O.A.C. assay is based on the determination of acidic matter which, by its insolubility in water, is obviously not chrysanthemumdicarboxylic acid. The 'pyrethrin II' figures obtained on pyrethrum flowers by determination of the water-soluble acidic matter, by the proposed method, are 6 to 8% lower than by the normal A.O.A.C. method. However, with some, but not all, samples of the so-called 'high test' flowers, the difference is greater. Sample 'HT 5' shows the biggest reduction (19%) of any sample so far examined. This sample had an unusually high ratio (1·4 to 1) of 'pyrethrin II' to 'pyrethrin I' as determined by the normal A.O.A.C. method, and should probably be regarded as atypical. In fact, it seems to us not unreasonable, pending fuller investigation and experience of them, to regard *all* 'high test' pyrethrum flowers as suspect, since our experience has been that numerous specimens have shown losses of pyrethrins, on storage, which are abnormally rapid and large. In the case of pyrethrum extracts (Table II), the reduction in the 'pyrethrin II' figures by the proposed modified method is about 10%, i.e. somewhat greater than with 'normal' flowers. It is noteworthy that the reduction was only 4% with a purified 'aerosol grade' product from which much of the extraneous matter had presumably been eliminated by extra treatment in manufacture.

That the water-soluble acid titrated in the proposed method is practically pure chrysanthemumdicarboxylic acid has been confirmed. The titration liquids from a series of assays were bulked, and the acidic matter was recovered by the usual methods. It was crystalline, m.p. 160–163°, and by titration showed a purity of 95%. The *p*-phenylphenacyl ester was obtained, by the normal method, in a yield of over 80%, and had m.p. 154° (corr. ; not depressed on admixture with an authentic specimen).

Discussion

It is recommended that the procedure described be included as an additional stage in the A.O.A.C. (or Seil) method. The extra time and manipulation required are not considerable, and the accuracy of the ' pyrethrin II ' determination is improved. It seems possible that reduction in inter-laboratory error (usually arising mainly in the ' pyrethrin II ' determinations) might thereby be reduced.

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VARIATION OF ULTIMATE pH WITHIN PIG MUSCLES

By J. F. SCAIFE

The muscles of cattle and sheep have been examined with a view to elucidating the effect which connective tissue, oxygen and proximity to bone have upon the ultimate pH attained by a muscle. Pig muscles show variability in their ultimate pH values and in the distribution of total pigment within individual muscles. This variation of ultimate pH and total pigment in pig muscles is related ; the higher the concentration of total pigment then the higher is the ultimate pH.

Introduction

The chemical changes occurring in muscle within a few hours of death have been studied extensively, both in relation to the metabolism of living muscle,[1] and to the onset of *rigor mortis*.[2] The *post-mortem* formation of lactic acid from muscle glycogen *via* the anaerobic glycolysis cycle is of considerable practical importance as a factor affecting the quality of meat, since the pH at which *rigor* is complete (the ultimate pH[3]) has a profound influence upon the colour, texture and keeping quality of the meat.[4]

The ultimate pH of a muscle has generally been assumed to be constant and uniform throughout the muscle, although slight variations ascribed to different causes have been reported. Thus it has been claimed that the proximity of a muscle to bone gives rise to a higher pH due to the neutralizing action of the bone substance on the muscle lactic acid.[5] Bate-Smith[4]

cited the variation in the connective-tissue content of a muscle as the main factor responsible for pH variations within the muscle. This effect however is mostly confined to the tendinous insertion of the muscle.

The present investigation describes the results of work on pig muscles, in which wide variations of ultimate pH were found within single muscles. These variations have been correlated with the distribution of myoglobin within the muscle. Unlike the muscles of many mammalian species most pig muscles show an uneven distribution of pigment throughout their mass.

Experimental

Treatment of muscles.—Muscles were either excised from the animal within half an hour of death or allowed to assume full *rigor* within the carcass or some part of it. In the former case the excised muscles were allowed to assume full *rigor* either aerobically or anaerobically (in nitrogen or in an evacuated plastic bag). Samples for pH determination were taken after at least 20 hours at room temperature, an interval considered to be adequate for the attainment of *rigor*. No figures exist for the anaerobic time-course of *rigor* in pig muscle, but the results of Marsh[6] on beef, and Bate-Smith & Bendall[2] on rabbit may be expected roughly to define the limits. In any case the homogenization of the sample for the determination of pH would cause the rapid attainment of ultimate conditions.[7]

Determination of ultimate pH.—1 g. of tissue was homogenized in 9 ml. of distilled water, in a Marsh–Snow homogenizer.[8] The pH of the resultant brei was determined by means of a glass electrode.

Determination of myoglobin. A modification of the alkaline haematin method of Lawrie[9] was used to determine total pigment.[10] The pigment was extracted from a sample (1–2 g.) by phosphate buffer and converted to alkaline haematin. The proportion of haemoglobin in the extracted pigment was determined from the position of the band of the carbon monoxide derivative. Haemoglobin and myoglobin for standardization of the instrument were prepared respectively from pig blood[11] and from muscle and heart.[12–14]

Results

To investigate the effect of atmospheric oxygen upon the ultimate pH reached by a muscle when excised from the carcass immediately after death, some experiments were made upon the long uniform neck muscles (*sternomandibularis*) of beef animals. Within one hour *post mortem* half of the muscle was placed in a flask containing nitrogen and the other half in a flask containing oxygen. Both flasks contained a few drops of toluene and were then kept under identical conditions until *rigor* was considered to be complete. Samples were then taken from the interior, and the outer surface layer to a depth of 2 mm. from each portion of the muscle and the ultimate pH determined. Results are given in Table I.

Table I

Comparison of the ultimate pH values for beef muscle attaining rigor *under anaerobic and aerobic conditions*

Anaerobic		Aerobic	
Surface	Interior	Surface	Interior
5·79–5·83	5·76–5·79	6·00–6·10	5·77–5·79
5·91–5·94	5·89–5·91	6·60–6·64	5·89–5·92
5·70–5·72	5·61–5·63	6·04–6·29	5·62–5·63

The penetration of oxygen into mammalian red muscle has been thoroughly studied by Hill[15] and Brooks.[16] Over the course of several hours the effects of oxygen penetration as shown by the formation of a layer of oxymyoglobin is restricted to a few mm. in depth. The interior of a large muscle thus goes into *rigor* under essentially anaerobic conditions even when exposed to an atmosphere of oxygen. This is also illustrated in Table I, where it is seen that the ultimate pH of the muscle is affected by the surrounding atmosphere only in the outer few mm. of surface. Under strictly anaerobic conditions the pH of all parts of a muscle were essentially the same. An examination of the interior muscles of a carcass which had reached full *rigor* showed that these

muscles also go into *rigor* under essentially anaerobic conditions. Similar results were obtained when sheep muscles were examined ; in addition the smaller muscles of the leg showed the effects of proximity to bone and proportion of connective tissue as reported by other workers. Thus on a single sheep leg muscle, the following range of values, due to the above effects, was found ; interior, 6·14–6·16 ; insertion, 6·40–6·46 ; adjacent to bone, 6·48–6·56.

An examination of pig muscles revealed that wide variation of ultimate pH could occur within a single muscle, which could not be accounted for by any of the above factors. These variations were observed, however, to be related to the proportions of myoglobin occurring in different parts of the pig muscle. This colour variation was found in the present investigation to be an anatomical feature of pig muscle and is constant in feature although variable in degree from one animal to another. Colour variations of muscles have been stated by Maximow & Bloom[17] to be due in most mammals to variations in the proportions of red and white fibres within the muscles. The red fibres contain more myoglobin than the white fibres. Where the muscle was visibly more red the ultimate pH of that portion was higher than that in a paler region.

During investigations of the above phenomenon ten pigs were slaughtered and the hind legs dissected approximately 48 h. after death, when *rigor* was complete. A preliminary examination revealed that the visible colour variation within the muscles was most marked in the three muscles *semimembranosus, semitendinosus* and *rectus femoris*. None of these is contiguous to bone, and all have a minimum of connective tissue at the ends. Accordingly each of these muscles was sampled in three places, for both pH and total-pigment determination, samples 1 and 2 internally at approximately $\frac{1}{4}$ the length of the muscle from the ends, bottom, and top, respectively, and sample 3 from the middle of the muscle but near the surface. For *semitendinosus* and *rectus femoris* muscles, this sample was taken near the outer paler surface of the muscle, while for the *semimembranosus* muscle it was from the flap on the inner redder surface.

Table II

Comparison of total pigment content (P) with ultimate pH, for three places within a muscle, for three pig muscles

Position		Animal number									
		1	2	3	4	5	6	7	8	9	10
						Semitendinosus					
1	pH	6·73	6·56	5·61	5·80	6·61	5·84	6·29	6·42	6·35	6·01
	P	0·19	0·29	0·51	0·29	0·28	0·50	0·29	0·35	0·31	0·30
2	pH	6·30	6·34	5·39	5·73	6·53	5·77	6·08	6·19	5·86	6·00
	P	0·10	0·15	0·28	0·26	0·25	0·39	0·28	0·28	0·26	0·29
3	pH	6·00	6·24	5·36	5·50	6·03	5·63	5·54	6·01	5·72	5·54
	P	0·09	0·13	0·29	0·15	0·12	0·21	0·21	0·27	0·15	0·21
						Semimembranosus					
3	pH	6·41	6·07	5·86	5·87	5·98	5·78	6·22	5·89	5·74	6·03
	P	0·45	0·46	0·55	0·37	0·39	0·60	0·34	0·44	0·40	0·42
1	pH	6·22	6·04	5·38	5·69	5·79	5·45	5·68	5·44	5·61	5·70
	P	0·19	0·19	0·36	0·23	0·21	0·42	0·27	0·20	0·21	0·20
2	pH	5·59	5·60	5·49	5·52	5·66	5·53	5·52	5·55	5·15	5·34
	P	0·16	0·17	0·36	0·17	0·21	0·34	0·26	0·23	0·18	0·17
						Rectus femoris					
1	pH	6·55	6·86	5·86	6·61	6·17	5·83	6·24	5·80	5·82	6·14
	P	0·33	0·39	0·50	0·30	0·30	0·53	0·31	0·34	0·36	0·30
2	pH	5·91	6·71	5·62	6·04	5·79	5·71	6·22	5·67	5·72	5·66
	P	0·18	0·20	0·36	0·19	0·20	0·29	0·29	0·28	0·19	0·20
3	pH	5·76	6·56	5·57	6·00	5·76	5·57	5·74	5·66	5·67	5·66
	P	0·16	0·14	0·33	0·15	0·15	0·29	0·20	0·24	0·17	0·20

It is evident from Table II that when the total pigment concentration is high in any part of a muscle sample the ultimate pH is also high, and that this parallelism exists for all of the three muscles. As an evaluation of the experimental variation of the sampling a longitudinal strip was cut from the *semimembranosus* muscle of one animal which showed a uniform pigment coloration throughout its length, and both pH and total pigment were determined along the strip.

The results of Table III illustrate that within the experimental limits the sampling variation was small.

Table III

Distribution of pigment and pH values along a longitudinal strip of the pig semimembranosus muscle

pH	5·49	5·50	5·50	5·52	5·52
P(% w/w)	0·37	0·37	0·37	0·38	0·38

These results also demonstrate that provided the pigment concentration remains constant within the muscle then the ultimate pH throughout that muscle does not vary significantly.

An examination of the muscle total-pigment extract for residual haemoglobin was made by means of the position of the absorption maximum of the α band of the carbon monoxide derivatives. The purified pig carbon monoxide haemoglobin gave an absorption band with a maximum at 568 mμ, while the purified myoglobin derivative showed an absorption maximum at 578 mμ. These values are in agreement with those reported in the literature.[18]

All the muscle extracts examined showed maximum absorption at 578 mμ, which with the sensitivity of the instruments employed indicated that any residual haemoglobin in the extracts must be present in concentrations less than 10% of the total pigment concentration. This is in agreement with the work of Lawrie,[9] who found that normal skeletal muscle contained not more than 5% of residual haemoglobin.

The pigs examined in this investigation were electrically stunned before killing, whereas the sheep and cattle were not. It was considered necessary therefore to investigate the possible effect of such treatment on ultimate pH and colour. Accordingly, a pig was bled to death without stunning and examined. Similar results to those found for the electrically stunned animals were obtained. As further confirmation, a sheep was electrically stunned before killing, and the muscles examined. The ultimate pH of each muscle examined was constant throughout, and no pigment variation within them was apparent, apart from a certain amount of 'blood splashing' due to spastic shock.

Discussion

The variation of the ultimate pH from one muscle to another within a carcass has been known for many years. Apart, however, from the slight variations reported by Callow[5] and Bate-Smith[4] within individual muscles, which they ascribed to the presence of connective tissue or proximity to bone, no reference has apparently been made to variations of ultimate pH within individual muscles.

Interesting correlations between age, species, myoglobin content, succinic dehydrogenase activity, and labile phosphate synthesis, have been made by Lawrie.[9, 19] This work is an extension of that of Whipple[20] and Biörck,[21] who related muscle function and activity to its myoglobin content. The part played by myoglobin as an oxygen reserve for the cytochrome–cytochrome oxidase system of muscle was elucidated by Keilin,[22] and its effect upon the rate of fall of pH as a muscle in minced form went into *rigor* was demonstrated by Lawrie.[19] While myoglobin and cytochrome act as oxygen reserves for a muscle during life it is difficult to see how they can affect the ultimate pH attained by a muscle once the supply of oxygen to it ceases after the death of the animal. The correlation between ultimate pH and myoglobin content in pig muscles is however evident from Table II. Statistical treatment of the data of Table II shows a highly significant correlation between pH and pigment concentration (P).

For the relation

$$P = b(\text{pH}) + \text{constant}$$

where b gives the relation between P (pigment) and pH it is found that the overall within-animal regression is $0{\cdot}47019 \pm 0{\cdot}05186$.

Hence applying the t test:

$$t = \frac{0{\cdot}47019}{0{\cdot}05186} = 9{\cdot}6$$

which is significant at the 0·1% level.

The increased amount of protein due to the higher myoglobin content in certain parts of a muscle will not affect the pH, since the buffering power of protein is in any case low.

In the sampling of muscles for pH determinations certain precautions are clearly necessary if fallacious values are not to be obtained. In the case of pig muscles an additional variation within individual muscles occurs over and above the effects of oxygen penetration and variations in connective tissue. Such variations in ultimate pH can be quite large and in view of these findings the significance of many of the deductions based upon the ultimate pH of pig muscles must be treated with some reserve.

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THE LIPOXIDASE ACTIVITY OF WHEAT

By J. A. BLAIN and J. P. TODD

The evaluation of lipoxidase activity in extracts of wheat constituents is described and some characteristics of the carotene-bleaching system used have been examined.

Information on the distribution of the enzyme in the wheat berry has been obtained.

Introduction

The catalytic peroxidation by soya-bean lipoxidase of linoleic acid and other fatty acids having methylene-interrupted double bonds is well known; the subject has recently been reviewed by Holman.[1] Manometric measurement of oxygen uptake, spectrophotometric assay of the diene conjugation of substrate, and measurement of secondary destruction of carotene by the oxidation products of the primary substrate have all been used to study the action of the enzyme system. But, while soya lipoxidase has been the subject of numerous studies, there have been

few observations on the lipoxidase activity of wheat. Sumner,[2] using an assay based on the conversion of ferrous to ferric iron in the presence of fatty peroxides, quoted a value for wheat germ, and Miller & Kummerow[3] examined the effect of baking on flour lipoxidase, and compared the activities of wheat mill fractions with that of soya-bean. Their assay procedure was based on carotene destruction. Irvine & Winkler[4] studied the effect of semolina lipoxidase on macaroni colour, and Irvine & Anderson,[5] measuring linoleate oxidation by a manometric technique, made a comprehensive kinetic study of the lipoxidase activity of semolina extracts.

The peroxidation of unsaturated fatty acids is catalysed also by haematin compounds[6] but the term lipoxidase is used in this paper to describe the active factor in wheat, as has been the practice of previous workers in this field. At present the evidence available is insufficient to exclude the possibility that haematin compounds may also be involved.

In this country, in recent years, certain techniques for making bread with unbleached, untreated flour have been used[7] or proposed.[8] Such techniques involve the coupled bleaching of flour carotenoids by lipoxidase-catalysed peroxidation of the unsaturated fat present in dough, and while soya-bean meal may be added, it appears that the lipoxidase present in flour plays a significant part in the process.

The work described in this paper concerns the application of an assay based on destruction of carotene to the evaluation of lipoxidase activity in wheat. A carotene–linoleate system developed for the assay of soya extracts is used.[9] This assay operates at a pH value at which the primary substrate, linoleate, and the enzyme are in different phases, and any natural surface-active factors occurring in crude extracts of lipoxidase may affect dispersion and reaction interface.

For this reason, when examining extracts of wheat fractions, it has been considered necessary to reassess certain characteristics of the assay as established previously for soya.

Experimental

Reagents and apparatus

Reagents and apparatus are as described previously[9] for the assay of soya lipoxidase with minor modifications which are referred to in the text.

Preparation of material

Commercial wheat germ consists of mill-flattened embryo with some scutellum and smaller inclusions of bran and endosperm. The term embryo is used here to describe the non-scutellar portion of germ. In an attempt to get an entity less subject to variation than the commercial product, uncrushed embryo was used in this work. Endosperm was also used in relatively large particles to aid separation.

Samples of wheat, normally 200 g., were crushed by passing through a Kenrick No. 3 hand mill, to an extent sufficient to release the embryo, then shaken on stainless steel sieves, the 20/30-mesh fractions being retained as embryo source. The remainder was then remilled and resieved until about 85% passed a 20-mesh sieve, the 30/40-mesh fraction being retained as endosperm source.

From these fractions most of the unattached bran was removed electrostatically by placing them on a vibrated metal plate. A polythene sheet, charged by friction, was lowered carefully over the plate several times, and the bran particles were attracted preferentially and thus removed.

The endosperm source was poured into a 7 : 93 v/v mixture of pentane and redistilled trichloroethylene in which endosperm particles sank, while the rest of the material, including scutellum, floated and was poured off. The endosperm fraction was then washed with pentane, air-dried, and ground in a mortar to pass a 60-mesh sieve, branny residue being discarded.

The embryo source was poured into a 15 : 85 v/v mixture of pentane and redistilled trichloroethylene. Only the embryo floated and this was poured off, washed with ether, further defatted by Soxhlet extraction with ether (6 hours), and air-dried.

For optimum separation by flotation it was sometimes necessary to vary the proportions of solvents in the flotation mixture according to the moisture content of the wheats used.

Preliminary tests indicated that, as noted by Irvine & Anderson[5] for semolina, defatting made no significant difference to the lipoxidase activity of endosperm.

It was found that if samples of commercial wheat germ were defatted by repeated cold extraction with trichloroethylene they were no less active than when defatted by Soxhlet extraction with pentane or ether, and it was thus assumed that exposure to trichloroethylene in separation would not cause lipoxidase destruction. Ether appears to remove the fat from embryo more rapidly than does pentane and for this reason was used in its Soxhlet extraction.

The embryo obtained by this method was mostly unbroken, and free of bran and endosperm, although traces of scutellum were adherent.

The endosperm was free of germ contaminants and contained a very small amount of attached bran which was considered to have a negligible effect on the assay.

It should be noted that for ease of separation an arbitrary range of particle size of endosperm was selected. Table II shows that particles small enough to pass a 40-mesh sieve exhibit higher activity, due possibly to inclusion of the more active components of the berry, since the solvent separation is less effective in the separation of finer particles. On the other hand the 30/40-mesh fraction appears not to differ in activity from the 20/30-mesh fraction.

Buffer extraction

Normally quantities of 1 g. for embryo and 5 g. for endosperm and whole wheat were ground intensively in a mortar with an equal weight of sand and from 2 to 10 ml. of 0·1N-acetate buffer of pH 4·5. The ground material was then washed into the extraction bottles with enough buffer to bring the volume of added buffer to 30 ml., and suspended with gentle agitation for 30 minutes.

Water or buffer could be used indifferently to extract endosperm, but if water were used as extraction solvent for embryo it was difficult to obtain clear extracts by centrifuging.

Extractions were carried out in 50-ml. centrifuge bottles with polythene-covered corks. The samples were then centrifuged at 3000 *g* for 15 minutes, and the supernatant liquid passed through Whatman No. 1 filter paper to remove floating particles before assay.

The buffer extraction of defatted soya flour was carried out in a similar fashion to that of embryo, subsequent dilution of about 1 in 10 being made with acetate buffer prior to assay.

Assay

The assay used is essentially as described previously for soya extracts.[9] The enzyme solution is added to a suspension of linoleate and carotene in buffer and allowed to act for a specified time, after which the reaction is stopped by the addition of sodium hydroxide and the residual carotene is estimated colorimetrically against a distilled water blank. A control, differing only in that the enzyme solution is added after the sodium hydroxide, is also read against distilled water, and carotene destruction found by subtraction.

In this work a reaction temperature of 20° has been used throughout.

Extracts of embryo are found to give high blanks caused by development of a yellow colour on addition of alkali. This may be attributed to the presence of water-soluble flavonoids, known to be present in wheat germ.[10] However, this colour reaches its maximum intensity very rapidly and is stable under assay conditions.

Data on the effect of pH on the activity of wheat extracts will be published in a separate paper. The responses are similar to that of soya in having optima between pH 5 and pH 6.

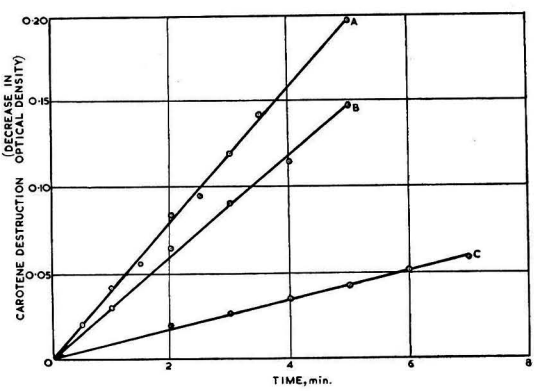

FIG. 1.—*Carotene destruction in relation to time for extracts of A. wheat embryo, B. whole wheat and C. wheat endosperm*

The nature of the pH effects on this assay system has been discussed previously.[9] The citrate–phosphate buffer of pH 5, which is used, gives in the weakly-buffered system a pH of 5·4 after normal additions have been made, and it is at this pH that the results described here have been obtained.

It can be seen from Fig. 1 that the rate of carotene destruction is linear with time for extracts of embryo, endosperm and whole wheat, over the assay periods used.

Figs. 2, 3 and 4 show that for these extracts the rates of carotene destruction are directly proportional to concentration of enzyme source over a limited range. With soya extracts at corresponding levels of carotene destruction, the diminished response to higher levels of enzyme

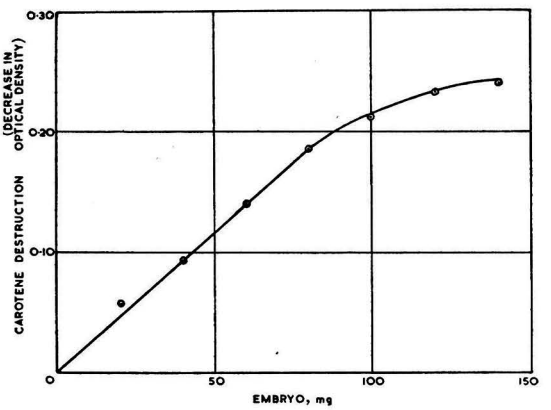

FIG. 2.—*Relation between carotene destruction and concentration of embryo lipoxidase (expressed as mg. of embryo) in system.—One-minute assay*

extract does not occur,[9] and the effect in wheat is more marked with endosperm than with embryo. With whole wheat, only low levels of carotene bleaching can be obtained on the linear portion of the curve. For assay purposes, it is therefore desirable to limit additions of crude extracts so that linearity of bleaching with volume of extract added is retained, and at the same time to have adequate carotene destruction. For this reason a 5-minute reaction time has been adopted in general. For embryo assay it is possible to use a 1-minute reaction period and obtain adequate carotene destruction over the linear portion of the curve, and it has been considered valid to do

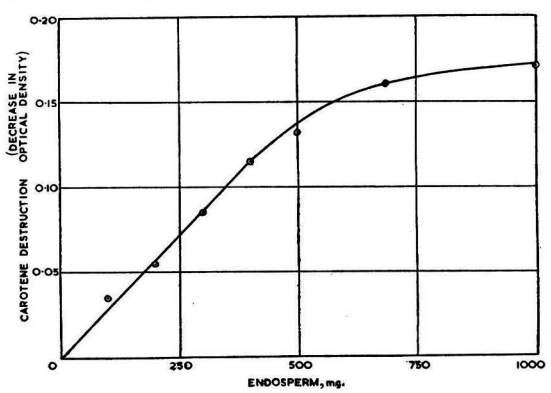

FIG. 3.—*Relation between carotene destruction and concentration of endosperm lipoxidase (expressed as mg. of endosperm) in system.—Five-minute assay*

this, and to calculate destruction of carotene over 5 minutes for comparison with the weaker sources of enzyme. Quantities of the order of 0·1 to 1 ml. of the buffer extracts prepared as described are added to the reaction mixture. Levels of carotene destruction are not normally more than 50% for embryo and not more than 30% for endosperm and whole wheat, under the conditions specified, the total carotene initially present being 0·03 mg.

Carotene destruction for three levels of added enzyme are plotted in the linear region of the

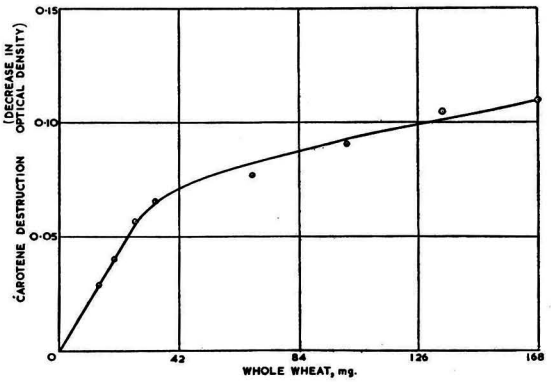

FIG. 4.—*Relation between carotene destruction and concentration of whole wheat lipoxidase (expressed as mg. of whole wheat) in system.—Five-minute assay*

curve and the level necessary to obtain 50% bleaching (corresponding to 0·015 mg. carotene) is found, extrapolating if necessary.

For the purpose of comparison of samples it has been convenient to define the unit of activity as that amount necessary to destroy 0·015 mg. carotene in 5 minutes at 20° under assay conditions.

Figs. 5, 6 and 7 illustrate the effect of substrate concentration on the activity of extracts of soya, embryo and endosperm.

The apparent inhibition of soya activity by excess substrate in a carotene–linoleate system was attributed by Balls *et al.*[11] to insufficiency of an activator present in soya extracts. They found also that the actual extent of development of fatty peroxides was reduced. Blain *et al.*[9] found a similar inhibition which, however, did not manifest itself when carotene was eliminated from the system and the extent of linoleate destruction was found by measurement of diene

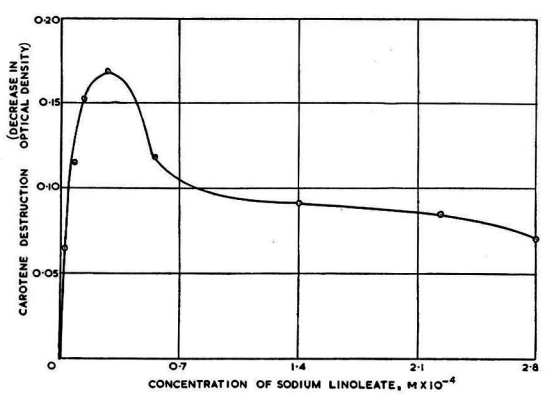

FIG. 5.—*Relation between carotene destruction by soya extract and concentration of sodium linoleate in assay system*

conjugation. Tentatively one might suppose that excess substrate interferes with either peroxidation of linoleate, or destruction of carotene by the peroxidizing linoleate, and that absorption of excess substrate by the higher levels of extraneous protein, or other material in extracts of endosperm and embryo, accounts for lack of apparent substrate inhibition.

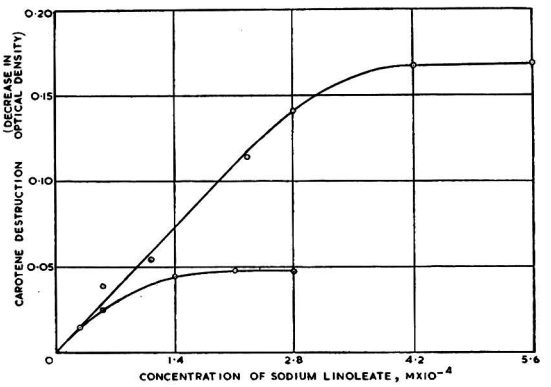

FIG. 6.—*Relation between carotene destruction by embryo extract at two levels and concentration of sodium linoleate in assay system*

This would also account for the fact that the level of substrate required by these extracts to obtain a comparable level of carotene destruction is greater than for soya.

In the assay of wheat constituents an addition of 1 ml. of substrate containing 4 mg. of linoleate is made, giving in the system a concentration of linoleate of $2{\cdot}8 \times 10^{-4}$M.

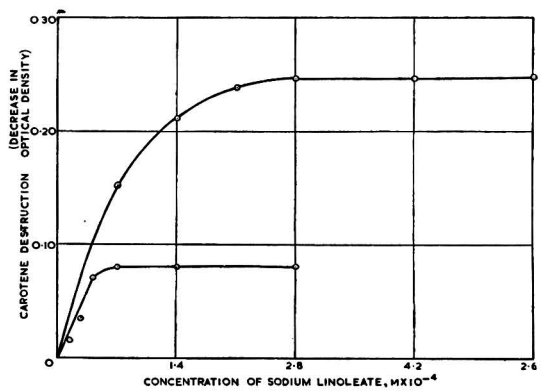

FIG. 7.—*Relation between carotene destruction by endosperm extract at two levels and concentration of sodium linoleate in assay system*

Results

The activities of the embryo and endosperm of samples of wheats commonly used for bread-making in this country have been examined, and results, along with those for two durum wheats, are given in Table I.

It is not intended here to make a comparison of the lipoxidase activities of different wheat types, but merely to indicate the range of variation of enzyme activity which may be encountered.

Results are expressed in units activity/g. dry fat-free weight in the case of embryo and units activity/g. dry weight in the case of endosperm.

Duplicate figures represent parallel assays on the same wheat sample.

Table I

Lipoxidase activity of various wheat types (units per g.)

Wheat type	Dry, fat-free embryo	Dry endosperm
Northern No. 1 (A)	60, 80	1·6
Northern No. 1 (B)	94, 90	2·0
Australian	56, 60	1·3, 1·4
Garnet	49, 55	—
Canadian Durum	40, 44	0·9, 1·1
Syrian Durum	40, 31	1·3, 1·3
Native Red	60, 60	—

Bran.—As obtained by milling, bran is of indefinite composition including a varying amount of aleurone layer along with the fibrous external coats of the berry. Attempts to measure its lipoxidase activity were unsuccessful, as a constant low level of bleaching was obtained for varying volumes of added extract over a wide range, and it was obvious that activity proportional to the added volume of extract at levels of carotene bleaching sufficiently high to obtain reproducible results could not be obtained. It was possible only to make estimates of minimal activity, which for the three wheats shown in Table II appeared to be about 10 units/g.

Indications of this high activity associated with the outer layers of the berry have been obtained indirectly, along with the activities of other portions of the berry, by hand dissection of three samples of wheat.

Dissected samples.—In each case a random selection of 100 grains was made and partial dissection carried out as follows. The outer layer covering the scutellum was scraped aside and the embryo levered out with a blunt scalpel. The scutellum was then removed using a spatula filed to have a $\frac{1}{16}$-in. wide, concave point. Thus an embryo fraction having a little attached scutellum, a scutellar fraction which included fragments of bran, and a residual portion of de-germed wheat was obtained. The percentage weights were as follows:

	Percentage weights		
	Embryo	Scutellum	Remainder
Northern No. 2	1·34%	1·08%	97·54%
Australian	1·27%	1·20%	97·53%
Garnet	1·49%	0·98%	97·52%

Endosperm from the same wheats was obtained as described previously. After flotation separation, the 20/30-mesh endosperm fraction and the 30/40-mesh endosperm fraction were each about 15% by weight of the total wheat. Fractions were also obtained by halving the berry at right angles to the long axis to obtain one half containing all the germ, and the other germ-free.

All these components were defatted and ground intensively in acetate buffer with sand before shaking to prepare buffer extracts. The assay results are shown in Table II.

Table II

Lipoxidase activity of wheat fractions (units per g.)

Fraction	Wheat		
	Northern No. 2	Australian	Garnet No. 3
Endosperm 20/30-mesh	1·8	1·3	1·3
Endosperm 30/40-mesh	1·8	1·4	1·1
Endosperm 40/60-mesh	2·8	2·6	2·1
Embryo	75	62	84
Scutellum	87	57	72
Entire wheat	11	7·4	7·7
De-germed wheat	7·5	6·0	6·2
Germ half	14	9·0	10
Germless half	7·0	5·1	5·8

Soya lipoxidase.—Since most processes utilizing the destruction of carotene in doughs by lipoxidase, to improve bread colour, have involved addition of soya, the definition of soya activity

in terms of the units used here to describe that of wheat would be interesting. However as has been indicated in Figs. 5, 6 and 7, while wheat activity reaches a steady value above a minimal level of substrate, soya activity shows a definite optimum and then decreases with excess substrate. Simple comparison would thus not be valid.

However, when the activities of extracts of three freshly-ground soya samples were measured at their substrate optimum (about one-quarter of the level used to assay wheats) values of 900, 1150 and 1400 units/g. were obtained. Thus it would appear that activity of soya is from 20 to 30 times that of the average for wheat embryo, measuring each at its own optimum substrate level on the system.

Measurement of soya at the optimum substrate level for wheat would flatter the relative activity for wheat at the level of carotene bleaching used here.

Discussion

It appears from the results quoted in Table II that the lipoxidase level of scutellum differs little from that of embryo. The proportion by wt. of embryo in wheat is usually between 1% and 1·5%. The corresponding value for scutellum is between 1·4% and 1·8%.[12]

In the tests described here, the separation of scutellum is incomplete, a small portion being retained on the berry and some adhering to the embryo. The high activity of degermed wheat compared with that of the germless half may be due to residual scutellum on the former fraction.

Several samples of commercial germ have also been assayed by the method described, and showed activities of between 50 and 100 units/g.

Although values for embryo or scutellum have not previously been published, Miller & Kummerow[3] have compared the activities of germ with various other fractions of the type obtained by commercial milling. They used the carotene-bleaching assay of Mitchell & King,[13] in which the lipoxidase acts on a Wesson Oil substrate in the presence of carotene for one hour at 36°, after which undestroyed carotene is extracted from the aqueous phase and assayed. They found germ to be about 20 times as active as patent flour, and our figures are in reasonable agreement with this. They also found bran to be just over three times as active as patent flour. The ratio of activity of degermed wheat to endosperm as indicated in Table II suggests that there is more activity in the external layers of the berry than is indicated by the value given by Miller and Kummerow. It may be that the aleurone layer is of high activity and that bran varies widely according to the amount of aleurone associated with it.

As far as could be found, the only other workers who have obtained values relating the lipoxidase activities of wheat components are Irvine & Anderson.[5] They compared values for whole wheat with those for 50% extraction semolina, and taking the mean of 89 samples found a ratio of about 4½ to 1.[14] The ratio was as high as 8 to 1 for wheats very low in lipoxidase. The relative values indicated here are in harmony with this figure, since the endosperm used would be expected to have lower activity than 50% extraction semolina.

Miller and Kummerow found the lipoxidase activity of soya to be about 200 times that of wheat germ, but Sumner, using a method of assay based on the oxidation of ferrous iron by fatty peroxides,[2] found soya to be only 40 times as active as wheat germ. Sumner's figure is of the same order as the values reported here. Although the characteristics of the carotene-bleaching system used by Miller & Kummerow are not described, it may be they resemble those described here in that soya and wheat extracts show optimum bleaching at very different substrate levels. Their available substrate level could be such as to elevate the relative value for soya.

In general it would seem that the carotene-bleaching assay for soya lipoxidase, which we have described previously,[9] can be applied successfully to products having much less activity than soya. It is plain however that to obtain valid results the characteristics of the assay must be examined in relation to the enzyme source. An inconvenient feature is the restricted range over which carotene destruction is proportional to enzyme concentration. The manometric method described by Irvine & Anderson does not appear to suffer from this limitation, which may be inherent in the use of carotene as a secondary substrate.

Nevertheless, in lipoxidase studies over a broader field, it is probable that valuable information can be obtained by a study of oxidation of secondary substrates such as carotene, in addition to that of the primary substrates, such as linoleate, by lipoxidase. This is stressed by the

observation of Tappel *et al.*,[15] that an antioxidant could be oxidized in the presence of linoleate and lipoxidase without the concurrent oxidation of linoleate. As has been pointed out by Holman,[1] this leads to the possible concept of linoleate functioning as a prosthetic group for the oxidation by lipoxidase of a variety of substrates.

Acknowledgment

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THE COLORIMETRIC DETERMINATION OF PHOSPHORUS IN PLANT MATERIALS

By A. J. CAVELL

The determination of phosphate in the presence of hydrochloric acid with Cavell's ammonium vanadate–ammonium molybdate reagent[2] has been studied and made quantitative.

When analysing plant materials which are not exceedingly high in phosphorus and low in calcium it is permissible to determine phosphorus in the hydrochloric acid extracts of the ashed materials.[1] It has now been shown that the phosphorus in such extracts can be speedily and accurately determined colorimetrically as the yellow phospho-vanado-molybdate using the mixed solution of ammonium vanadate and ammonium molybdate previously described.[2]

Variations in the amount of hydrochloric acid present in phosphate solutions containing 0–4 mg. of phosphorus pentoxide per 100 ml. may alter the transmittancies measured at 400 mμ. If the acidity of the solution to which an equal volume of the mixed reagent is to be added exceeds 2N the rate of colour development is retarded. Colour development to a constant transmittancy takes place in five minutes if the acidity lies between 0·5N and 1·5N.

Experiments with phosphate solutions containing added cations indicated that the following method would be suitable for the determination of phosphorus in the hydrochloric acid extracts of normal plant ashes. The error due to the absorption at 400 mμ by ferric chloride derived from a plant material containing 0·1% iron would be less than + 0·01 in the percentage of phosphate determined. The percentage of iron in plant materials is usually very much less than 0·1%.[3]

The reagent (R) used is an ammonium molybdate/ammonium vanadate mixture, prepared as follows: dissolve 5 g. of ammonium molybdate and 0·25 g. of ammonium vanadate in warm water, cool, dilute to 500 ml. and filter. The mixed reagent is very stable and batch differences are negligible.

Procedure

Ash 5 g. of the material at 550°. Moisten with 5 ml. of concentrated hydrochloric acid, evaporate to dryness and dehydrate the silica by baking at 105°. Moisten the residue with 5 ml. of concentrated hydrochloric acid, and gently boil for 2 minutes: add 25 ml. of water, boil, filter through a 9-cm. Whatman No. 30 paper and make the filtrate and washings up to 250 ml. The acidity of this solution (A) will not exceed 0·25N.

Pipette 10 ml. of A into a 50-ml. graduated flask, add 10 ml. of 5N-hydrochloric acid and dilute to 50 ml. with water to give a final acidity of approx. N. Pipette 5 ml. of this solution into a dry glass-stoppered tube, leave in a water-bath at 20° for 5 minutes and then add exactly 5 ml. of the mixed reagent (R), also at 20°. Mix the solutions by inverting the tubes several times, allow the tube to remain in the water-bath at 20° for at least 5 minutes and then measure the transmittancy at 400 mμ with an SP. 600 Unicam Spectrophotometer, using 1-cm. cells: read off the percentage of phosphorus pentoxide in the plant material from a calibration curve.

A standard graph may be obtained by plotting the transmittancy at 400 mμ of solutions made by mixing 5 ml. of the mixed reagent with 5 ml. of solutions containing up to 4 mg. of phosphorus pentoxide in 100 ml. of N-hydrochloric acid. The range is suitable for plant materials containing up to 1% of phosphorus pentoxide.

Results of analyses

The figures given in the Table show the agreement between results for various plant materials obtained by this method and by the volumetric method of Richards & Godden.[4]

Plant material	% Phosphorus as P_2O_5	
	Volumetric method	Colorimetric method
Clover	0·55	0·53
Grass	1·06	1·07
Hay 1	0·56	0·58
Hay 2	0·68	0·68
Hops	1·61	1·65
Kale leaves	0·70	0·68
Kale stems	0·73	0·70
Lucerne 1	0·78	0·78
Lucerne 2	0·67	0·68
Lucerne 3	1·49	1·50
Pea meal	1·06	1·03
Potatoes	0·40	0·40

National Agricultural Advisory Service
West Midland Province
Wolverhampton

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

AUGUST, 1955

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

INDEX OF AUTHORS' NAMES

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

AUGUST, 1955

I.—AGRICULTURE AND HORTICULTURE

General, Soils, and Fertilisers

Pennsylvania Agricultural Experiment Station, 67th Annual Reports. Anon. (*Pa. agric. Exp. Sta.*, 1954, *Bull.* 578, 20 pp.).—The annual reports for the year ended June 30, 1954.
A. H. CORNFIELD.

Natural classification of soils. J. Tokarski (*Roczn. Glebozn.*, 1954, **3**, 57—105).—A quant. method of soil classification based on mechanical composition and the sand, clay, humus, and carbonate contents, is discussed. The mineral content of the soil is determined by a thermal method based on losses at 150° (dehydration of montmorillonite), 400° (burning of humus), 500° (dehydration of kaolin), and 900° (dissociation of calcite). The mechanical composition is determined by microscopy.
SOILS & FERT. (A. G. P.).

Proposed classification of the accumulation zones of free sesquioxides on a genetic basis. J. d'Hoore (*Afr. Soils*, 1954, **3**, 66—81).—Conditions necessary for accumulation and induration, and the morphology of absolute and relative accumulation zones are discussed. The framework of the proposed classification is explained.
SOILS & FERT. (A. G. P.).

The laterites of Gumbi (Lower Congo). G. Waegemans (*Publ. Inst. nat. agron. Congo Belge, Ser. Sci.*, 1954, No. 60, 27 pp.).—In a method of analysis described, the Fe sesquioxide cement is dissolved without interference with the accompanying minerals. The determinations of quartz, kaolinite, free Al oxides, and hydration index of free Fe oxides are described. The composition, structure, and development of granular and slag-like formations are discussed.
SOILS & FERT. (A. G. P.).

Characterisation and classification of soils in the White Sugar Belt of Bihar. IV. Profile distribution of manganese. K. L. Khanna, P. B. Bhattacharya, and K. L. Joneja (*J. Indian Soc. Soil Sci.*, 1954, **2**, 105—109).—In the highly calcareous (40% $CaCO_3$) light-textured types of soil exchangeable and reducible Mn (Leeper's extracts, *Soil Sci.*, 1947, **63**, 79) decreased with depth of profile. In the slightly calcareous (2% $CaCO_3$) light-medium textured type exchangeable Mn decreased and reducible Mn increased with depth. In the slightly calcareous heavy clay type both exchangeable and reducible Mn remained approx. constant with depth. Exchangeable Mn was of the same order for all three soil types, but reducible Mn was much higher in the slightly calcareous than in the highly calcareous types. Negligible amounts of water-sol. Mn were present in all three types.
A. H. CORNFIELD.

Soil textural classes. H. Segeberg (*Z. PflErnähr. Düng.*, 1955, **68**, 237—239).—Some changes in the grouping of soils according to textural classes are discussed in relation to both the tabular and graphical (triangular co-ordinate) methods of presentation.
A. H. CORNFIELD.

Bulk density of stony and gravelly soils. E. Vetterlein (*Z. PflErnähr. Düng.*, 1955, **68**, 193—203).—A method of determining the bulk density and pore vol. of stony and gravelly soils, to which soils the core sampling method is not applicable, is described. An undisturbed cube of soil is sprayed with molten paraffin wax to stabilise it during vol. measurements by the hydrostatic method. Changes in pore space with depth for a no. of gravelly and stony soils are presented.
A. H. CORNFIELD.

Determining porosity, specific gravity, and air capacity of soils. Cz. Swięcicki (*Roczn. Glebozn.*, 1954, **3**, 175—202).—An air pycnometer, giving readings independent of changes in atm. pressure, is described. A method for determining water-resistant structural clods makes it possible to establish relationships between the sum of water-stable aggregates (0·25—10·0 m.m.) of non-humus soils and the ratio of mechanical fractions (<0·005 : 0·05—0·005) determined by the aërometric method. Good structure with high porosity is related to non-acid humus with a high 0·05—0·005 fraction.
SOILS & FERT. (A. G. P.).

Specific gravity of peat. H. Segeberg (*Z. PflErnähr. Düng.*, 1955, **68**, 233—237).—Practical details for the sampling, drying, and sp. gr. determination of peats are described. More satisfactory results are obtained if the determination is done on the air-dried than on the field-moist sample.
A. H. CORNFIELD.

Soil structure studies with synthetic conditioners. R. B. Alderfer (*Pa. agric. Exp. Sta.*, 1954, *Bull.* 586, 46 pp.).—Pre-sowing applications of hydrolysed *poly*acrylonitrile or vinyl acetate–maleic acid copolymer (0·025—0·2%) to soils of poor structure usually resulted in improved aggregation of soils and increased yields of most, but not of all, of the vegetable crops tested. Crops yields in treated soils were usually closely correlated with aggregation and to a lesser extent with aeration porosity and permeability. Yields of crops were also generally increased by applications of solutions of soil conditioners over the row after sowing. In greenhouse tests structural improvement due to conditioner treatments was not accompanied by increased tomato yields. Resistance to compaction of turf soils was increased by treatment with conditioners in solution. At equal rates of application of conditioner, aggregation in soils of very similar texture was altered to considerably different extents. Extent of aggregation and size of aggregates were usually correlated with the amount of conditioner applied. The treatments had no effect on water holding capacity or wilting point of the soils. The aggregating effectiveness of conditioners was not altered by steam sterilising the soils after treatment.
A. H. CORNFIELD.

Water stability of CRD-treated soils as influenced by leaching. J. Hagin (*Bull. Res. Counc. Israel*, 1954, **4**, 297—299).—Coarse-textured and fine-textured soils were treated with 0·1% of CRD-186 (Krilium) polyelectrolyte soil conditioner and then leached with 1N-solutions of $BaCl_2$, NH_4 acetate and K_2HPO_4. Leaching did not affect the water stability of the soil aggregates. The results confirm earlier findings that the bonds between soil particles and the polymer particles are not of an ion-exchange character. It is suggested that working the soil after application of the conditioner may improve aggregation.
G. HELMS.

Aggregate stability in a clay soil in relation to cacao cultivation. G. Havord, T. E. Wasowicz, and R. G. White (*Trop. Agriculture, Trin.*, 1954, **31**, 233—241).— Cacao seedlings were grown in pots in soil which had been air-dried and sieved into aggregate fractions (<1, 2—4, and 4—12 mm.). Stem and leaf growth and root development were generally superior in the coarsest fraction. The % of nutrient elements in leaf ash was not greatly affected by the size of soil aggregates in which the plant grew.
A. G. POLLARD.

Acidoid value and surface area of soil separates. B. R. Puri, J. Singh, and L. R. Sharma (*Soil Sci.*, 1955, **79**, 199—205).—Various soils were separated into fractions of particle sizes between 0·4 and <0·0002 mm. diameter. Portions of the fractions were used to determine the acidoid val. by titration with $Ba(OH)_2$, the water content and the mechanical analysis, as well as the moisture content after the materials had been equilibrated with atmospheres of known R.H.. Surface area was calculated from the results of the mechanical analysis, from the moisture content–vapour pressure data or from the Orchiston formula for the water adsorption. In general, surface area calculations agreed with each other. On progressive drying it was found that aggregation increased, the aggregates being water resistant. A close relationship was established between surface area and acidoid value.
T. G. MORRIS.

Adsorption of water vapour. IV. Characterisation of expanding lattice minerals at 25. H. D. Orchiston (*Soil Sci.*, 1955, **79**, 221—224).—Two methods are discussed for the characterisation of clay minerals of the montmorillonite, bentonite, and illite types. One is based on the adsorption of water vapour, under defined conditions, before and after heating the mineral to 600°, and the other measures the ratios of Ca/K adsorption with relative pressure for water vapour. The first method requires the removal of impurities. Both methods suffer from the fact that mixed adsorbents do not display water-vapour adsorption of an additive nature.
T. G. MORRIS.

Erosion and its control. B. Mansingh (*J. Indian Soc. Soil Sci.*, 1954, **2**, 135—139).—A general discussion with particular reference to conditions in India.
A. H. CORNFIELD.

Soil acidity and basicity in the light of the latest research. J. di Gléria (*Acta agron. hung.*, 1954, **4**, 175—202).—A reinterpretation

of the principles of base exchange and associated phenomena in soil in terms of the modern theory of electrolytes. A. G. POLLARD.

Redox potential of soils. W. Flaig, K. Scharrer, and G. K. Judel (*Z. PflErnähr. Düng.*, 1955, **68**, 97—122).—Methods of determining the redox potential of soils are described. A. H. CORNFIELD.

Redox potential of soil profiles. W. Flaig, K. Scharrer, and G. K. Judel (*Z. PflErnähr. Düng.*, 1955, **68**, 203—218).—Redox potential and redox titration values, through the profile of six soils, both arable and natural, are presented. A. H. CORNFIELD.

Kinetics of ion exchange between two adsorbents. III. Hydrogen and hydroxyl systems. C. Krishnamoorthy and A. D. Desai (*Soil Sci.*, 1955, **79**, 215—220).—The problem of the behaviour of the H ion in exchange systems is discussed and equations are derived for the reaction between Ion-X (H-form) and IR-100 (NH_4-form) when it is assumed that (*a*) the entire H present is held by pure electrostatic forces on the adsorbent, and (*b*) only a portion of the H is so held. Exchange of H on Ion-X, IR-100, and clay was studied by the interaction of the H- and NH_4-forms of these adsorbents. The data indicate that monovalent ion systems involving H essentially follow film diffusion, slight deviations being traceable to incomplete dissociation of RH. Similar conclusions were drawn with regard to systems involving OH ions. T. G. MORRIS.

Base-exchange studies with Agra soil and Rajmahal clay. K. Zutshi and A. K. Bhattacharya (*J. Indian Soc. Soil Sci.*, 1954, **2**, 131—133).—In both soil and clay there were considerable differences in total exchangeable bases and base saturation when these values were determined by different methods. It is suggested that the best value for total exchangeable bases is that obtained by summing the values for individual bases. A. H. CORNFIELD.

Variation of cation exchange between colloidal clays and resins with concentration of the disperse phase. A. Chatterjee (*J. Indian Soc. Soil Sci.*, 1954, **2**, 111—114).—Cellophane bags containing varying amounts of H-resin were allowed to come to equilibrium with clay suspensions of varying concn. saturated with Na, K, or Ca, and the exchange of cations between the resins and clay was then determined. The exchange of Na was unaffected, that of K decreased slightly, whilst that of Ca decreased to a considerable extent with increasing concn. of clay. Exchange of all cations at all clay concn. increased considerably with the amount of resin present. Results are discussed in relation to the extent of hydration of the cations. A. H. CORNFIELD.

Silica gel as a microbiological medium: potentialities and a new method of preparation. J. M. Kingsbury and E. S. Barghoorn (*Appl. Microbiol.*, 1954, **2**, 5—8).—A series of tested formulæ and methods of preparation of nutrient gels based on colloidal silica (Ludox) are presented. The silica may be purified by the use of an ion-exchange system utilising both anion- and cation-exchange, final autoclaving producing a sterile gel. E. G. BRICKELL.

Adsorption of cetyltrimethylammonium bromide and Krilium by bentonite. H. Mukherjee (*J. Indian Soc. Soil Sci.*, 1954, **2**, 99—103).—The extent of adsorption of cetyltrimethylammonium bromide (**I**) by H-bentonite increased with the amount of **I** added. The adsorbed **I** was recovered to only a small extent by 0·1N-HCl. Adsorption of **I** was not restricted to base exchange sites. The extent of adsorption of Krilium (hydrolysed *poly*acrylonitrile) by both H- and Ca-bentonites increased with the amount of Krilium added. With any given level of Krilium, adsorption was greater on the Ca- than on the H-bentonite. Treatment with **I** reduced the hydrophilic and increased the organophilic nature of the clay. Krilium increased the hydrophilic and had no effect on the organophilic nature of the clay. A. H. CORNFIELD.

Electrochemical studies of pure clay minerals and their mixtures. S. K. Chakravarti (*J. Indian Soc. Soil Sci.*, 1954, **2**, 127—130).—pH–titration curves, using baryta, of two samples each of kaolinite and montmorillonite and of mixtures of the two types of minerals are presented. The buffer action increased and one of the two inflexion points due to kaolinite became more masked as the proportion of montmorillonite in the mixture increased. There were linear relationships between the % of montmorillonite in the mixture and both base-exchange capacity and $1/\tan\theta$ (where $\tan\theta$ is the slope of the straight line joining the origin to the point on the titration curve at pH 6·0). A. H. CORNFIELD.

Ammonia fixation by residual soil from crystalline schists at Yahatahama. T. Harada and K. Kutsuna (*Bull. nat. Inst. agric. Sci. Nishigahara*, 1954, Ser. B, No. 3, 17—41).—In moist soil the fixation of NH_4^+ in non-exchangeable form increased with temp. (8—40°). The absorption–time curve was logarithmic. A sigmoid relationship was demonstrated between the amount of NH_4^+ added to soil and the amount fixed. The fixation of NH_4^+ is associated with a decrease of base-exchange capacity. The presence of exchangeable K^+ lowered the amount of NH_4^+ fixed by occupying some of the positions in the clay lattice which would otherwise have been taken up by NH_4^+. The amount of NH_4^+ fixed was increased by drying the soil after treatment. At temp. >200° soil lost its capacity to fix NH_4^+. NH_4^+ fixed by moist soil was partly replaced by cations from N neutral salt solutions and completely displaced by N-HCl. Substantially all NH_4^+ fixed was held by particles 2—200 μ. in diameter, and mainly in those of 2—30 μ. A preliminary examination of soil minerals capable of fixing NH_4^+ is recorded. A. G. POLLARD.

Influence of lime and fertilisers on the mineralisation of peat nitrogen in incubation experiments. A. Kaila, S. Soini, and E. Kivinen (*Maataloust. Arhak.*, 1954, **26**, 79—95).—Samples examined were from virgin fen peats and bog peats and were only slightly decomposed. The accumulation of NO_3'-N during 8—10 months' incubation at 15—22° was relatively high in all limed samples, but was marked also in most unlimed samples except, notably, in a *Sphagnum fuscum* peat. During incubation at lower temp. lime stimulated ammonification in *S. fuscum* peat during the first month, but later limed and unlimed samples showed similar accumulations of mineral N. K, P, N, $CuSO_4$, $ZnCl_2$, and NH_4 molybdate had no effect. SOILS & FERT. (A. G. P.).

Modified colorimetric method for field determination of soil nitrate. W. R. Horne and O. T. Denmead (*J. Aust. Inst. agric. Sci.*, 1955, **21**, 34—36).—A simple and rapid colorimetric method, based on the brucine method suggested by Morgan (*Conn. agric. Exp. Stn Bull.*, 1941, 450) and described by Peech and English (*Soil Sci.*, 1944, **57**, 167), is described for the field determination of soil nitrate-N. The method is accurate to 1 p.p.m. and recovery of added NO_3' averaged 98%. Nitrite-N is included in the results. S. C. JOLLY.

Organic phosphorus in New Zealand soils under pasture. I. Conversion of applied phosphorus into organic forms. R. H. Jackman (*Soil Sci.*, 1955, **79**, 207—213).—Estimations were made of the total and org. P contents at different depths of a variety of soils carrying grass or clover or both, and receiving lime or superphosphate alone or together. Conversion of added P into the org. form apparently occurred in most soils at all depths but was statistically significant in only the top 1 in. in yellow brown loams; in podsolic soils the conversion was up to 33%. Lime appeared to have had little effect on these changes. On a silt loam in which sheep dung and urine were returned to the soil, and which was also treated with superphosphate and lime, and then cropped with clover, little conversion of added P took place. A similar trial on a mature podsolic sand gave similar results, except that some fertiliser P was leached. T. G. MORRIS.

Fertilisation of a laterite with soluble phosphate and the effect of soil amendments on phosphorus availability. G. P. Gokhale, M. M. Kibe, N. Narayana, and H. G. Pandya (*J. Indian Soc. Soil Sci.*, 1954, **2**, 141—147).—The P status (as measured by H_2CO_3 extraction) of a lateritic soil of pH 5·2 was not improved materially by addition of sol. PO_4'''. Addition of 0·5—2·0% of $CaCO_3$ prior to PO_4''' application resulted in a higher P status. Addition of 5—20% of Na_2SiO_3 prior to PO_4''' application resulted in even higher P status, but also brought the soil to a high pH. Addition of org. manure was ineffective in maintaining applied PO_4''' in an available condition. A. H. CORNFIELD.

Determination of phosphoric acid in soil with use of sodium acetate buffer solution as solvent. J. Benjaminsen (*Tidsskr. Planteavl*, 1955, **58**, 627—650).—With solvents of concn. 0·2—1·0N-acetic acid + 0·2—1·0N-Na acetate, used in a ratio soil to solvent of 1 : 10, the pH is very nearly always maintained at 4·7 during 8 hr.; during the first hr., >80% of the total extractable PO_4''' is dissolved. The effect of variations in the amount of soil used in a constant vol. of solvent is satisfactorily expressed by the Bondorff equation. With a soil to solvent ratio greater than 1 : 5, the amount of PO_4''' dissolved is almost always an increasing function of the buffer concn., but with closer ratios, the results are unpredictable. The abnormal behaviour of isolated samples is discussed. P. S. ARUP.

Effect of procedures for extraction of free oxides of iron in soils on the iron compounds present therein. O. Robichet (*C. R. Acad. Sci., Paris*, 1955, **240**, 1354—1355).—The different standard methods for extraction of specific Fe oxides in soil minerals are compared by making two extractions by each procedure on 100 mg. of sample (⩾0·2 mm. particle-size). For one extraction, the Drosdoff–Truog method (reduction with H_2S and dissolution of FeS with HCl) is most effective, but with two extractions Deb's method (*J. Soil Sci.*, 1950, **1**, 212) (reduction by $Na_2S_2O_4$ followed by solution in HCl) yields the highest % of Fe (70—98) from all minerals except chlorites and siderite, for which the Fe extraction is only 5·4%. Tamm's method (dissolution with org. acid and complexing of Fe with an oxalate-buffer at pH 3·2) is useless both as regards specificity and effectiveness (<10% Fe yield with two extractions). W. J. BAKER.

Movement of iron in the development of loess-derived brunizem soils. R. M. Swenson and F. F. Riecken (*Soil Sci.*, 1955, **79**, 177—186).—Samples of soil from five horizons of two loess-derived soils, one slightly and the other highly weathered, were mechanically divided into $<0{\cdot}2\mu.$, $0{\cdot}2$—$2\mu.$, 2—$5\mu.$, 5—$20\mu.$, and $>20\mu.$ fractions. In general, the total Fe content decreased as the particle size decreased; in most cases the total Fe content was similar throughout the profile depth. In the slightly weathered profile the Fe content of the clay fractions increased with depth, probably due to accumulation from the upper horizons. The average Fe content of the highly weathered profile was higher than that of the slightly weathered, and variations within the profile suggest a redistribution during soil formation. In the slightly weathered profile, the $0{\cdot}2$—$2\mu.$ fraction contained a larger percentage of total Fe than did the $<0{\cdot}2\mu.$ fraction, whilst in the other profile the reverse was true. The $>20\mu.$ fraction of the highly weathered soil contained more free Fe than did the corresponding fraction of the other soil, the free Fe contents of the 2—$5\mu.$ and 5—$20\mu.$ fractions of both soils were similar, and the free Fe content of the finer fractions of the slightly weathered soil was the higher. The difference between total and free Fe is taken as lattice Fe: above 12 in. in the highly weathered profile the Fe content was less, and below 12 in. more than in the other profile. Below 12 in. more lattice Fe was found in the $<0{\cdot}02\mu.$ fraction of the highly weathered profile than in all the other fractions combined. T. G. MORRIS.

Soil manganese. I. Use of disodium calcium Versenate for extraction of divalent manganese from soils. R. S. Beckwith (*Aust. J. agric. Res.*, 1955, **6**, 299—307).—The soil is extracted at pH 8·0 with Ca and NH_4 acetate containing 1% of Na_2Ca Versenate. The extractant has little effect on three pptd. Mn oxides having respectively the structures of hausmannite, manganite, and manganous manganite; the attack on the oxides is not increased in the presence of soils. The procedure is of value for the removal of Mn^{++} formed by treatment of soils with reducing agents. Results with three pptd. oxides indicate that the degree of attack by quinol. in the presence of the extractant at pH 8 reflects the availability of these oxides to oats grown on a fen soil. R. H. HURST.

Rapid manometric method for determining soil carbonate. A. E. Martin and R. Reeve (*Soil Sci.*, 1955, **79**, 187—197).—The soil is shaken mechanically with HCl in a sealed bottle, for three periods of 5 min. After standing to equilibrate the temp., the bottle is connected to a mercury manometer, unsealed, and the pressure read off directly. The manometer bottles were calibrated with $CaCO_3$. This method and the titrimetric method on 67 soils gave virtually identical results. Reproducibility was not so good as was that of the titrimetric method for soils low in carbonate. Variations were reduced by grinding samples to pass a 0·5-mm. sieve. The effect of MnO_2 on the recovery of $CaCO_3$ added to org. non-calcareous soils was examined. On a soil with 4% of total C up to 4% of MnO_2 had little effect, but when the total C content was 10% this amount of MnO_2 raised the apparent $CaCO_3$ content by 10%. Addition of $FeCl_3$ reduced this interference to a low figure. Decarboxylation of soil org. matter by the HCl used had little effect on the $CaCO_3$ figure. T. G. MORRIS.

Humic acids in decomposed plant roots. H. E. Freytag (*Z. PflErnähr. Düng.*, 1955, **68**, 123—132).—Spectral adsorption curves of extracts of the humic acid fraction of decomposed lucerne roots are presented and compared with those of similar extracts from a black earth. A. H. CORNFIELD.

Distribution and nitrogen-fixing activity of *Azotobacter* in the rhizosphere of perennial herbage. M. V. Federov and V. F. Nepomiluev (*Mikrobiologiya*, 1954, **23**, 275—282).—Strains of *Azotobacter*, present in the rhizosphere of clover and timothy of all ages, when isolated had low N-fixing and oxidising activity. Numbers of *Azotobacter* in the rhizosphere declined with advancing age of the herbage and were higher during spring growth than during budding and flowering. If soil was inoculated with *Azotobacter* when the herbage was sown, the organisms became partially acclimatised to the rhizosphere with a loss of N-fixing capacity unless org. fertilisers were applied. SOILS & FERT. (A. G. P.).

Distribution of pectin-decomposing micro-organisms in nature. K. T. Wieringa (*Landbouwk. Tijdschr., 's Grav.*, 1954, **66**, 292—299).—Bacteria and fungi producing pectin-splitting enzymes are listed. Two species of snail produce a similar enzyme. Effects of different rates of marling on the distribution of the pectin-splitting microflora of soil are described. SOILS & FERT. (A. G. P.).

Climate, plant, and soil. H. Glathe (*Landb.-Forsch.*, 1954, **4**, 44—45).—Four soils were placed in frames in different localities and planted with potatoes. Results indicated that climatic conditions and their possible influence on microbial activity rather than the soil itself were the main factors determining production. SOILS & FERT. (A. G. P.).

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Effects of BHC on soil micro-organisms. III. Heterotrophic bacteria. P. H. H. Gray (*Appl. Microbiol.*, 1954, **2**, 37—40).—BHC and its γ-isomer were studied. Thirty-five strains did not grow in presence of BHC but were not affected by the γ-isomer, five species of phenol-decomposing bacteria were inhibited, starch hydrolysis was prevented in four species of amylolytic bacteria and reduced in three others, and growth of *Cytophaga*, growth and nitrate, reduction by *B. mesentericus* and nitrate reduction but not growth of *Micrococcus pyogenes* var. *aureus* were prevented. γ-BHC prevented the reduction of SO_4'' by a mixed soil microflora in Elion's medium. E. G. BRICKELL.

Production of mycorrhiza in oak seedlings. M. V. Aleskovskiĭ (*Mikrobiologiya.*, 1954, **23**, 297—303).—The morphology of six types of mycorrhiza is described. The type of mycorrhiza usually varies with the soil. Introduction into soil of mycorrhizal fungi from soil of another district may not be very effective in establishing a mycorrhiza SOILS & FERT. (A. G. P.).

Mycorrhiza of *Pseudotsuga taxifolia*, Britt. G. Linnemann (*Zbl. Bakt.*, 1955, **108**, II, 398—410).—The nature of the mycorrhiza is described and the manner of invasion of the roots by the fungi is examined. Mycorrhiza are not necessarily present in the initial growth stages: they develop mainly during the first or second period of active growth of the seedlings and may occur on relatively deep roots. A. G. POLLARD.

Use of pot tests in research on the fertility of sugar-cane soils. III. Loss of effectiveness of ammonium sulphate and superphosphate. H. Schroo. **IV. Effect of phosphate and lime on growth, yield, and quality of sugar cane.** H. Schroo and N. O. Schmidt. **V. Relative effectiveness of forms and placements of phosphatic fertilisers. VI. Effect of increasing phosphate dressings and various methods of placement on the phosphate status of a red ferruginous clay and on the growth of cane.** H. Schroo (*Trop. Agriculture, Trin.*, 1954, **31**, 71—78, 161—172, 242—250, 327—341).—III. Losses of efficiency of superphosphate in a limed soil during a four-month period of alternate wetting and drying ("ageing") were examined in pot tests using Sudan grass as test plant. The ageing process lowered the response to N by approx. 20%. Excessive liming caused heavy loss of N, probably as NH_3. The availability of superphosphate was not altered by ageing. Reduction in yields due to heavy liming could not be attributed to lowered availability of P.

IV. In an acid, P-deficient soil the effects of various dressings of superphosphate and of liming were examined. The soil "fixed" considerable amounts of P and liming increased this action. Data for increased growth of cane, P uptake (leaf analysis), and cane quality are recorded. The results demonstrate the value of "pot" tests carried out in 40-gal. drums. Important precautions in such experiments are noted.

V. In further pot tests the placement of $Ca(H_2PO_4)_2$ in a band immediately below the seed pieces and admixture with the bulk of the soil produced similar responses with moderate dressings. With heavy dressings admixture with soil produced the better growth (no. of tillers, total shoot length, total fresh wt.). When mixed with soil to a depth of 6 in. $Ca(H_2PO_4)_2$ was more effective than $CaHPO_4$ or $Ca_3(PO_4)_2$, whether or not the soil was limed. The depressing effect of liming on yields was attributed to loss of NH_3 from applications of $(NH_4)_2SO_4$. The efficiency of P fertilisers is regarded as dependent on the total area of contact of roots and phosphated soil and on the actual dilution of the added P with the bulk of soil.

VI. Thorough mixing of P fertiliser with the whole of the soil in 40-gal. drums produced better growth responses in the early stages than did admixture with only the upper layer of soil or placement 2 in. below the seed pieces. Differences, however, tended to disappear in later stages. Corresponding trends appeared in leaf analysis data. Liming depressed growth in the early stages but produced beneficial effects (stem length, no. of tillers) later in the season: the P content of the filtered juice was increased but ease of clarification was adversely affected when the P dressing was heavy. A mathematical relationship was established between water-sol. and available (Truog) P in the soil. A. G. POLLARD.

Foliar analysis. I. Application of chemical analysis of the leaf and of the method of visual diagnosis to the investigation of mineral deficiencies in cultivated soils. R. Dios Vidal and J. M. Albareda Herrera (*An. Edafol. Fisiol. veg.*, 1954, **13**, 339—418).—Deficiencies in acid and calcareous soils are examined. Interactions N/P, K/Mg, and Fe/Mn are discussed. Leaves visibly deficient in Fe and Mn were richer than normal in N + P + K. SOILS & FERT. (A. G. P.).

Leaf analyses of differentially cover-cropped deciduous fruit trees. E. L. Proebsting and J. G. Brown (*Hilgardia*, 1954, **23**, 125—153).—The N, P, K, Ca, and Mg contents of the basal leaves of prunes, apricots, pears, and peaches were examined at monthly intervals

during the growing period for three years. The total N content was high in spring and decreased during the season. Cover-cropping with legumes had little effect on the values. The K content of the leaves varied during the season more than did that of the N. The size of the crop had more effect on the K levels in prune leaves than did the cover crop treatment although lucerne tended to give lower K levels. The spring levels of K were high for prunes, peaches, and pears but low for apricots. The changes in P content were similar to those of the N content and treatment had little effect. In the case of apricots the levels tended to rise during the season and levels generally were higher than with the other fruits. Ca and Mg levels were low initially and tended to rise during the season, with few differences that could be ascribed to either season, species, or treatment. T. G. MORRIS.

Use of foliar analysis in oil palm cultivation. H. Broeshart (*Trop. Agriculture, Trin.*, 1954, **31**, 251—260).—Analyses of the ash of the first fully-developed leaf (sampled from mid-parts of leaflets and middle of frond) are utilised. The Ca, Mg, and P contents (actual % and ratios between elements) serve as an index of the nutrient status of the soil and thence of fertiliser requirements. Ranges of optimum values are determined. A. G. POLLARD.

Crop response to fused tricalcium phosphate. L. F. Seatz, S. L. Tisdale, and E. Winters (*Agron. J.*, 1954, **46**, 574—580).—In comparison with conc. superphosphate, " fused tricalcium phosphate " (FTP) was a poor source of P for all crops tested on alkaline soils. On acid soils, FTP was as satisfactory as superphosphate in increasing yields of soya-beans, lucerne, sericea, pasture crops, cereals, red and crimson clover, and vetch, but was less satisfactory than superphosphate for maize, soya-bean hay, cotton, and vegetable crops. Response to FTP was slightly less on limed than on unlimed soils. Finely-ground FTP (< 80 mesh) usually gave only slightly better results than did the coarsest grade (< 6 mesh). The residual effects of FTP were not consistently different from those of superphosphate. A. H. CORNFIELD.

Critical evaluation of inoculums in composting. C. G. Golueke, B. J. Card, and P. H. McGauhey (*Appl. Microbiol.*, 1954, **2**, 45—53).—Garden soil, horse manure, partially decomposed org. material, and a commercial prep. of special bacterial cultures did not affect the course of temp., increase in ash, or decrease in C in the composting of garbage and mixed municipal refuse. E. G. BRICKELL.

Application of liquid manure and potassium nitrate to crops during autumn, winter, and spring, 1941—53. K. Iversen (*Tidsskr. Planteavl*, 1955, **58**, 574—605; *Rept. No.* 497, *Danish State exp. Sta. for Plant Culture*).—The efficacy, as judged by increases in root and cereal crop yields, of liquid manure and NO_3' is greater in the Islands than in Jutland, due to smaller leaching losses in the Islands, where the average rainfall is comparatively lower. Differences arising from the time of application are much smaller in the Islands than in Jutland, where the highest increases are obtained from spring, and the lowest from autumn applications. Liquid manure is more efficient than NO_3', probably due to the absorption of the NH_3 by the soil; no nitrification of NH_3 occurs at temp. below 3—5°. Loss of NH_3 due to unfavourable weather conditions is reduced by drilling or harrowing the liquid manure into the soil; applications on frozen soil, especially in presence of snow, cause injury to crops. P. S. ARUP.

" Interlaced " field experiments with nitrogen, phosphorus, and potassium fertilisers. K. Dorph-Petersen (*Tidsskr. Planteavl*, 1955, **58**, 553—573; *Rept. No.* 496, *Danish State exp. Sta. for Plant Culture*).—A special design for the economical layout of plots is described in which the effects of three-stage applications of N, P, and K can be estimated as in three independent experiments with a min. of mutual interference. Results of 45 experiments with various crops demonstrate the practicability of the system. P. S. ARUP.

Effects of differential fertiliser treatments on a Coloso silty clay. G. Samuels and P. Landrau, jun. (*J. Agric. Puerto Rico*, 1954, **38**, 179—187).—Some soil properties after nine years differential fertiliser treatments (up to 250 lb. of N and up to 300 lb. each of P_2O_5 and K_2O per acre per annum in varying combinations) of a Coloso silty clay loam under sugar cane were studied. Soil pH and org. C, total N, and available P contents were unaffected by the fertiliser treatments when compared with the control soil, but available K was increased on K-treated soils. A. H. CORNFIELD.

Plant Physiology, Nutrition, Biochemistry

Preservation of botanical specimens in natural form by a thin plastic cast (not embedment). M. P. Burton (*N.Z. J. Sci. Tech.*, 1955, **36**, 479—487).—Preliminary work on spraying of specimens with a solution of acrylic plastic resin in an organic solvent is described. Optimum results were obtained with leaves and flowers of medium texture, or with stiff small-leafed species. E. G. BRICKELL.

Microcalorimetric study of the growth acceleration of cereal grains treated ultrasonically. R. G. Busnel and G. Obolensky (*C. R. Acad. Sci., Paris*, 1955, **240**, 1358—1360).—Measurements made with Calvet's microcalorimeter show that grains of barley exposed initially to ultrasonic vibrations (2—5 w. per sq. cm.) are characterised by subsequent increased rates of growth and of heat evolution during germination, in comparison with untreated grains. The measurements were made on 1 g. of grains immersed in 1 ml. of water; the thermal evolution curves are discussed in relation to growth metabolism. The effect, which persists for weeks or even months after treatment, is probably associated with mechanical rupture of teguments, thus favouring penetration of water. For equal ultrasonic intensities, the accelerated growth is independent of frequency (80—960 kHz.). W. J. BAKER.

Flower initiation in pasture legumes. I. Factors affecting flower initiation in *Trifolium subterraneum*, L. II. Geographical implications of cold temperature requirements of varieties of *T. subterraneum*, L. III. Flower initiation in *Medicago tribuloides*, Desr., and other annual medics. Y. Aitken (*Aust. J. agric. Res.*, 1955, **6**, 212—244, 245—257, 258—264).—I. At any time of sowing the length of the growing season depends greatly on the response to temp. and on the photoperiod of the first few weeks after germination. Flower initiation is accelerated by a period of low temp. In later varieties, the initiation is prevented by an insufficient period of low temp. The length of the necessary low-temp. period is shortened under longer photoperiod. Early varieties flower early because they do not require so long a low-temp. period or so low a temp. as do late varieties.

II. The low-temp. requirements of varieties of the clover, lengthen the growing season and result in greater productivity than is usual in northern Europe. The low-temp. requirement reduces the value of the species as a self-regenerating annual where temp. in both summer and winter are too high for flower initiation. Summer temp. in the tropics is probably too high even for the short low-temp. requirement of early flowering species. If water supply is available during the winter, flower initiation is possible, though retarded. Thus, in northern Australia only early flowering varieties may be of use in pasture.

III. When barrel medic was sown in the field throughout the year, flower initiation of the winter sowings was the most rapid and that of late summer sowing the most delayed. The delay was due to the absence of sufficiently low temp. following germination. Low temp. and long photoperiods accelerate flower initiation in barrel medic as in subterranean clover. R. H. HURST.

Types of lignification and reactions for lignification. J. Ullrich (*Ber. dtsch. bot Ges.*, 1955, **68**, 93—104).—Three different types of lignification are exemplified by *Sambucus*, *Clematis*, and *Halesia*, respectively. With the *Halesia* type, the phloroglucinol–HCl reaction fails in the initial stages. The Mäule reaction may be inhibited by either a marked thickening of lignified cell-walls, or by a protective incrustation of wood-gum; on removal of the gum, the reaction proceeds. P. S. ARUP.

Protective tissues in roots of alpine plants. M. Luhan (*Ber. dtsch. bot. Ges.*, 1955, **68**, 87—92).—Descriptions are given of eight types of specialised cork tissue found in the roots of a no. of alpine plants. P. S. ARUP.

Fertilisation and post-fertilisation development in wheat. J. W. Morrison (*Canad. J. Bot.*, 1955, **33**, 168—176).—Using a dissection squash technique, the fusion process in fertilisation in wheat is examined. The male gamete is in the resting stage when it comes into contact with the egg cell nucleus. After penetrating into the nucleus it becomes diffused, stains less densely, and eventually is indistinguishable from the female nuclear contents. These enter prophase and the chromosomes resolve on one spindle. The fusion process in the fertilisation of the two polar nuclei proceeds similarly but much more rapidly. The embryology of wheat is described. R. H. HURST.

Influence of light and darkness on metabolism of radioactive glucose and glutamine in wheat leaves. R. G. S. Bidwell, G. Krotkov, and G. B. Reed (*Canad. J. Bot.*, 1955, **33**, 189—196).—Wheat leaves absorbed ^{14}C-labelled glucose or glutamine in light or darkness. Complete oxidation of glutamine to CO_2 was twice as rapid in light as in darkness. In light, glucose was largely prevented from reaching the pyruvic acid stage. The light-block of carbohydrate respiration did not operate when NH_4NO_3 was supplied to the leaves. Light has no effect on the respiration of glucose by *Staphylococcus aureus*. R. H. HURST.

Portable apparatus for comparison of photosynthetic activity by the ^{14}C technique. S. Larsen and G. Nielsen (*Tidsskr. Planteavl*, 1955, **58**, 651—656).—The apparatus consists essentially of a closed

chamber with ample window space, which contains a holder for eight leaves, and through which air can be circulated; the air circuit includes a generating system consisting of three interconnected test-tubes, in which CO_2 is evolved by the admixture of HCl and $BaCO_3$ containing ^{14}C, and then washed through water. After exposure during 20—30 min. to a circulating atm. containing 0·03% of CO_2, the dried and pulverised leaves are compared for radioactivity. Mean deviations in results are ±4·6% for different leaves from the same tree, and ±1·5% for radioactive leaf-powder. P. S. ARUP.

Biosynthesis of vitamin C. II. Biochemistry and physiology of vitamin-C synthesis. W. Franke (*Planta*, 1955, **45**, 166—197; cf. *ibid.*, 1954, **44**, 437).—A review and discussion of the literature relating to the biochemistry of the synthesis, the possibility of any relationship between the synthesis and photosynthesis, and the influence of external factors on the synthesis (or decomposition) of the vitamin. (59 references.) P. S. ARUP.

Time-effects of temperature changes on carbon dioxide output of potato parenchyma. G. Rosenstock (*Planta*, 1955, **45**, 208—212).—A comparatively rapid increase in the temp. of thin potato slices (kept in the dark in a current of moist air) from 20·5 to 25·5°, followed after 10 hr., by a rapid decrease to the original temp. causes "temp.-shock" effects manifested by a temporary rise and fall (respectively) above and below the constant values (differing by ~40%) which are established at the two temp. With gradual changes in temp., the constant values for CO_2-output are established more slowly without shock effects. P. S. ARUP.

Connexion between longitudinal growth and protein synthesis. W. Schumacher and H. Matthaei (*Planta*, 1955, **45**, 213—216).—No positive relationship is found between protein synthesis and the increase in area of flower-corollæ or the elongation of the sporogonium of *Pellia*. P. S. ARUP.

Separation of acidic amino-compounds using a sulphonated polystyrene resin. M. K. Hamdy, W. J. Harper, and H. H. Weiser (*J. Dairy Sci.*, 1955, **38**, 147—154).—By altering the buffer pH from 3·42 to 4·0 and the concn. from 0·1M to 0·06M in the ion-exchange chromatographic method of Moore and Stein (*J. biol. Chem.*, 1951, **192**, 663), the column can be operated at 20—30°, and the procedure shortened by ~75%. By this method 11 acidic amino-compounds, together with serine phosphate and glutamine, were separated. S. C. JOLLY.

Elimination of amino-acids in pure and mixed cultures of plant species. H. F. Lenskens and R. Knapp (*Planta*, 1955, **45**, 106—117).—Various amino-acids were excreted by roots of *Trifolium repens*, *Lolium perenne*, and *Artemesia absinthium*, grown separately or in mixed culture in sand under sterile or in non-sterile conditions. Varying amounts of alanine, aminobutyric acid (**I**), valine, leucine, serine, aspartic acid, glutamic acid, and tryptophan (**II**) were liberated under the different conditions examined. **I** and **II** were produced only in mixed cultures and under sterile conditions. A. G. POLLARD.

Water-uptake by cultivated plants. K. Kreeb (*Ber. dtsch. bot. Ges.*, 1955, **68**, 71—86).—The wider variations in concn. of the leaf press-juice of cultivated, as compared with wild plants necessitate greater attention to sampling. Observations on cereals, potatoes and sugar beet show regular increases in concn. for all the plants at all seasons, from morning to noon, and decreases during the afternoon, with (for potatoes) an established min. shortly after sunset. All the plants show the same seasonal variations, with min. during the Feb. frosts, and max. at harvest time, but with varietal and environmental differences between the actual values. The extent of frost damage to leaves of cereals bears a positive relation to the observed concn. The observed effects of the watering of cereals on the reduction of leaf press-juice concn. are slight but significant. P. S. ARUP.

Movement of moisture in large woody stems. K. N. H. Greenidge (*Canad. J. Bot.*, 1955, **33**, 202—221).—The movement of moisture, under normal conditions of uptake, is described. Consideration is also given to the aberrant patterns of staining characters of the stems after partial injection of lower boles of standing trees. Staining patterns after drastic disruption of the normal channels of water conduction are discussed. R. H. HURST.

Osmotic and non-osmotic uptake by diatoms. H. J. Bogen and G. Follmann (*Planta*, 1955, **45**, 125—146).—Non-osmotic uptake from sucrose solutions is demonstrated for two species of *Melosira* by the considerable decreases (11—18% within 2 min.) in uptake observed on the addition in low concn. of the protoplasmic poisons Na azide or dinitrophenol, osmotic conditions remaining unaffected. Similar reductions (max. 30—60%) in the "permeation constants" are found on replacing the sucrose by erythritol, glycerol, or other readily permeating substances. The unusually high uptake of sucrose etc. by diatoms is largely conditioned by a metabolic peculiarity resulting in a high non-osmotic uptake. P. S. ARUP.

Effcet of composition and concentration of the nutrient solution on the growth of tomato and kohl-rabi in culture tests. E. Rauterberg and G. Lenhard (*Z. PflErnähr. Düng.*, 1955, **68**, 132—141).—Pot culture tests with tomato and kohl-rabi grown in pumice gravel with nutrient solutions of varying concn. and varying ratios of N : P : K are described. Highest yields of tomato fruit were usually obtained with a solution containing 0·2% of sol. salts and a N : P_2O_5 : K_2O ratio of 1 : 0·69 : 1. Max. yields of kohlrabi were obtained with a similar solution, the concn. of which was increased from 0·127% in the first week to 0·762% in the sixth and later weeks. A. H. CORNFIELD.

Uptake of organic matter by plants. M. H. van Raalte (*Landbouwk. Tijdschr. 's Grav.*, 1954, **66**, 356—363).—A review. Root absorption of sugars, amino-acids, metabolic products of bacteria, extracts of peat, vitamin B, yeast, auximones, cellulose, and poultry manure are discussed. SOILS & FERT. (A. G. P.).

Rôle of bicarbonate ion in lime-induced chlorosis. P. Baxter and R. Belcher (*J. Aust. Inst. agric. Sci.*, 1955, **21**, 32—34).—In chlorotic citrus plants the roots absorb HCO_3' as well as Ca^{++}, and the internal environment is disturbed by accumulation of HCO_3'. The effect of this accumulation on CO_2 excretion, which is accompanied by a rise in internal pH and in buffer capacity, is probably the main cause of the general metabolic disturbance associated with iron-deficiency symptoms (chlorosis) in plants. S. C. JOLLY.

Uptake of magnesium by sugar beet in relation to that of calcium, phosphorus, and potassium. H. Lüdecke and I. Paulsen (*Z. PflErnähr. Düng.*, 1955, **68**, 240—255).—The Mg, Ca, P, and K contents of the tops and roots of four strains of sugar beet through the season over three years were determined. Yields of tops and roots through the season in relation to total uptake of nutrients are also reported. The Ca : Mg ratio of the tops, but not of the roots, varied considerably from one season to another. The P : Mg ratio of the tops increased, whilst that of the roots remained fairly constant, with age of plant. The K : Mg ratio of the tops increased, whilst that of the roots decreased, with age of the plant. Uptake of all nutrients increased up to August, but little or any of them was taken up thereafter. Root yields through the season increased with uptake of all nutrients, and were particularly closely correlated with K content of the roots. There were little differences in nutrient uptake and contents between the four strains. A. H. CORNFIELD.

"Extra-root" manganese top-dressings of agricultural plants. P. A. Vlasyuk, L. D. Lendenskaya, and A. P. Kibalenko (*Izv. Akad. Nauk, Ser. biol.*, 1954, No. 3, 19—31).—Spraying with aq. 0·05—0·1% $MnSO_4$ and a 1 : 10 suspension of superphosphate (800 l. per hectare) increased potato and sugar-beet yields and the sugar content of the beet. With $MnSO_4$ potato yields were increased to a somewhat smaller extent and sugar-beet yields to a larger extent than with the double spray. SOILS & FERT. (A. G. P.).

Further experiments with molybdenum. A. Sorteberg (*Forskn. Fors. Landbr.*, 1954, **5**, 161—198).—On peat soils application of NH_4 molybdate (1 kg. per hectare) or liming to pH 5·85 produced healthy lettuce plants. Yields were lowered by heavy liming and by applications of $MnSO_4$ (210 kg. per hectare) but a simultaneous application of NH_4 molybdate (1 kg.) improved the yields. The effect of Mo increased with increasing water supply. SOILS & FERT. (A. G. P.).

Autumn application of boron sprays as a control for blossom blast and twig dieback of pears. Folke Johnson, D. F. Allmendinger, V. L. Miller, and D. Polley (*Phytopathology*, 1955, **45**, 110—114).—Effects of the disease are described. The disorder was corrected by spraying with aq. borax (3 lb. per 100 gal.) in autumn. Midsummer applications were less satisfactory. Spraying increased leaf-B, differences in levels between treated and untreated trees being most apparent in early spring. A. G. POLLARD.

Plant growth regulators. I. Influence of side-chain length on the activity of ω-(2-naphthyloxy)-*n*-alkylcarboxylic acids for the induction of parthenocarpy in tomatoes. L. C. Luckwill and D. Woodcock (*J. hort. Sci.*, 1955, **30**, 109—115).—Ten members of the homologous series of ω-(2-naphthyloxy)-*n*-alkylcarboxylic acids and six members of the corresponding 3-chloro-2-naphthyloxy series were synthesised, and the ability of the Na salts to induce parthenocarpy in tomatoes was compared. Acids with an odd no. of C atoms in the side-chain were completely inactive. Acids with 4 or 6 C atoms were as active as 2-naphthyloxyacetic acid and those with 8, 10, and 12 C atoms showed definite though reduced activity, probably due to the decreased penetration of the larger mol. The introduction of Cl in position 3 in the 4-, 6-, 8-, 10-, and 12-C acids completely destroyed their activity, indicating that oxidation at the β-position (blocked by the introduction of the Cl atom) plays a part in the process. T. G. MORRIS.

Characterisation of substances with plant hormone activity in peat. J. Niggemann (*Naturwissenschaften*, 1955, **42**, 98).—When peat (white or black) was heated with tryptophan, acetic acid, and buffer (pH 6·8) at 100° for several hr., the mixture evaporated to dryness at 80° and extracted with ether, β-indolylacetic acid (IAA) could be demonstrated on paper chromatograms of the extract. In the absence of peat, and in partially decomposed IAA, substances giving the colour reaction of IAA, but differing in R_f value, were present. Using bioassays (root formation etc.), substances producing stimulation and inhibition of growth were located on paper chromatograms (butanol–ammonia solvent) of aq. extracts of white peat.
P. G. STANLEY.

Spray thinning of apples. D. D. Hemphill (*Amer. Fruit Gr.*, 1954, **74**, No. 2, 36—39).—Naphthylacetamide (30—50 p.p.m.) applied at petal-fall produced the desired thinning effects without causing foliage damage. HORT. ABSTR. (A. G. P.).

Chemical thinning sprays on apple trees. A. H. Thompson (*St. hort. Ass. Pa. News*, 1954, **33**, No. 1, 29—38).—Best results with naphthylacetamide were obtained with concn. 25—60 p.p.m. applied 9—16 days after full bloom according to variety.
HORT. ABSTR. (A. G. P.).

Chemical defoliation of cotton. III. Study of seed and fibre from cotton plants treated with aminotriazole. L. C. Brown and A. H. Hyer (*Agron. J.*, 1954, **46**, 580—581).—Application of the chemical defoliant aminotriazole (3-amino-1 : 2 : 4-triazole ; 0·25—1·0 lb. per acre) either to the whole or to the lower parts of the cotton plant had no significant effect on seed germination, boll size, lint %, seed or lint index, no. of seeds per boll or length, strength, or fineness of fibre. A. H. CORNFIELD.

Effects of foliar sprays of maleic hydrazide on the storage and respiration of vegetable crops. F. M. R. Isenberg, M. L. Odland, and C. O. Jensen (*Pa. agric. Exp. Sta.*, 1954, *Bull.* 584, 12 pp.).—Foliage sprays of maleic hydrazide (**I**) (3000—4000 p.p.m.) applied 16 days prior to harvest were effective in preventing sprouting and maintaining the quality of onions during storage. Applications of **I** sprays (4000 p.p.m.) to potato foliage 30 days prior to harvest were very effective in reducing sprouting of tubers during storage for six months at 7·3°. The treatments were ineffective in prolonging the storage life of snap beans or carrots. Application of **I** at low concn. increased, whilst application at high concn. inhibited, respiratory activity. A. H. CORNFIELD.

Effect of 2 : 4-dinitrophenol on nitrate reduction and respiration in green algæ. E. Kessler (*Planta*, 1955, **45**, 94—105).—Within critical ranges of pH and concn. dinitrophenol (**I**) inhibited the reduction of NO_2' in *Ankistrodesmus braunii* in darkness, the reduction of NO_3' to NO_2' becoming quantitative. In concn. below the critical range **I** stimulated respiratory activity : in concn. above this range respiration was diminished. Somewhat similar effects were produced in *Chlorella*. Relationships between respiration and reduction of NO_2' are discussed. A. G. POLLARD.

Crops and Cropping

Crop forecasts and estimates with particular reference to permanent crops in the tropics. W. H. Beckett (*Trop. Agriculture, Trin.*, 1954, **31**, 292—302).—The collection of suitable data and methods used in its application to the forecasting of crop yields are discussed.
A. G. POLLARD.

Survey of factors affecting wheat yields in some parts of Canterbury. Jean G. Miller (*N.Z. J. Sci. Tech.*, 1954, **36, A**, 401—415).—A survey at Christchurch for the period 1945—50 shows that better yields were obtained from wheat as the first or second crop after grass than from wheat after two or more other crops. In a survey from 1947 to 1952, in Ashburton County, soil type was the most important factor affecting yields which were greatest with wheat sown after peas irrespective of whether that crop followed pasture or another crop. G. HELMS.

Protein content of wheat varieties grown in standard plots and spaced rows. H. J. Sims (*J. Aust. Inst. agric. Sci.*, 1955, **21**, 38—39).—The protein content of grain grown in spaced rows (14 in. apart) was higher than that of grain in standard plots. The correlation between the protein content of grain in plots and rows is sufficiently high to allow the use of spaced rows in selection for high protein content. S. C. JOLLY.

Differential varietal responses of winter wheat germination and early growth to controlled limited moisture conditions. R. H. Helmerick and R. P. Pfeifer (*Agron. J.*, 1954, **46**, 560—562).—Field observations showed that certain varieties of winter wheat produced consistently better autumn stands than did other varieties. Germination tests with two varieties in solutions of varying osmotic pressure as well as in soil at varying moisture tensions gave the same trends as did field observations. Varietal differences in field stands are probably due to differences in germination tolerance to high moisture stress. A. H. CORNFIELD.

Wheat fertilisation studies in Western Oklahoma. H. V. Eck and B. A. Stewart (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 432, 16 pp.).—Applications of N increased grain yields at seven out of the eight locations in the 1951—2 season but at only one location in the next season. Yields and % of protein in the grain increased with the amount of N applied (up to 80 lb. per acre). Yield and protein increases from spring applications of N were superior to those from autumn applications in the first, but not in the second season. Application of P increased yields at most of the locations in both seasons. Twenty lb. of P_2O_5 per acre was sufficient to give max. yield increases. A. H. CORNFIELD.

Quality of wheat as affected by manures and fertilisers. I. Chemical composition. Y. P. Gupta and N. B. Das (*J. Indian Soc. Soil Sci.*, 1954, **2**, 121—125).—The chemical composition of wheat receiving varying fertiliser treatments in different types of rotations was studied. Rape cake, N, K, NK, green manure, and green manure with legume in the rotation increased, whilst P, NK, PK, and NPK decreased, the % of protein in the grain. Applications of N tended to reduce whilst all other treatments tended to increase the % of P in the grain. The % of Ca in the grain was decreased by green manure and legumes, with or without added P, in the rotation, and increased by manure, rape cake, NPK or NP fertilisers.
A. H. CORNFIELD.

Response of winter oat varieties from winter and early spring seeding. A. M. Schlehuber and R. M. Oswalt (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 435, 15 pp.).—Certain winter oat varieties produced higher grain yields and test wt. from Jan. and early Feb. seedings than did spring oats from any seeding date. However, highest yields were obtained when the winter varieties were sown in the autumn (Sept. 15—Oct. 15). Winter oats sown in Jan.—Feb. were less subject to lodging than when sown in autumn.
A. H. CORNFIELD.

Effect of Israeli climatic conditions and soils upon the free and total β-amylase of three varieties of barley. J. Gutstein (*Bull. Res. Counc. Israel*, 1954, **4**, 300—304).—Randomised replicate experiments during two consecutive years with three types of barley grown in four locations showed that the contents of free and total β-amylase (i.e., free + bound amylase) in the kernels are a varietal characteristic, and are also affected by type of soil, growing conditions, and particularly by rainfall distribution over the season. Dry spells promote production of free β-amylase ; adequate moisture during early development with inadequate supplies during the development of the stalk and ear causes an increase in the total β-amylase. The significance of the results is discussed.
G. HELMS.

Post-harvest handling and marketing of garden-fresh sweet corn. E. K. Alban and R. C. Scott (*Ohio agric. Exp. Sta.*, 1954, *Res. Circ.* 23, 31 pp.).—The quality of sweet corn (as measured by consumer preference, total and reducing sugars, and respiration rate) was higher when the cobs were mixed with ice prior to storage and then refrigerated than when they were stored at normal temp. The economics of the two storage processes are discussed.
A. H. CORNFIELD.

Performance of winter grains alone and with winter vetch for supplemental forage. G. H. Ahlgren, M. Pool, and H. W. Gausman (*Agron. J.*, 1954, **46**, 563—565).—Sowing winter vetch with winter cereals increased the yields of protein, but not of dry matter, as compared with pure stands of cereals. Dry matter yields of winter cereals sown alone were highest during the milk or dough stages, whilst the % of protein in both pure and mixed stands was highest during the heading or milk stages. Yields of protein in mixed stands were highest during the milk or dough stages.
A. H. CORNFIELD.

Pasture studies on coastal lowlands of subtropical Queensland. I. Introduction and initial plant nutrient studies. C. S. Andrew and W. W. Bryan. **II. Interrelation of legumes, *Rhizobium*, and calcium.** W. W. Bryan and C. S. Andrew (*Aust. J. agric. Res.*), 1955, **6**, 265—290, 291—298).—I. The underdeveloped coastal lowlands of southern Queensland are described ; location, soils, vegetation, climate, present use, and problems to be investigated are dealt with. The limiting plant nutrients, in descending order of importance are : P, N, Ca, K, Cu, Zn, Mo, and B. The nutrients to which responses are obtained are in very low supply in the soils of the area named. The effect of over-liming on the change of soil pH and the availability of Cu and Fe is discussed. Deficiency symptoms of Ca, K, and Cu, for certain species are discussed. Max. plant growth is obtainable only if all limiting nutrients are supplied.

II. Initial attempts to grow clovers on the soils failed because available strains of *Rhizobium* were not effective. Without effective strains, legumes do not, under local conditions, respond normally

to nutrients. In particular, responses to Ca may be quite abnormal. Effective strains of *Rhizobium* were successfully applied to stands of white clover on three soil types. R. H. HURST.

Pasture establishment studies. IV. Comparison of mixtures containing short rotation rye-grass, perennial rye-grass, or both as the grass component. R. W. Brougham (*N.Z. J. Sci. Tech.*, 1954, **36, A,** 365—374).—The individual and mixed grasses were sown at total levels of 15 and 40 lb. per acre in conjunction with broad red clover at 4 lb. and white clover at 3 lb. per acre. Herbage yields and clover yields were determined eight months after sowing. The short-rotation grass was more aggressive than the perennial rye-grass, and caused decreased clover growth but better suppression of weeds. Light sowings of short-rotation grass yielded pasture establishment similar to that obtained with heavy sowing of perennial rye-grass. The practical applications of the results from Parts I—IV are discussed (*ibid.*, 1954, **36, A,** 47; J.S.F.A. Abstr., 1954, ii, 215). G. HELMS.

Root growth in pastures. A. Könekamp and E. Zimmer (*Z. PflErnähr. Düng.*, 1955, **68,** 158—169).—Through the grazing season the wt. of root material was positively correlated with that of above-ground material for grass–clover, rye–vetch, and fodder rye swards. In permanent grassland root wt. per unit area increased rapidly from April to May, then decreased somewhat to June, increased again to August, and then fell off again. Mean root wt. of *Lolium perenne* during the second season was higher when sowing was carried out during May than in July. With *Lolium italicum* mean root wt. during the second season was similar for both sowing dates. A. H. CORNFIELD.

Turf grasses. W. C. Elder (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 425, 32 pp.).—A general account of the development and maintenance of turf grasses in Oklahoma. The chemical control of undesirable weeds and grasses is discussed briefly. A. H. CORNFIELD.

Effects of some agricultural practices on grass production at Mandan, N. Dakota. J. L. McWilliams (*U.S. Dep. Agric. Tech. Bull.* 1097, 28 pp.).—Grass seed production is favoured by row spacing of 30 in., except for Russian wildrye, which requires 36 in. When grasses are 1—3 years old, satisfactory yields are obtained from 18-in. rows. Seeds should be sown $\gtrsim \frac{1}{2}$ in. deep in the warm season and $\gtrsim 1$ in. deep in the cool season.
L. G. L. UNSTEAD-JOSS.

Midland Bermuda grass, a new variety for Oklahoma pastures. J. R. Harlan, G. W. Burton, and W. C. Elder (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 416, 10 pp.).—The origin, characteristics, and performance of the new strain, which is similar to Coastal, but more winter-hardy, are described. A. H. CORNFIELD.

Cutting techniques in grassland experiments. P. B. Lynch and N. S. Mountier (*N.Z. J. Sci. Tech.*, 1954, **36, A,** 375—386).—Five cutting techniques for measuring pasture production are compared. In the light of all the factors involved, the "Standard" method using a pre-trimming cut and a mower to cut the herbage was preferred for most types of investigation. G. HELMS.

Influence of depth of sowing and temperature on pre-emergence weight changes in subterranean clover (*Trifolium subterraneum*, L.). J. N. Black (*Aust. J. agric. Res.*, 1955, **6,** 203—211).—Plants were sown at depths of 0·5, 1; 1·5, and 2 in. and exposed to temp. of 28, 21, 14, and 7°. No difference was detected in total plant wt., or in the proportions of the parts, between plants sown at different depths under a particular temp. treatment. The rate of transfer of material from the cotyledons increased with temp. The extent of cotyledonary reserves remaining on emergence diminished with depth of sowing. The optimum temp. for hypocotyl extension per unit of cotyledonary material translocated was 21°. Cotyledon wt. on emergence varied from 33 to 61% of total plant wt.
R. H. HURST.

Effect of harvest practices on the performance of lucerne. H. O. Graumann, J. E. Webster, C. L. Canode, and H. F. Murphy (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 433, 57 pp.).—Vigour, yield, composition, and root reserves of lucerne as affected by harvesting at various stages of maturity are reported. A. H. CORNFIELD.

Penngift crown vetch for slope control on Pennsylvania highways. H. B. Musser, W. L. Hottenstein, and J. P. Stanford (*Pa. agric. Exp. Sta.*, 1954, *Bull.* 576, 21 pp.).—The results of practical trials over a no. of years using Penngift crown vetch for the control of highway slopes are reported. Dehulling and seed scarification are necessary to secure good stands in a reasonable time. Rate of establishment is slow but rye-grass and red fescue are suitable companion crops for providing early protective cover. Established crown vetch produced a cover that excelled the best perennial grasses in persistence and quality of slope protection.
A. H. CORNFIELD.

Control of biennial bearing in fruit trees by chemical thinning. W. Wuzler *et al.* (*Rev. romande Agric. Vitic.*, 1954, **10,** 32—34).—Spraying with α-naphthylacetic acid at petal-fall or 14 days later resulted in lighter but better quality crops and had no ill-effects on keeping quality. The treatment also induced regular bearing in some varieties of apples and pears.
HORT. ABSTR. (A. G. P.).

Biennial bearing of apples and means of overcoming it. F. Lalatta (*Ann. sper. agrar.*, 1954, **8,** 575—587).—Regular bearing can be induced by the following treatment in an "on" year: (i) thinning by spraying with 20% DNOC at 0·15% just before full blossom and again three days later, (ii) hand-thinning within 3 weeks of full blossom to give a fruit : leaf ratio of 1 : 20—40, and (iii) ringing the main branches during flowering.
HORT. ABSTR. (A. G. P.).

Rejuvenation of an old appled orchard by means of fertilisers, mulches, and cover crops. F. H. Hewetson (*Pa. agric. Exp. Sta.*, 1954, *Bull.* 585, 33 pp.).—The effects of varying levels of inorg. fertiliser in combination with straw and pea vine mulching and ladino clover and bluegrass cover crops on development of trees in an old appled orchard were studied. The fertiliser treatments increased trunk area and leaf wt. in proportion to the amounts applied, although yields were not greatly increased. Pea vine mulching was the most effective of the cultural treatments tested. Straw mulching and bluegrass cover crops were ineffective in increasing yields where no fertilisers were applied. A. H. CORNFIELD.

Effect of soil structure on growth of apple seedlings. K. Weissenborn (*Mitt. ObstbVersuchsringes Jork*, 1954, **9,** 47—51).—In pot trials addition of CaO to heavy acid soil poor in humus had deleterious effects on apple seedlings. It is suggested that the pH of such soils should be raised gradually, preferably by adding a mixture of CaO and finely granulated peat.
HORT. ABSTR. (A. G. P.).

Influence of source and growth substance on the behaviour of apple and plum cuttings. R. J. Garner and E. S. J. Hatcher (*J. hort. Sci.*, 1955, **30,** 116—128).—A comparison was made of the source of Myrobalan B plum cuttings, some from a hedge and some from layer shoots, planted on a loam, or on dry or wet delta soils. Rotting of the basal tissues, especially of upper cuttings from thin shoots, occurred on the wetter soil. On the loam soil most cuttings were healthy, and all the cuttings rooted well. With thick shoots it was found that there was an optimum girth above which rooting declines, but this level depends on conditions. The effects of the growth substance varied, in general, the easier rooting Myrobalan did not benefit as much as the difficultly rooting Crab C.
T. G. MORRIS.

Influence of orchard nutrition on the acidity relationships in Cortland apples. C. A. Eaves and J. S. Leefe (*J. hort. Sci.*, 1955, **30,** 86—96).—Apples grown on soil receiving dolomitic limestone, with and without superphosphate, superhosphate alone and no additions, all plots receiving superimposed annual treatments of N, K, and P, were examined for changes in the acid content on storage. The acid content of the apples decreased significantly over a period of 12 days at harvest time. Dolomitic limestone depressed the acid content at harvest and after storage at 18·5° for a week. Superphosphate had no effect. Annual applications of K increased the acid content at harvest and after storage for one week at 18·5° more than did those of N; complete fertiliser also gave a significantly higher acid content. A marked reduction was found in the P content of leaves from limed plots without N additions, particularly those receiving no P. This effect was negligible on the K and N plots. After storage for one week at 18·5° the apples were then stored for five months at 1° and then for one week at 18·5°, when acid loss was determined. No differences in acid loss during long storage due to time of picking or limestone treatments were apparent. Fruit from untreated trees showed a significantly lower acid loss than that from trees receiving N only (all pickings), or that from those receiving P (first two pickings). Acid loss was positively associated with the N : K ratio in the leaves. T. G. MORRIS.

Effect of nitrogen and phosphate manuring on the content of some valuable constituents of fruit. Anon. (*A. R. Bundesanst. Qualitätsforsch. pflanzl. Erzeugn., Geisenheim*, 1953/4, 14).—N-deficient red currants had an ascorbic acid content of 64 and those from plants supplied with a high dosage of N 50 mg. per 100 g. (fresh wt.). Sugar and total acid contents were unaffected by the manurial treatment. P fertilisers did not affect the composition of cherries.
HORT. ABSTR. (A. G. P.).

Potassium deficiency in vines cured by leaf sprays. F. Ciferri (*Nat. Mal. Piante*, 1954, No. 26, 11—14).—Spraying vine leaves with aq. 1% K_2SO_4 increased leaf-K by 35% and cured K-deficiency. Absorption of K was increased by addition to the solution of urea, a wetting agent or Bordeaux mixture.
HORT. ABSTR. (A. G. P.)

Chemical composition of strawberry leaves from a factorial manurial trial. B. Ljones (*Forskn. Landbruk.*, 1954, **5**, 141—154).—Leaf samples were taken on June 13 and on July 22. In both series of leaves manuring with $Ca(NO_3)_2$ increased the N and Mg contents; that with K_2SO_4 increased the K and lowered the Mg content, and application of dolomite increased the Mg and lowered the K content of the leaves. HORT. ABSTR. (A. G. P.).

Growing better strawberry plants. C. A. Reimer and J. H. Davidson (*Amer. Fruit Gr.*, 1954, **74**, No. 4, 14—15, 39—40).—To produce nematode-free stock plants, mother-plants are grown in cans which are placed in the fumigated plant bed so that runners will grow over the edge of the can and root in the treated soil. For fumigation MeBr containing 2% of chloropicrin is used at the rate of 1 lb. per 100 sq. ft. under a gas-proof cover.
HORT. ABSTR. (A. G. P.).

Chloride injury to strawberries. B. Ljones and K. Refsdal (*Frukt og Boer*, 1954, **7**, 73—80).—In field trials, KCl (25 kg. of K per 1000 sq. m.) applied in May caused symptoms of Cl′ injury in July. The Cl′ content of leaves did not correspond closely with the amount applied. In pot trials Na, K, and Ca chlorides were applied in the irrigation water un concn. equivalent to 0—0·4% Cl′. All caused severe injury KCl being the most harmful. Symptoms of injury appear when the leaf-Cl′ is about 0·5%.
HORT. ABSTR. (A. G. P.).

The content of soluble sugars in various parts of the vine during ripening of the grapes. M. G. Marteau (*C. R. Acad. Agric. Fr.*, 1955, **41**, 193—198).—The importance of the time of gathering the grapes in obtaining wines of maximum quality is discussed. Tests were based on ratios of contents, glucose/fructose, tartaric acid/total organic acids, sugars/acidity, and a further consideration, probably a varietal constant, that the grape contains most sugar at the time of max. wt. The influence of chlorophyll synthesis in leaves, shoots, and stalks is considered. The intensity of migration of sugars to the berry is governed by the intensity of chlorophyll assimilation, i.e., by sugar reserves accumulated in the leaves. Maturity of the grape is marked by a rapid decline in the soluble sugar content in the stalk which coincides with cessation of sugar metabolism in the berry and an accumulation of soluble sugars in the leaves, shoots, and stalks. E. M. J.

Nutrition of the sultana bunch. A. J. Antcliff (*J. Aust. Inst. agric. Sci.*, 1955, **21**, 39—40).—Food material in the vine was as readily available to bunches of fruit on stubs of truncated and defoliated branches as on control branches. Some of the experimental shoots matured into canes suitable for retention at pruning, except that they were too short, so that this maturation was apparently unaffected by the treatment. S. C. JOLLY.

Relative value of repeated annual fertiliser applications on hops, *Humulus lupulus*, L. K. R. Keller (*Agron. J.*, 1954, **46**, 535—537).—Yields of strobiles over five years from application to irrigated hop vines of six levels each of N and P_2O_5, ranging from 0 to 375 lb. per acre, alone and in all possible combinations are reported. There was a greater response to N than to P applications. Yields from the heavier N applications were not significantly greater than where 75 lb. of N per acre was applied. Increased yields due to P applications were greater with the intermediate, than with the low or high rates, of N. A. H. CORNFIELD.

Effect of position on the bines, of fertilisers, and of season, on the mineral and nitrogen contents of hop leaves and cones. H. O. Askew, J. Hodgson, and G. Ward (*N.Z. J. Sci. Tech.*, 1955, **36, B**, 495—510).—The position of the leaf on the plant greatly affects the composition, younger leaves being richer in P, K, and N, but poorer in Ca, Mg, and SiO_2, than older ones. Increases in standard fertiliser mixture had little effect on mineral and N content except to increase sol. ash and potash in the dry matter, but heavy applications of K_2SO_4 significantly increased the K content of leaves and cones, and decreased the Mg content of the former but not of the latter. Seasonal effects, except for N and Mg, were small. The larger uptake of nutrients by the hop plant (especially in the cones) is stressed. E. G. BRICKELL.

Effect of weather and climate on the sucrose content of sugar cane. M. A. Lugo-López and B. G. Capó (*J. Agric. Puerto Rico*, 1954, **38**, 149—169).—Highest yields of sucrose were obtained on irrigated soils where rainfall was low and where irrigation was stopped 45—60 days prior to harvest. Yields were usually reduced if rainfall was high during the 3—4 months prior to harvest. Cool nights favoured high yields and yields were highest in areas having the highest diurnal temp. variation. Yields were directly correlated with hours of sunlight and were low in areas of high wind. A. H. CORNFIELD.

Effect of potassium on the yield and sucrose content of sugar cane. G. Samuels and P. Landrau, jun. (*J. Agric. Puerto Rico*, 1954, **38**, 170—178).—Potash increased cane yields mainly on the red and yellow podsolic soil of the humid area and on a planosol of the semi-arid area. Little response to K occurred in irrigated areas. Response to K increased with increasing ratoons. Sucrose % in the cane and the polarisation value of the juice increased only when cane yields increased. Brix and % extractability were not affected by K treatments. Response to K applications in the humid area was obtained when the leaf contained less than 1·8% of K (dry matter basis). A. H. CORNFIELD.

Factors affecting cotton planting for mechanised production. J. G. Porterfield, E. W. Schroeder, and E. M. Smith (*Okla. agric. Exp. Sta.*, 1954, *Tech. Bull.* 50, 27 pp.).—A progress report for 1948—52. A. H. CORNFIELD.

Growth of coffee in Africa. H. Jacques-Felix (*J. trop. Agric. appl. Bot., Paris*, 1954, **1**, 118—122).—A general account of the problems arising in coffee growing with particular reference to the problems of the degradation of the coffee plant and pests.
A. H. CORNFIELD.

Effect of some fertility treatments on the yield and fibre quality of sansevieria in south Florida. E. O. Gangstad, J. F. Joyner, and C. C. Seale (*Trop. Agriculture, Trin.*, 1954, **31**, 321—326).—In a light sandy soil (moderate P, low K) yields of sansevieria (total green matter and dry fibre) were increased by use of N and K fertilisers, split applications (every 4 months) giving best results. Differences in fibre quality due to fertilisers were apparent though probably not commercially important; N and K tended to lower the resistance to wear and to flex more than that to strength or shear.
A. G. POLLARD.

Pepper culture with special reference to Sarawak. J. S. Blacklock (*Trop. Agriculture, Trin.*, 1954, **31**, 40—56).—The culture, propagation, and manuring of pepper in this area is described. Pests and diseases of this crop are listed and control measures are indicated.
A. G. POLLARD.

Growth of chinchona and the production of quinine in the French Cameroons. L. Gerin (*J. trop. Agric. appl. Bot., Paris*, 1954, **1**, 21—40).—A general account. A. H. CORNFIELD.

Oklahoma brush-type cotton stripper. E. W. Schroeder and J. G. Porterfield (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 422, 14 pp.).—The stripper is described and its performance is compared with that of other types of strippers. A. H. CORNFIELD.

Design and operation of freezing and frozen storage facilities for research with agricultural products. J. E. Nicholas and M. D. Shaw (*Pa. agric. Exp. Sta.*, 1954, *Bull.*, 579, 33 pp.).—The construction and performance of freezing and frozen storage rooms are described. A. H. CORNFIELD.

Animal Husbandry

Relation of size of growing cattle to pasture intake and its use as an index of palatability. C. M. Martin, W. F. Brannon, and J. T. Reid (*J. Dairy Sci.*, 1955, **38**, 181—185).—A highly significant correlation occurred between body wt. of growing cattle and dry-matter intake. Providing sufficient herbage is available to satisfy appetite dry-matter intake increased by 3·5 lb. per day per 100 lb. body-wt. increase. An index of palatability may possibly be derived from the effect of varying body wt. on the absolute dry-matter intake. S. C. JOLLY.

Rate and efficiency of gains in beef cattle. I. Response to injected testosterone. M. J. Burris, R. Bogart, A. W. Oliver, A. O. Mackey, and J. E. Oldfield. **II. Factors affecting performance testing.** C. D. Pierce, H. G. Avery, M. Burris, and R. Bogart. **III. Factors affecting weight and effectiveness of selection for gains in weight.** G. H. Hitchcock, W. A. Sawyer, R. Bogart, and L. Calvin (*Ore. agric. Exp. Sta.*, 1954, *Tech. Bull.* 31, 35 pp.; 33, 32 pp.; 1955, 34, 22 pp.).—I. Weekly intramuscular injections of testosterone (0·001 g. per kg. of body-wt.) resulted in increased wt. gains and feed efficiency of both heifers and steers, although steers showed the most marked response. Treated calves developed pronounced masculine appearance and behaviour in comparison with control calves. The treatment increased the thyrotropic hormone content of the pituitaries and the wt. of the thyroid and adrenal glands. Palatability and cooking quality of the meat was unaffected by the treatment.

II. Multiple correlations of the effects of four independent variables (birth wt., suckling gains, wt. on test, and age on test) on three dependent variables (gain on test, economy of gain, and gain per day) are presented for both pure-bred and grade Hereford calves over three winters.

III. A study was made of the possibility of attaining heavier sale weights on a low level of nutrition by selecting beef cattle for this characteristic. A. H. CORNFIELD.

Heat tolerance of two breeds of calves from one to twelve months of age. G. H. Klemm and Kathleen W. Robinson (*Aust. J. agric. Res.*, 1955, **6**, 350—364).—For air temp. about 95°F. the rise in rectal temp. was greater, and equilibrium was reached more slowly, in Illawarra Shorthorns than in Zebu-Herefords. Increase in humidity at temp. over 80°F. produced more stress in the animals than did increase of dry-bulb temp. Pulse rates changed little during heat exposure but the rate fell with age in all circumstances. As the calves increased in age the panting rate decreased for a given hot environment. Evaporative loss increased as dry bulb temp. was raised. The Zebu-Herefords showed high transcutaneous water loss soon after birth, with continued increase up to 12 months. Skin evaporation was relatively small in the Illawarra Shorthorns at 1—3 months, increasing at 6—8 months. In the Zebu-Herefords, sweat glands became active at skin temp. of ~36°; with the onset of sweating, the skin temp. fell. R. H. HURST.

Biological effect of ionising rays. (Short theoretical survey.). W. Minder (*Mitt. Lebensm. Hyg., Bern*, 1955, **46**, 76—90).—Physical and chemical, in addition to the biological, effects are considered. Diagrammatic representation is made of a linear, four-atom mol. *n*-butane and the splitting after ionisation by electron impact. The general behaviour of biological effects caused by rays is summarised, *e.g.*, generally the effect is increased with increase of dose; for ionising rays there is no specific biological effect; for any one effect (death), very different doses are required for different biological species, 500 r. for unweaned animals, 50,000 r. for insect imagines, etc. Physical effects consist of ionisation, stimulation and raising of the thermal energy of the mol., dissociation to form more stable split products, building of more stable end products, resulting in the animal organism in death, mutation, cessation of growth, sterility, and necrosis. These changes are discussed in detail. E. M. J.

Methionine deficiency: a possible cause of liver injury in sheep. J. W. Groenewald, J. D. Smit, and T. F. Adelaar (*J. S. Afr. vet. med. Ass.*, 1954, **25**, No. 4, 29—33).—Emaciation and liver damage in sheep which have grazed on lupin fields is not due entirely to alkaloid poisoning but is related also to methionine deficiency. Bitter and sweet varieties of lupin provide only 20% of the methionine requirement of sheep. Bitter varieties have lower contents of crude protein, methionine, Cu, and P than have sweet varieties. A. G. POLLARD.

Mammary elimination of radio-iodine. W. E. Wright, J. E. Christian, and F. N. Andrews (*J. Dairy Sci.*, 1955, **38**, 131—136).—Following oral administration of ^{131}I-labelled iodocasein to a lactating rabbit, radio-activity was detected in mono- and di-iodotyrosine and iodide of the milk; in lactating goats ~15·5% of the iodine in a single dose was excreted in the milk as iodide. Following oral administration of $Na^{131}I$ to goats an average of 50% was excreted in the milk as iodide; in a single experiment 45·3, 40·7, and 2·3% of the iodine was eliminated in the milk, urine, and faeces, respectively, and 31·2% of a single dose of carrier-free $Na^{131}I$ was excreted in the milk as iodide. I compounds other than iodide were not detected in skim milk from goats. S. C. JOLLY.

Toxicity of trichloroethylene-extracted soya-bean oil meal. J. C. Picken, jun., N. L. Jacobson, R. S. Allen, H. E. Biester, P. C. Bennett, L. L. McKinney, and J. C. Cowan (*J. Agric. Food Chem.*, 1955, **3**, 420—424).—Indications of the possible autoxidative decomposition of trichloroethylene during the processing of soya-beans are presented. Using young calves as test animals, soya-beans, defatted soya-beans, soya-bean protein, and casein, treated with these autoxidation products of trichloroethylene, were assayed for toxicity. The typical toxicity disease was not produced except by two of the preparations, although several produced isolated manifestations of the disease syndrome. Detailed experimental results are tabulated, and discussed. (12 references.) E. M. J.

Autoxidation products of trichloroethylene. L. L. McKinney, E. H. Uhing, J. L. White, and J. C. Picken, jun. (*J. Agric. Food Chem.*, 1955, **3**, 413—419).—Outbreaks of a refractory, hæmorrhagic, aplastic anæmia occurred in cattle fed trichloroethylene-extracted soya-bean oil meal in 1947/52 and the meal was toxic to sheep. Autoxidation products of trichloroethylene were studied to find the reaction of these substances under controlled conditions with soya-beans and with soya-bean oil flakes. Autoxidation with O_2 at 45—70° yielded about 95% of a liquid, composed of about equal parts of dichloroacetyl chloride and a reactive isomer which appeared to be trichloroethylene epoxide, and 5% of a gas mixture consisting of phosgene, CO, and HCl. The epoxide was not isolated and identified, but was hydrolysed to glyoxylic and formic acids, CO and HCl. (50 references.) E. M. J.

Proving dairy sires and dams. J. L. Lush and L. D. McGilliard (*J. Dairy Sci.*, 1955, **38**, 163—180).—Possible errors in "proving" and methods of reducing these errors are discussed. S. C. JOLLY.

Effect of semen dilution with a yolk-containing extender on oxygen uptake and motility of bull spermatozoa. M. W. H. Bishop and G. W. Salisbury (*J. Dairy Sci.*, 1955, **38**, 202—207).—Dilution of semen with yolk–saline–phosphate extender increased O_2 uptake by bovine spermatozoa at 37°, the stimulation being more sustained by the addition of catalase. Motility was decreased by dilution and not significantly improved by catalase. S. C. JOLLY.

Comparison of the fertility of bull semen diluted in egg-yolk–citrate and homogenised milk. J. R. Perkins, M. C. Carpenter, and D. M. Seath (*J. Dairy Sci.*, 1955, **38**, 155—158).—The 60- to 90-day non-returns were not significantly different for bull semen diluted with homogenised milk, previously heated at 97° for 10 min., or standard yolk–citrate diluter. Highly significant differences occurred between 2- and 3-day-old semen, milk diluter being best for the former and yolk–citrate for the latter. Bulls of low fertility had better non-return rates when milk diluter was used. S. C. JOLLY.

Effect of forced exercise on bull fertility. J. W. Snyder and N. P. Ralston (*J. Dairy Sci.*, 1955, **38**, 125—130).—Daily exercise for six months did not affect significantly the semen characteristics of Holstein and Guernsey bulls. The 60- to 90-day non-returns of high-fertility Guernsey bulls were apparently improved, but high-fertility Holstein and low-fertility Guernsey and Holstein bulls were unaffected. Libido, appetites, and general well-being were not affected. S. C. JOLLY.

Production of hyperkeratosis in calves with topically applied base-oils for use in livestock sprays. W. G. Hoekstra, R. J. Dicke, and P. H. Phillips (*J. Dairy Sci.*, 1955, **38**, 186—196).—Hyperkeratosis was produced in calves to varying degrees by the application of a mineral seal oil depending on the rate and frequency of topical application. The ability of the oil in 11 different oil preparations to produce hyperkeratosis was not related to source, viscosity, unsulphonated residue, b.p., or the acid used in refinement of the oil. The hyperkeratosis was indistinguishable from that of chronic bovine hyperkeratosis (X-disease); the skin condition was the only symptom consistently produced by the oils. A temporary depression of blood plasma-vitamin A occurred. Oral administration of a hyperkeratosis-producing oil caused no symptoms of X-disease and no plasma-vitamin A depression. S. C. JOLLY.

Aluminium in cow's milk. J. G. Archibald (*J. Dairy Sci.*, 1955, **38**, 159—162).—Milk from cows fed for four months on a control ration and on the ration supplemented with potash alum contained on average 0·46 and 0·81 mg. of Al per l., respectively; the difference was significant at the 5% level. S. C. JOLLY.

Winter rations for beef cows. A. B. Nelson, O. B. Ross, A. E. Darlow, W. D. Campbell, and R. W. MacVicar (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 418, 22 pp.).—Growth of cows (*a*) grazed on native pastures throughout the year and fed cottonseed cake during the winter months, or (*b*) grazed for seven months and fed prairie hay and cottonseed cake during the winter was considered satisfactory. A system in which cows were grazed throughout the year and fed lucerne hay during the winter was better than seven months' grazing and feeding lucerne hay and prairie hay during the winter. Lucerne hay satisfactorily replaced cottonseed cake as the winter protein supplement in system (*a*), although three times as much of the former was required. A. H. CORNFIELD.

Prairie hay for milk production. M. Ronning and A. H. Kuhlman (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 423, 3 pp.).—Satisfactory milk production was obtained with good quality prairie hay supplemented with 20% protein concentrate. Results compared favourably with those obtained by feeding lucerne hay + 15% protein concentrate. For best results the hay should be cut whilst the grasses are still in active growth. If cut when mature it should be supplemented with carotene in addition to protein concentrate. When the hay is grown on soils of low Ca status, extra Ca should be supplied in the feed. A. H. CORNFIELD.

Relative value of three grades of feeder steers when wintered, grazed, and fed grain on pasture. P. Gerlaugh, E. W. Klosterman, and L. E. Kunkle (*Ohio agric. Exp. Sta.*, 1954, *Res. Circ.* 26, 10 pp.).—The relative value of choice Herefords, medium Shorthorns, and common Holsteins when wintered, grazed, and fed grain on pasture was studied. The three grades made similar gains when wintered and grazed without grain. The Holsteins gained significantly faster when fed grain on pasture than did the other two breeds, but these gains were economical in only one out of two tests. Profitability increased in the order Herefords, Shorthorns, Holsteins. Holstein carcasses had a lower dressing % than did Herefords, but there was no difference in tenderness of meat between the two grades. A. H. CORNFIELD.

Beef cattle feeding investigations. L. S. Pope, R. D. Humphrey, A. E. Darlow, V. G. Heller, O. B. Ross, and R. A. Long (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 428, 20 pp.).—Addition of 1·0—1·5 lb. of cottonseed cake per head daily to the diet was sufficient for the growth requirements of fattening calves fed a maize–sorghum silage–lucerne hay diet. Addition of crude carotene concentrate did not improve wt.-gains or feed efficiency. Wt.-gains and feed efficiency of calves given pre-press solvent-extracted cottonseed meal were slightly less than those receiving hydraulic-pressed meal. A. H. CORNFIELD.

Relative importance of heredity and environment in body-weight increments at different ages in Australian Merino sheep. G. C. Taneja (*Aust. J. agric. Res.*, 1955, **6**, 343—349).—The growth rates of ewe lambs born in spring, 1951, and of others born in autumn, 1952, are analysed. There is a negative correlation between the two rates due to environment in the two periods. The genetic correlation between the growth rates is zero, indicating that different sets of growth genes are operating during the two periods. The heritability for the periods of growth is 0·27 and 0·31 in one group and 0·04 and 0·22 in the other. Environment plays a greater part than heredity in causing variation in growth rate. R. H. HURST.

Maintenance rations for Merino sheep. II. Performance of weaners fed daily and weekly on rations of wheat and wheaten chaff at maintenance levels and effect thereon of vitamin-A supplements. M. C. Franklin, G. L. McClymont, P. K. Briggs, and B. L. Campbell (*Aust. J. agric. Res.*, 1955, **6**, 324—342).—Weaners could be fed for long periods with maintenance levels of the simplest of cereal rations, provided the Ca- and vitamin-A-deficiencies are corrected. Live-wt. changes in daily fed and weekly fed groups were similar, and there was no significant difference in mortality rate. The addition of NaCl did not appear to benefit the whole-wheat group, despite the very low Na content of the diet. Plasma-vitamin-A levels of the weaners which were not treated with vitamin A fell to very low values and the death rate was 63·0%, compared with 16·7% among those which received the vitamin supplement. There was no great difference in wool production of the various groups irrespective of the different drought rations, the method of feeding, or treatment with vitamin A. Feeding *ad lib.* significantly increased the wool production and the rate of eruption of incisors. The beneficial effects of a single large dose (500,000 i.u.) of vitamin A administered orally were still evident in plasma-vitamin-A content and survival rate nearly six months later. R. H. HURST.

Papers and feeders *versus* feeders alone for starting chicks. R. H. Thayer, G. F. Godfrey, G. W. Newall, and R. B. Thompson (*Okla. agric. Exp. Sta.*, 1954, *Bull.* 424, 8 pp.).—No advantage in growth rate, mortality, and feed conversion to 10 weeks of age resulted from supplying chicks during their first 72 hr. with feed scattered over newspapers and egg flats as well as in feeders as compared with supplying the feed in feeders alone. A. H. CORNFIELD.

Control of mastitis in dairy cattle. J. O. Schnautz (*Ore. agric. Exp. Sta.*, 1954, *Bull.* 545, 11 pp.).—The causes, symptoms, and control of mastitis are described. A. H. CORNFIELD.

Flies on cattle and their control. L. E. Adams and S. G. Gesell (*Pa. agric. Exp. Sta.*, 1954, *Circ.* 432, 6 pp.).—Control of flies on cattle with chemical sprays is discussed. The treadle sprayer is one of the most effective methods of controlling all types of flies attacking cattle. A. H. CORNFIELD.

Spraying cattle to control ticks: field application in Ankole District, Uganda. H. C. Clifford (*Trop. Agriculture, Trin.*, 1954, **31**, 19—26).—The satisfactory operation of a scheme of spraying cattle (BHC prep.) at regular intervals, *e.g.*, weekly, is described. A. G. POLLARD.

Cattle grubs and their control in South Dakota. J. A. Lofgren, I. H. Roberts, W. L. Berndt, and K. Rasmussen (*S. Dakota agric. Exp. Sta.*, 1954, *Bull.* 435, 32 pp.).—Two species of cattle grub are widely distributed in S. Dakota, although *Hypoderma bovis* (northern grub) is found under a wider range of environmental conditions than is *H. lineatum* (common grub). Time and degree of infestation varied considerably from year to year. Of a no. of materials tested rotenone dusts, sprays, and washes have given the best control. Correct timing of the application is important and must be determined each season. Wt. gains of infested cattle were no different from those of grub-free cattle, but the value of the hides from infested cattle was greatly reduced. A. H. CORNFIELD.

Chemotherapy of calf paratyphoid. M. W. Henning (*J. S. Afr. vet. med. Ass.*, 1954, **25**, No. 4, 1—7).—Calves artificially inoculated with *Salmonella dublin* were treated with (*a*) chloromycetin, intramuscularly, or (*b*) furazolidone, by mouth. Both drugs lowered the temp. in 24—48 hr. The diarrhœa was corrected the more quickly by (*b*), which, however, caused some temporary toxic symptoms. A. G. POLLARD.

Safe use of Dieldrin dust for sheep ked control. R. E. Pfadt (*J. econ. Ent.*, 1955, **48**, 195—198).—Sheep treated twice with a 1% Dieldrin dust showed only small amounts of Dieldrin in the renal fat, omental fat, liver, and flesh nine days after treatment. After 86 and 191 days no traces of toxicant were found in the tissues but very small quantities were determined in the wool. No harm to sheep resulted from dusting with Dieldrin and consumers of lamb or mutton would not be endangered by toxicant residues. A. A. MARSDEN.

Lice control on chickens with chlorinated hydrocarbon insecticides. H. E. Fairchild and P. A. Dahm (*J. econ. Ent.*, 1955, **48**, 141—146).—Five insecticides were tested as dusts for control of the chicken body louse, *Eomenacanthus stramineus*, the fluff louse, *Goniocotes gallinæ*, and the shaft louse, *Menopon gallinæ*. Toxicity and tissue residue studies with Dieldrin and lindane are reported. Rapid and effective lice control was obtained with a rotary duster of Aldrin (1·5), chlordane (5), Dieldrin (1), heptachlor (1·5), and lindane (1%) dusts, applied over roosting chickens (approx. 15 g. of dust per bird). Lice control was also obtained by scattering Aldrin, chlordane, and lindane dusts on to the litter of poultry houses. No toxic effects from the use of Dieldrin or lindane were noted during a 12-week experimental period. A. A. MARSDEN.

Pest Control

Antibiotics and agriculture. J. Dufrenoy (*J. trop. Agric. appl. Bot., Paris*, 1954, **1**, 9—20).—A review of the production of antibiotics and their applications to plant protection and animal feeding. A. H. CORNFIELD.

Internal medication of plants for the control of insects. R. F. Anderson (*J. econ. Ent.*, 1955, **48**, 187—190).—Quassia extracts applied to the soil of potted plants affected seven species of insects feeding on the plants, but not the two-spotted spider mite, potato- and green peach-aphids, or imported cabbage-worms. Parathion affected Mexican bean beetles and the eastern tent caterpillar but not the two-spotted spider mite. A. A. MARSDEN.

Improved deposits for controlling insects outdoors. W. N. Sullivan, I. Hornstein, A. H. Yeomans, and C-H. Tsao (*J. econ. Ent.*, 1955, **48**, 153—155).—Addition of a chlorinated terphenyl greatly extended the residual life of lindane and Aldrin but not that of DDT. The insecticides were applied in methyl ethyl ketone solutions on pine twigs and tested against houseflies. Surface residues from the ketone solutions of DDT lasted longer than did those from fuel oil solutions. Evaporation from conc. solutions of insecticide and chlorinated terphenyl left residues which dispersed well and adhered tenaciously without penetrating the surface under test. A. A. MARSDEN.

The hazard of DDT treatment of potatoes. M. V. Sharangapi and S. V. Pingale (*Bull. centr. Food technol. Res., Mysore*, 1954, **4**, 57).—In 12 samples of potatoes purchased in the open market in Mysore, a range of 134—169 (average 151·8 ± 3) p.p.m. of DDT was found. The potatoes had been treated excessively with the insecticide. As DDT can cause liver disorders, the necessity of safeguarding its indiscriminate use is pointed out. L. G. L. UNSTEAD-JOSS.

Toxicity of certain chlorinated hydrocarbon insecticides to laboratory animals with special reference to Aldrin and Dieldrin. J. F. Treon and F. P. Cleveland (*J. Agric. Food Chem.*, 1955, **3**, 402—408).—Details are given of toxicity tests of Aldrin, Isodrin, Dieldrin, and Eldrin on rats, rabbits, and dogs. The relation between the toxicity and spatial configuration is indicated. In young rats of both sexes the females appear to be more susceptible to a single oral dose. Diets containing Aldrin, Dieldrin, or DDT in concentrations of 2·5, 12·5, and 25·0 p.p.m. respectively, fed during two years to rats of either sex, resulted in no significant increase in mortality, or decrease in growth rates, as compared with controls. In rabbits, dry powdered Aldrin and Dieldrin, applied to intact skin, are more toxic than DDT, but less toxic than Endrin. Dogs are more susceptible than rats to Aldrin or Dieldrin. E. M. J.

Phytotoxicity of hydrocarbons. H. B. Currier and S. A. Peoples (*Hilgardia*, 1954, **23**, 155—173).—Barley and carrots were exposed in the greenhouse to varying concn. of vapours of hydrocarbons. Plant response was the same with all materials, the two plants showing similar symptoms except that carrots invariably recovered by producing new growth from the crown unless they were very young, and barley less than five days old sometimes continued to grow after the aërial portions had died. Carrots were more resistant than barley to all hydrocarbons, resistance increasing with age; the age of the barley had no influence on resistance. Toxicity increased on a molar basis in the order hexene, hexane, *cyclo*hexane, *cyclo*hexene, and benzene. There was no simple time–concn. relation in the acute toxicity. In other tests with *Anacharis canadensis*

(elodea) the toxicity increased in the order hexane, hexene, *cyclo*hexane, *cyclo*hexene, benzene. T. G. MORRIS.

Effect of sprays on growth and photosynthetic activity of apple trees. S. Dalbro and G. Nielsen (*Tidsskr. Planteavl*, 1955, **58,** 657—682; *Rept.* No. 499, *Danish State exp. Sta. for Plant Culture*).—In greenhouse experiments, spraying with Bordeaux mixture, lime–S, and with a prep. containing Hg, shoot-growth and photosynthetic activity were decreased (the latter temporarily) by the two first-mentioned materials only, but trunk diameters, total shoot-wt., no. of leaves, and total leaf area were decreased by all three prep. (without leaf-scorch). In field experiments, adverse effects on growth were observed with Bordeaux mixture for one variety, and with Bordeaux mixture and lime–S for the two varieties of trees under test; the latter spray decreased photosynthesis, whilst the former caused leaf-scorch in both varieties. A photo-electric apparatus for measuring leaf-area is described. (30 references.) P. S. ARUP.

Antibiosis and red rot disease of sugar cane. B. A. Bourne (*Phyto-pathology*, 1955, **45,** 37—38).—The yeast *Candida intermedia* which is found associated internally and externally with the sugar-cane moth borer *Diatræa saccharalis*, Fabr. (and with one of its parasites) is strongly antibiotic against the red rot fungus *Physalospora tucumanensis*, but not against an associated red rot organism *Fusarium moniliforme*. The possibility of using cultures of the yeast for protecting cane cuttings from red rot is suggested. P. S. ARUP.

Effect of seed-maturing on inhibition of Southern bean mosaic virus in bean. Pen Ching Cheo (*Phytopathology*, 1955, **45,** 17—21).—During the maturing of the seed, the concn. of the virus (originally present in all parts of systematically infected bean plants) increases in the embryo, and decreases in the seed-coat and pod. During the drying of the seed, the virus in the embryo is rapidly inhibited, due to the formation of a heat-, acid-, and alkali-resisting substance which can be extracted in greater concn. from the mature than from the immature seed. The presence of the inhibitor does not affect the susceptibility of host-cells to infection by the virus. P. S. ARUP.

Toxic metabolic products in culture filtrates of *Botrytis cinerea*, Pers. W. Sauthoff (*Phytopath. Z.*, 1955, **23,** 1—36).—The culture filtrates are toxic to shoots of peas and other plants; the toxin is very probably identical with that which is estimated in germination inhibition tests on *Ustilago* spores. The toxin can be produced by cultivating the mould in Fries II medium (+ 100 μg. per l. of aneurin), in which the NH_4 tartrate (replaceable by NH_4 citrate with moderate success) is an essential constituent; the addition to the medium of agar (1%) greatly enhances toxin production. Toxin formation is limited to the period of active mycelium growth, and is accompanied by a reduction in the surface tension of the medium. In ageing cultures, the toxin is inactivated or decomposed. (34 references.) P. S. ARUP.

Influence of nutrition on sensitivity of tomato plants to toxins. H. Zähner (*Phytopath. Z.*, 1955, **23,** 49—88).—Tomato plants of medium sensitivity to *Fusarium*-wilt show max. sensitivity to fusarinic acid, lycomarasmin, and Fe-lycomarasmin when grown in culture solutions of normal composition, and reduced sensitivity under conditions of N-deficiency (but not of P-deficiency) or over-nutrition (high concn. of nutrient salts). These results have bearings similar to those found by other workers with respect to susceptibility to infection. The physiological effects of fusarinic acid in presence of varying supplies of N are not directly traceable to inhibition of respiration or to protein coagulation, but to increased permeability of the plasma, an effect which is counteracted by N-deficiency. (96 references.) P. S. ARUP.

Effect of toxic wilt on excretion from tomato leaves. H. F. Linskens (*Phytopath. Z.*, 1955, **23,** 89—106).—Conductivity measurements and analyses of the wash-waters from the leaves show that the addition of lycomarasmin or fusarinic acid to the water in which tomato-shoots are placed increases appreciably the excretion from the leaves of salts and amino-acids. The effects can be observed before the appearance of wilt symptoms and on neighbouring non-necrotic tissue. The results support the supposition that the operative effect of the toxins is to increase the permeability of the plasma. P. S. ARUP.

Production and rôle of extracellular pectic enzymes of *Fusarium oxysporum* f. *lycopersici*. P. E. Waggoner and A. E. Dimond (*Phyto-pathology*, 1955, **45,** 79—87).—The organism produces a pectin methyl esterase (*M*) and a polygalacturonase (*G*) on a pectin medium; on glucose media only *M* is formed. Characteristics of the enzymes are recorded and the mechanism of their action in *Fusarium* wilt infection in tomatoes is discussed. (40 references). A. G. POLLARD.

Accumulation of chemicals in diseased areas of leaves. C. E. Yarwood and L. Jacobson (*Phytopathology*, 1955, **45,** 43—48).—Considerable accumulations of S, P, and C (supplied as H_2S, H_3PO_4, and sucrose, respectively) in the infected areas of leaves, as compared with the normal tissue, are demonstrated by applications of the ^{35}S, ^{32}P, and ^{14}C techniques for a majority of the host-pathogen (viruses and fungi) combinations under examination. In the case of rusted bean leaves, much more ^{14}C and ^{32}P are accumulated by way of translocation than by direct application of solutions to the leaves; selective accumulation is reduced, but not eliminated, after killing the rust in the living bean leaf. The accumulations are probably connected with the high metabolic activity of the infected tissue. P. S. ARUP.

Scald-resistance in pears. III. Material basis of resistance to leaf-scald. E. Siebs (*Phytopath. Z.*, 1955, **23,** 37—48; cf. *ibid.*, 1954, **22,** 437).—Neither the leaf-tannins nor arbutin (as such) are toxic to *Ventura pirina*, or especially associated with resistance to leaf-scald. Quinol is, however, toxic (in concn. 1 : 1000) to the fungus, and can be qual. demonstrated in resistant, but not in non-resistant leaves. The importance of arbutin as a probable source (by hydrolysis) of quinol is discussed. P. S. ARUP.

Differential susceptibility of the sexes and developmental stages of the American cockroach to several insecticides. D. G. Cochran (*J. econ. Ent.*, 1955, **48,** 131—133).—Adult females of *Blattella germanica* were normally less susceptible than were adult males to the effects of the following insecticides: lindane, Dieldrin, DDT, toxaphene, chlordane, and methoxychlor. The % of superiority of the female varied with the toxicant. Last instar nymphs showed no differences in susceptibility between the sexes. Adult females, male and female nymphs showed no differences in their susceptibility to DDT. A. A. MARSDEN.

Isolation of pathogens from clover seed. C. M. Leach (*Phyto-pathology*, 1955, **45,** 94—96).—Apparatus is described for disinfecting the outside surface of the seeds with hypochlorite, drying the seed aseptically, transferring the seeds, by means of a vac. seed counter, to a 2% malt–agar medium in which pathogens within the seed can develop and be isolated. An untreated sample with surface pathogens is also similarly plated. A. G. POLLARD.

Flavour evaluation and residue analysis of Malathion-treated lettuce. L. Hopkins, I. K. Abu Yaman, and L. A. Carruth (*J. econ. Ent.*, 1955, **48,** 151—152).—No significant residues or objectionable flavour were found on lettuces harvested 3—12 days after treatment with recommended dosages of Malathion. A. A. MARSDEN.

Effect of fertilisers on the resistance of winter and spring varieties to the wheat stem sawfly. P. Luginbill, jun., and F. H. McNeal (*Agron. J.*, 1954, **46,** 570—573).—Application of P or PN at planting time increased the extent of sawfly damage to both winter and spring wheats. Application of NPK decreased, whilst that of N or K alone had no effect on, the degree of damage. Fertilisers applied to spring wheat at times other than seeding had little effect on the damage. A. H. CORNFIELD.

Specificity in effect of high temperature on adult plant reaction of wheat varieties to races of stem rust. G. J. Green and T. Johnson (*Canad. J. Bot.*, 1955, **23,** 197—201).—The reactions of wheat varieties to races of stem rust were determined at 60°F. and 80°F. Two varieties were resistant to all races at both temp. One was resistant to most races at both temp., but susceptible to some. Other varieties were more susceptible at the higher temp. to one or more races to which they were resistant at the lower temp. R. H. HURST.

Tests with acaricides against the brown wheat mite. C. F. Henderson and E. W. Tilton (*J. econ. Ent.*, 1955, **48,** 157—161).—Of 18 sprays tested against the brown wheat mite, *Petrobia latens*, Demeton and parathion (0·25—0·5 lb. per acre) gave the best control based on both residual and initial effectiveness. S compounds and chlorinated hydrocarbons gave little control, and methyl parathion was much less effective than parathion. An acaricide with better residual qualities and with greater efficiency at low temp. would be preferable to Demeton and parathion. A. A. MARSDEN.

Backcrossing for disease resistance in cereals. A. T. Pugsley (*J. Aust. Inst. agric. Sci.*, 1955, **21,** 16—20).—The results obtained using this technique are outlined and its value in cereal breeding is emphasised. S. C. JOLLY.

Insecticides against the maize earworm in New Brunswick. D. D. Pond (*J. econ. Ent.*, 1955, **48,** 198—199).—Light to medium infestations of *Heliothis armigera* on sweet maize were satisfactorily controlled with mineral oil, DDT (5), DDD (3), or ryania (40%) dusts. Good control of a heavy population was obtained with a single

application of 3% DDD dust, although two applications of this dust the following year failed to give satisfactory control.
A. A. MARSDEN.

Distribution and relative abundance of wireworms in potato-growing areas of the Southeastern States. O. T. Deen and F. P. Cuthbert, jun., (*J. econ. Ent.*, 1955, **48**, 191—193).—Studies of the economic importance and distribution of wireworms in the coastal areas of the Southeast States are reported. A. A. MARSDEN.

Colorado beetle (*Leptinotarsa decemlineata*, **Say**). B. Schaerffenberg (*Z. PflKrankh.*, 1955, **62**, 67—75).—The beetles may survive indefinitely in dry soil but are killed by floods of even short duration. Destruction of humus and deterioration of structure in soil increased the susceptibility of potato to attack by the beetle. Plants grown in compost were much less frequently attacked. A. G. POLLARD.

Control of the clover root curculio on lucerne with notes on life history and habits. G. W. Underhill, E. C. Turner, jun., and R. G. Henderson (*J. econ. Ent.*, 1955, **48**, 184—187).—Aldrin (1—4), Dieldrin (1—4), and chlordane (2·5—10 lb. per acre) gave good control of clover root curculio, *Sitona hispidula*, when applied prior to sowing or later during the autumn. Spring applications gave poor control with the exception of a preliminary experiment with heptachlor 2—8 lb. per acre). A. A. MARSDEN.

Physiological differences between a normal and a degenerate strain of *Sclerotina trifoliorum*. V. M. Held (*Phytopathology*, 1955, **45**, 39—42).—The normal strain differs from the degenerate one in showing greater growth responses to various sources of N or to B vitamins, in secreting less protopectinase, and in secreting a substance which causes wilting of rapidly transpiring clover leaves. Na *poly*pectate increases the secretion of protopectinase in both strains. P. S. ARUP.

Band placement of insecticides for clover root borer control. C. R. Weaver and J. L. Haynes (*J. econ. Ent.*, 1955, **48**, 190—191).—Aldrin (0·25—1·5 lb.), and γ-$C_6H_6Cl_6$ (0·75—2 lb. per acre) mixed with a fertiliser and placed in a band under red clover seed gave good control of the clover root borer, *Hylastinus obscurus*, at lower rates than were required for control by surface application. Yields of clover at second cutting increased 34—82% over untreated plots.
A. A. MARSDEN.

Apple scab. M. Gaudineau *et al.* (*Arboric. fruit.*, 1954, No. 2, 3—8).—One pre-blossom and three post-blossom applications of sprays were given. Best control of scab with least damage to fruit resulted from use of 1% Bordeaux mixture, 0·2% dichloronaphthaquinone and 0·3% Captan. HORT. ABSTR. (A. G. P.).

Some factors affecting the balance of phytophagous and predacious mites on apple in south-east England. E. Collyer and A. H. M. Kirby (*J. hort. Sci.*, 1955, **30**, 97—108).—Cox's Orange and Worcester Pearmain trees were sprayed at green cluster, pink bud, petal-fall, and fruitlet stages with either lime–S, dispersible S, 341 SC (2-heptadecylglyoxalidine), or SR 406 (*N*-trichloromethylthiotetrahydrophthalimide) for three years, no winter washes being used. At the start of the experiment considerable populations of *Metatetranychus ulmi*, Koch alone were present, with bronzing (least on the lime-S plots) of the foliage in July. By Sept. trees receiving lime–S carried significantly larger populations than those treated with SR 406, the other treatments having intermediate effects. The first count of *Typhlodromid* mites was low but populations on leaves increased markedly during the summer and there were large differences by the autumn, those on lime–S-treated trees being much the lowest. The ratio between the no. of *M. ulmi* and *Typhlodromid* mites is important if the latter are to control the former, effective control is only possible if this ratio is >10 to 1. This was attained only on plots treated with org. fungicide. T. G. MORRIS.

Antibiotics used in controlling fireblight. A. E. Murneek (*Bett. Fruit*, 1954, **48**, No. 9, 6—7).—Antibiotics, sprayed on apples when 30—50% were in bloom gave complete control of fireblight (*Erwinia amylovora*). Streptomycin and Terramycin, singly or combined (100—150 p.p.m.) with Cellosolve or Carbowax (1%) as penetrants were the most effective. HORT. ABSTR. (A. G. P.).

Fireblight on pears and apples. R. S. Kirby and A. H. Bauer (*Pa. agric. Exp. Sta.*, 1954, *Circ.* 436, 8 pp.).—Symptoms of fireblight are described and illustrated. Host plants are listed. Apple varieties differ widely in their resistance to fireblight, but most pear varieties are susceptible to the disease. Control measures include spraying with streptomycin (100 p.p.m.), Bordeaux mixture (1 : 3 : 100), or zineb (2 lb. per 100 gal.). Cultural control measures are also discussed. A. H. CORNFIELD.

Raspberry and blackberry disease control. L. P. Nichols and A. H. Bauer (*Pa. agric. Exp. Sta.*, 1954, *Circ.* 431, 13 pp.).—Symptoms of fungus, virus, and bacterial diseases are described and methods of control, both cultural and chemical, are discussed.
A. H. CORNFIELD.

The raspberry cane midge (*Thomasiniana theobaldi*, **Barnes**). **III. Control.** R. S. Pitcher (*J. hort. Sci.*, 1955, **30**, 73—85).—Raspberry canes were sprayed with either wettable powder or emulsions of BHC or DDT and with parathion emulsion and then exposed to active midges. Emulsions of BHC and DDT gave some control of oviposition, but wettable powders did not. Adequate protection was not given by any treatment. Parathion at concn. $\leq$0·005% and above was very toxic to eggs and larvæ already present on the canes. BHC was moderately toxic in the spring and DDT not at all. Soil treatments with tar oil and naphthalene gave some control of the first midge generation but no treatment controlled the second. In field trials using young canes DDT wettable powder at 0·1% was effective only if the soil was sprayed also. 0·1% BHC wettable powder applied twice gave good control, and was superior to BHC emulsion. 5% dusts of BHC and DDT were less effective than wettable powders. Parathion gave variable results.
T. G. MORRIS.

Preliminary field trials to control blast of stone fruit *Pseudomonas syringæ*, **van Hall**). D. W. Dye (*N.Z. J. Sci. Tech.*, 1954, **36, A**, 331—334).—Small field trials were carried out mid-winter, late winter, and early spring to test the efficacy of four therapeutants for preventing infection of peach trees by *Pseudomonas syringæ*. The trees were wounded, in the bark, then sprayed with therapeutant, and, when dry, sprayed with a broth culture of a pathogenic strain of *P. syringæ*. Streptomycin sulphate was outstandingly superior to the other three therapeutants, of which Bordeaux mixture was sufficiently good to merit further trial, whereas Flit 406 afforded only slight protection in two of the trials and Dithane Z78 yielded anomalous results. G. HELMS.

Brown rot of stone fruits. II. Differences in incidence in the peach variety, Levis Cling, on different soil types. H. R. Angell (*J. Aust. Inst. agric. Sci.*, 1955, **21**, 30—31).—Excessive soil moisture, which is differentially associated with soil types during and after wet weather, is the main environmental factor causing brown rot in peaches.
S. C. JOLLY.

Ant control in citrus orchards. F. J. Stofberg (*Fmg S. Afr.*, 1954, **29**, 529—531).—*Pheidœ megacephala*, Fab. is controlled by Dieldrin or Endrin at 1·5, DDT at 2, and chlordane at 2% emulsion or as wettable powder concn., the former remaining effective for at least a year whereas DDT and chlordane only give protection for six months. E. G. BRICKELL.

Biology and control of *Polistes exclamans*, **Viereck in Arizona citrus groves.** L. Hopkins (*J. econ. Ent.*, 1955, **48**, 161—163).—Of 12 insecticidal formulations tested against *Polistes exclamans*, Thanite (1%)–kerosene was the most effective and economical spray tested, giving a quick knockdown of wasps and good control of the nest brood. Nests treated with this spray were not reactivated.
A. A. MARSDEN.

Vapour heat sterilisation of California citrus and avocado fruits against fruit-fly insects. W. B. Sinclair and D. L. Lindgren (*J. econ. Ent.*, 1955, **48**, 133—138).—Citrus and avocado fruits were treated by moist heat (saturated vapour) at 43·3 and 49°. Navel oranges were severely injured and lemons showed surface injuries after this treatment; the storage life of both fruits was also significantly reduced. Valencia oranges, unless picked late in the season, showed little or no damage, whilst grapefruit withstood the 43·3° treatment. Heat treatments produced a marked reduction in titratable acidity and ascorbic acid of navel and Valencia oranges, grapefruit and lemons. Vapour-heat treatments altered the taste of citrus fruits and produced off-flavours which were very marked in lemons and navel oranges. Avocado fruits did not tolerate these treatments.
A. A. MARSDEN.

Ovicidal action of insecticides on phyloscera eggs. O. Jancke and H. Becker (*Z. PflKrankh.*, 1955, **62**, 61—67).—Among a number of insecticides examined prep. of nicotine, derris, BHC, and Systox gave the best results. The action of most of the insecticides was not influenced by temp. in the range 18—30°. Toxaphene and a Systox prep. were more effective at 30° than at 18°. Humidity had no apparent effect on ovicidal activity. A. G. POLLARD.

Fungicides for the control of *Cercospora musæ*, **in Guadeloupe.** M. Merny (*J. trop. Agric. appl. Bot., Paris*, 1954, **1**, 61—70).—The effectiveness of fungicides against *Cercospora musæ* was determined in the field by spraying four circular areas on a banana leaf with different test materials and noting the no. of lesions arising from natural infection. Zineb was superior to, whilst tetramethylthiuram disulphide, *N*-trichloromethylthiotetrahydrophthalimide, salicylanilide, and $CuSO_4 + ZnSO_4$ were as effective as $CuOCl_2$ in controlling *Cercospora* infection. A. H. CORNFIELD.

Control of *Hyphantria cunea*, Drury. C. Reichart and L. Szalay-Marzsó (*Acta agron. hung.*, 1954, **4**, 279—312).—Spraying of mulberry trees with a 10% oil emulsion containing up to 10% of DDT is more effective than dusting, and also kills adult larvæ. Control should, however, be directed as far as possible against larval populations when in the less developed and more susceptible stages.
P. S. ARUP.

Leaf-spot of tomato caused by *Stemphylium floridanum*, nov. sp. C. I. Hannon and George F. Weber (*Phytopathology*, 1955, **45**, 11—16).—The symptoms produced by the fungus on tomato and other plants, and its morphological and cultural characteristics are described.
P. S. ARUP.

Mosaic virus of cucumber (*Cucumis virus I*, Doolittle). G. Roland (*Parasitica*, 1955, **11**, 3—9).—An anti-serum to the virus can be prepared by intravenous injection of rabbits with centrifuged press-juice from infected cucumber plants. The serum gives positive results by the author's serological micro-method for infected tobacco or tomato plants, but not for infected cucumber or dahlia plants. Variations ranging from extreme susceptibility to non-susceptibility to the virus are recorded for several likely host-plants.
P. S. ARUP.

Ohio MR25, a pickling cucumber highly tolerant to mosaic. J. D. Wilson, C. A. John, and F. Myrice (*Ohio agric. Exp. Sta.*, 1954, *Res. Circ.* 25, 8 pp.).—The pedigree and characteristics of the variety, which is also resistant to angular leaf spot, are described.
A. H. CORNFIELD.

Schradan content in field grown peas in relation to pea aphid control. T. B. Davich and J. W. Apple (*J. econ. Ent.*, 1955, **48**, 180—181).—Foliage applications of schradan at 4 and 8 lb. per acre gave very high initial control of *Macrosiphum pisi*; residues 2 days after treatment were 109·2 and 266·8 p.p.m. respectively. Aphids increased after 14 days, although 19·8 p,p.m. of schradan were detected in plants treated at the higher rate. Shelled peas harvested 21 days after spraying contained 3·2 and 6·5 p.p.m. of schradan, respectively. At a dosage of 1 lb. of toxicant per acre 90% kill of aphids was obtained 9 days after treatment; no residues were found in the shelled peas at harvesting but plants contained 31·5 p.p.m. of schradan 9 days after treatment. A 0·25 lb. dosage was inadequate for good aphide control.
A. A. MARSDEN.

Pea aphid control with Demeton in relation to pea plant maturity. J. W. Apple and R. Martin (*J. econ. Ent.*, 1955, **48**, 193—195).—Peas within three weeks of harvest were protected from aphids for at least 10 days by Demeton (2 oz. per acre). Dosages of 4 and 8 oz. of Demeton on young plants gave good protection for 18 days; single treatments on young plants gave insufficient protection until harvest due to the rapid degradation of this toxicant within pea plants. Residue analyses on plants of different sizes after treatment with various dosages of Demeton (1—8 oz. per acre) are tabulated.
A. A. MARSDEN.

Control of the two-spotted spider mite on bush beans. S. Togashi and R. L. Parker (*J. econ. Ent.*, 1955, **48**, 177—179).—Single treatments of three varieties of bush beans with Aramite and Ovotran wettable powder sprays gave <99% kill after 19 days; Malathion produced only good initial kill. In tests over 9 days using potted plants, Aramite, Malathion, Ovotran, and Chlorobenzilate gave >91% control. All three varieties of beans used showed the same susceptibility to mites. The leaves of one variety showed some yellowing after treatment with a 25% Malathion spray.
A. A. MARSDEN.

Streptomycin sulphate for the reduction of bacterial soft rot of packaged spinach. Wilson L. Smith, jun. (*Phytopathology*, 1955, **45**, 88—90).—Applications of streptomycin either by spraying plants before harvesting or by dipping leaves after collecting protected the leaves from soft rot for several days.
A. G. POLLARD.

***Phytophthora* root rot diseases of Lawson cypress and other ornamentals.** D. C. Torgeson, R. A. Young, and J. A. Milbrath (*Ore. agric. Exp. Sta.*, 1954, *Bull.* 537, 18 pp.).—The cypress fungus (*Phytophthora lateralis*) attacks only *Chamæcyparis* spp., whilst the cinnamon fungus (*P. cinnamoni*) attacks a wide range of ornamental conifers and broad-leaved plants. The resistance or susceptibility of a wide range of plants to both species of fungus are reported. Chemical methods of soil treatment have not usually been successful in controlling the disease. Cultural methods of control are discussed.
A. H. CORNFIELD.

Tests with BHC emulsion sprays to keep boring insects out of pine logs in Massachusetts. W. B. Becker (*J. econ. Ent.*, 1955, **48**, 163—167).—Lindane (0·2 and 0·4%) emulsion sprays gave good to excellent protection of unseasoned pine logs from wood and bark boring beetles. The higher concn. caused no visible injury to living pines, hemlock, or deciduous plants when applied in early spring, but foliage injury resulted when broad-leaved deciduous hardwood trees were sprayed with 0·1—0·4% γ-isomer spray emulsions.
A. A. MARSDEN.

Cotton production, insect and disease control. Anon. (*S. Carolina agric. Exp. Sta.*, 1954, *Circ.* 393, 16 pp.).—Practical recommendations for growing cotton and measures for controlling insects and diseases are described.
A. H. CORNFIELD.

Response of moths of the pink bollworm and other cotton insects to certain ultraviolet and visible radiation. P. A. Glick and J. P. Hollingworth (*J. econ. Ent.*, 1955, **48**, 173—177).—In field tests with traps a near-ultraviolet lamp (single 15-w. black-light fluorescent lamp) was the most efficient light source for collecting moths of pink bollworms and other cotton insects. In most tests, a black-light blue fluorescent lamp was nearly as efficient. Little difference was noted in the no. of male and female moths caught. Lamps with their principal radiation in the visible portion of the spectrum attracted few moths.
A. A. MARSDEN.

Electron microscopy of a bacteriophage attacking *Xanthomonas malvacearum*. D. W. Rosberg and A. L. Parrack (*Phytopathology*, 1955, **45**, 49—51).—The bacteriophage could be isolated from old dried, but not from fresh blight-infected cotton plant leaves; its morphology, cultural characteristics, and mode of attack on the bacterial cells are described.
P. S. ARUP.

Anthracnose: a serious disease of tobacco nurseries in N. Rhodesia caused by *Colletotrichum tabacum*, Boning. E. A. Riley (*Trop agriculture, Trin.*, 1954, **31**, 307—311).—In N. Rhodesia the disease is spread by wind and by soil rather than by seed, and is associated with alkaline conditions in soil. Sparse sowing in seed-beds and repeated spraying with Bordeaux mixture failed to provide complete protection.
A. G. POLLARD.

Strains of sugar-cane mosaic in Puerto Rico. G. W. Bruehl (*J. Agric. Puerto Rico*, 1954, **38**, 188—198).—Three strains (A, B, and D; similar to Louisiana strains) of sugar-cane mosaic predominated in Puerto Rico. There was no marked geographic pattern of strain distribution. Strain A was the most easily transmitted mechanically, but strain B predominated in the field. There were varietal differences in the resistance of sugar-cane to the different strains of mosaic.
A. H. CORNFIELD.

Chemical composition of sorghum roots and its relation to chinch bug injury. J. E. Webster, F. Davies, and J. Sieglinger (*Okla. agric. Exp. Sta.*, 1954, *Tech. Bull.* 49, 9 pp.).—Differences in the chemical composition (ash-free solids, total sugars, sucrose, and total and sol. N) of the roots of four varieties of sorghum during early growth were not related to varying resistance of these varieties to chinch bug injury.
A. H. CORNFIELD.

Peppermint diseases. C. E. Horner (*Ore. agric. Exp. Sta.*, 1955, *Bull.* 547, 16 pp.).—The symptoms of rust (*Puccinia menthæ*, Pers.), verticillium wilt, nematode and root rot damage, and some other minor diseases of peppermint are described. A peppermint disease control programme is presented.
A. H. CORNFIELD.

Diseases of ornamental shrubs and vines. L. P. Nichols (*Pa. agric. Exp. Sta.*, 1954, *Circ.* 429, 24 pp.).—The diseases, mainly fungal, of 50 species of ornamental shrubs and vines are described. Control measures are discussed.
A. H. CORNFIELD.

Diseases of commercial florist crops. R. S. Kirby, O. D. Burke, and L. P. Nichols (*Pa. agric. Exp. Sta.*, 1954, *Circ.* 423, 36 pp.).—Disease symptoms on a wide variety of flowering plants and methods of control are described.
A. H. CORNFIELD.

Symptoms induced in chrysanthemums on inoculation with the viruses of mosaics, aspermy, and flower distortion. P. Brierly (*Phytopathology*, 1955, **45**, 2—7).—The symptoms induced in three varieties of chrysanthemums are described.
P. S. ARUP.

Pests of stored products in New Zealand. I. Family *Phycitidæ* (*Lepidoptera*). K. A. J. Wise (*N.Z. J. Sci. Tech.*, 1955, **36**, 523—530).—The species concerned are *Ephestia sericarium*, *E. elutella*, *E. cautella*, and *Plodia interpunctella*. Original and N.Z. references and records of occurrence are listed together with notes on synonymy, habits, and morphological characteristics.
E. G. BRICKELL.

Insect infestation of West African groundnuts. P. L. K. Fairchild, W. D. Raymond, and R. G. W. Spickett (*Colon. Plant Anim. Prod.*, 1954, **4**, 330—333).—The effect of varying degrees of attack by *Tribolium castaneum*, *Trogoderma granarium*, and *Caryedon fucus* on the amount and quality of oil and protein in groundnuts is reported. In general, large losses of oil and protein occur only with heavy infestations. Acidity of extracted oil increases with extent of infestation, and this increase is associated with fragmentation of the nuts by the insects.
S. C. JOLLY.

Control of adult tabanids by aërial spraying. A. W. A. Brown and P. E. Morrison (*J. econ. Ent.*, 1955, **48**, 125—129).—In laboratory tests lindane, DDT, and Dieldrin were all toxic to adults of several forest species of *Tabanus* and *Chrysops*: lindane was the fastest

material in action. In the field aërial applications, lindane (0·5 lb. per acre) temporarily eliminated tabanids in open but not in dense Canadian forest. Neither DDT nor Dieldrin was satisfactory in these field tests. A. A. MARSDEN.

Chemical weed control in farm crops, pastures, and brush. G. H. Bergren, S. M. Raleigh, J. S. Cobb, D. E. H. Frear, B. S. Horne, R. E. Patterson, W. C. Bramble, and J. O. Pepper (*Pa. agric. Exp. Sta.*, 1954, *Circ.* 440, 21 pp.).—Practical recommendations are given for the use of a wide variety of selective and " blunderbuss "-type weed killers. A. H. CORNFIELD.

Chemical weed control recommendations. V. H. Freed, W. R. Furtick, E. R. Laning, jun., and R. Warren (*Ore. agric. Exp. Sta.*, 1954, *Bull.* 539, 23 pp.).—Practical recommendations for the chemical control of weeds in berried fruit, trees, ornamentals, legumes, small grain, annual and perennial grasses, pastures, and vegetable crops are given. Control of aquatic weeds, brush and " blunderbuss " methods of killing vegetation are also discussed. A. H. CORNFIELD.

Spraying to control big sagebrush, *Artemisia tridentata*. D. N. Hyder (*Ore. agric. Exp. Sta.*, 1954, *Bull.* 538, 12 pp.).—The butyl ester of 2 : 4-D (1·0—1·5 lb. of 2 : 4-D acid equiv. + wetting agent in 5—6 gal. of water per acre) is recommended for the control of big sagebrush. Practical spraying, including aircraft spraying, measures are given. A. H. CORNFIELD.

Absorption, translocation, and metabolism of radio-active 3-(*p*-chlorophenyl)-1 : 1-dimethylurea (CMU) by bean plants. S. C. Fang, V. H. Freed, R. H. Johnson, and D. R. Coffee (*J. Agric. Food Chem.*, 1955, **3**, 400—402).—Carbonyl-^{14}C-labelled CMU applied to the leaves of bean plants is absorbed and translocated to all parts of the leaves where >90% of the radioactivity remains. Two days after treatment chlorosis was observed. Two radioactive compounds were found after a chromatographic separation of an 80% alcohol extract, (*a*) unchanged CMU, R_F value between 0·84 and 0·87 in butanol–acetic acid–water solvent, and (*b*) an unknown with R_F value between 0·62 and 0·66. E. M. J.

Effects of 2 : 4-dichlorophenoxyacetic acid on sixty woody plants. H. J. Dittmer (*Agron. J.*, 1954, **46**, 581).—Heavy applications of 2 : 4-D to lawns resulted in damage to many shrubs and trees growing nearby. This was probably due to adsorption of 2 : 4-D through the surface roots of these species. The extent of injury to the various species is described. A. H. CORNFIELD.

Differential varietal response of oat varieties to 2 : 4-dichlorophenoxyacetic acid. J. H. Williams (*Agron. J.*, 1954, **46**, 565—569).—There were varietal differences in yields, no. of spikelets per panicle, and no. of kernels per spikelet of oats due to application of 2 : 4-D (1 lb. per acre) during floral primordia initiation. The greatest yield reductions occurred when applications were made 34 days after planting, but applications at all dates resulted in lower grain yields than those obtained from check plots. Yield reductions were closely associated with reductions in no. of kernels per spikelet and no. of spikelets per panicle. Differential response of varieties at any one treatment date could usually, but not always, be explained on the basis of different degree of development at the time of treatment. A. H. CORNFIELD.

Chromotropic acid method for determining 2 : 4-D residues in rinses. L. C. Erickson and B. L. Brannaman (*Hilgardia*, 1954, **23**, 175—184).—An examination of the probable hazard of the residues of 2 : 4-D in spray equipment is reported. Pieces of galvanised Fe, Cu, Sn, Fe, Al, and glass, after immersion in aq. 2 : 4-D for 24 hr., were rapidly rinsed with 4 × 50 ml. amounts of water followed by rinses which remained in contact for 24 hr. The 2 : 4-D was determined in the rinses using the chromotropic acid method. Nearly all the 2 : 4-D was removed by the first of the four rapid rinses. The 24-hr. rinses showed that 2 : 4-D was absorbed by the metals (except Sn) and slowly released. Cu and glass retained only traces, but Fe and Zn retained considerable amounts. No 2 : 4-D was found on Al after three rapid rinses but after a further five days contact some was present. Tarnished Zn retained several times the amount held by bright Zn. Rapid rinsing with aq. NH_3 (0·027N) did not remove 2 : 4-D but prolonged soaking facilitated removal. Water was ineffective in removing 2 : 4-D esters from any of the materials and acetone dissolved only part of the ester. T. G. MORRIS.

Factors affecting the performance of treadle sprayers. W. N. Bruce and G. C. Decker (*J. econ. Ent.*, 1955, **48**, 167—169).—Animal routine was a highly important factor in the efficiency of spray concentrates applied by treadle sprayer ; the animals should have access to the sprayer at that time of day when horse fly population is highest. Design and operation of sprayer units and insecticide formulation were also controlling factors in the degree of control of *Tabanus sulcifrons* with treadle sprayers. A. A. MARSDEN.

New method for control of nozzles of low and high consumption. R. Caussin (*Parasitica*, 1955, **11**, 10—15).—The apparatus described consists essentially in a universally adjustable mounting for the nozzle, and a mechanical obturator formed by a pair of partly overlapping and rotating semicircular metal discs ; the angle of the open sector produced by the overlap, and the rate of rotation are adjustable. The quant. distribution (longitudinal and transverse) of spraying liquid is determined in relation to the type of nozzle, the pressure employed, and the time of spraying, by spraying 3% aq. methylene blue on to glass plates (16·4 × 12 cm.) which are disposed horizontally near to the ground at various distances and angles in relation to the spray ; the actual evaluation of results can be made by colorimetric determination of the dye collected from each (wet) plate, and by microscopical counts and measurements of the droplets dried on the plates. P. S. ARUP.

2.—FOODS

Grain substitutes. V. Nutritive value of synthetic rice. V. Subrahmanyan, G. S. Bains, M. Swaminathan, and D. S. Bhatia (*Bull. centr. Food technol. Res. Inst., Mysore*, 1954, **4**, 55—57).—Synthetic rice prepared from tapioca flour (65), groundnut (15), and wheat (25%) was coated with Ca caseinate. Tests on rats showed that both types of rice were superior to raw milled rice in overall growth-promoting value, and the animals fed on the synthetic rice reproduced satisfactorily whereas those fed on raw milled rice did not. H. S. R.

Effect of storage on the chemical composition and nutritive value of groundnut flour, tapioca flour, and their blends. V. Subrahmanyan, G. Rama Rao, H. B. N. Murthy, and M. Swaminathan (*Bull. centr. Food technol. Res. Inst., Mysore*, 1954, **4**, 31—33).—Tapioca flour, expeller pressed groundnut cake flour, and blends of the two are stored at 72—91°F. and 98·6°F. for five months and the effects of storage studied. A slight loss of thiamine occurs in all samples ; groundnut flour samples and blends show an increase in the free-fatty-acid and peroxide value of the fat present ; groundnut flour alone develops slight rancidity. No diminution of the nutritive value of the blends is observed. N. M. WALLER.

Preparation and chemical characteristics of the cohesive proteins of wheat, barley, rye, and oats. D. K. Cunningham, W. F. Geddes, and J. A. Anderson (*Cereal Chem.*, 1955, **32**, 91—106).—Extraction of visco-elastic proteins from wheat, barley, rye, and oat flours is effected using dilute formic, oxalic, or citric acids (0·01N-formic acid is the most efficient for wheat and oats). Since powerful shearing forces are required to solubilise the proteins a Waring Blendor is used. Proteins from wheat, barley, and rye flours are pptd. by neutralising the formic acid extract with saturated $Ca(OH)_2$ solution, but oat proteins so pptd. are clay-like rather than cohesive. The N and amide-N contents, water-absorbing powers, and appearances of the four protein preparations are recorded. N. M. WALLER.

The effect of temperature on the rate of penetration of moisture within damped wheat grains. J. D. Campbell and C. R. Jones (*Cereal Chem.*, 1955, **32**, 132—139).—The rate of moisture penetration to the centres of damped Manitoba wheat grains is determined using a method based on the micro-determination of endosperm density. The rate is increased three-fold by a rise of 12° between 20 and 43·5° ; 85% completion of moisture movement occurs in 24 hr. at 20° and 2·6 hr. at 43·5°. Initial warming of damp grain to 43·5° for at least 1 hr. shortens the subsequent period required for moisture distribution at lower temp. N. M. WALLER.

Inhibition of wheat lipoxidase by cyanide. G. N. Irvine and J. A. Anderson (*Cereal Chem.*, 1955, **32**, 140—143).—Crude wheat lipoxidase is inhibited by as much as 63% by cyanide concn. of the order 5 × 10^{-3}M. The reaction is sluggish and is best observed if 15 min. equilibration of enzyme and cyanide is permitted before the substrate is added. The degree of inhibition is dependent on the enzyme level and on the total fluid volume used. N. M. WALLER.

Viscosity *vs.* protein and ash content of western wheat varieties. C. R. Bresson and M. A. Barmore (*Cereal Chem.*, 1955, **32**, 144—152).—Data on viscosity of flour suspensions as related to protein and ash content of 17 commercial wheat varieties are summarised by the calculation of correlation coeff. and regression equations. Highly significant correlation coeff. (0·7—0·95) are obtained between viscosity of flour suspension and flour protein content for all varieties. N. M. WALLER.

Bacterial aspects of soda cracker fermentation. J. Micka (*Cereal Chem.*, 1955, **32**, 125—131).—The relative growth of yeast and bacteria and their effect on fermentation, pH, temp. increase of soda cracker sponge, and quality of the finished product are studied.

Proportions of yeast above 0·5% retard development of acidity to such an extent that an undesirable flavour and abnormally high pH values result. N. M. WALLER.

Gas pressure in fermented doughs. C. H. Bailey (*Cereal Chem.*, 1955, **32**, 152—156).—A device is described for measuring the pressure of gas released by fermentation in the vesicles of bread dough. In a common type of dough made with hard wheat flour containing 11·3% crude protein the measured pressure averages 1·032 atm. N. M. WALLER.

Scientific and technical progress in yeast and bread production. C. N. Frey (*Food Technol.*, 1955, **9**, 211—218).—The development of science is reviewed from 600 B.C., dealing with: the scientific spirit in historical perspective, the scientist's environment today, the federation of science and industry, science and industry take epochal strides, a challenging problem (to grow yeast in molasses 1917/19), understanding and controlling yeast production, obtaining control over flours, strengthening the "staff of life." E. M. J.

Storage stability of vacuum-packed active dry yeast. A. R. Felsher, R. B. Koch, and R. A. Larsen (*Cereal Chem.*, 1955, **32**, 117—124).—Samples of granular and pelleted active dry yeast packed in vacuum are stored at temp. ranging from −20 to 120°F. As the storage temp. increases the storage life of the yeast decreases from two years to eight days. Alternating periods of freezing and thawing temp. have no detrimental effect, and no significant difference between the behaviour of granular and pelleted forms is observed. N. M. WALLER.

Food yeast. V. Subrahmanyan, Gowri Sur, and M. Swaminathan (*Bull. centr. Food technol. Res. Inst., Mysore*, 1954, **4**, 14—18).—A review of the use and value of yeast as a food supplement in human diets. (76 references.) N. M. WALLER.

Alcohol production and growth of aërobically cultivated bakers' yeast. M. Lemoigne, J. -P. Aubert, and J. Millet (*Rev. Ferment. Industr. aliment.*, 1955, **10**, 17—24).—Formation of EtOH is not suppressed in aerated glucose media; after the period of active growth (second to sixth hr.), all the glucose is found to have disappeared, approx. 75% thereof having been converted into EtOH, which is then consumed by the yeast at a slower rate of growth. Uniform fermentation, with direct consumption of the glucose, can be achieved only by considerably reducing the concn. of glucose in the medium. These results are discussed in relation to the findings of other investigators. P. S. ARUP.

Simple method for determination of optimum proportion of water for dough making. C. J. Wensveen and H. de Miranda (*Conserva*, 1955, **3**, 296—300).—The Halton "Research Water Absorption Meter" is described, with minor modifications for the adaptation of the apparatus to Dutch practice. Reproducible results are obtainable with unfermented dough only; the tests should be made without the addition of yeast, and soon after kneading for 3 min. in the Swanson mixograph. A correction curve is given by means of which the amount of water added may be adjusted to the optimum proportion by reference to the extrusion time found in a single test. P. S. ARUP.

Physical properties of cake as affected by method of butter manufacture and addition of emulsifying agent. F. E. Hunt and M. E. Green (*Food Technol.*, 1955, **9**, 241—246).—Butter made by two different methods had little effect on the batter or cake. Addition of an emulsifying agent to either kind of butter resulted in cakes of slightly bigger volume. The addition of a mono- or diglyceride type of emulsifying agent to either kind of butter helped in dispersion of fat and of gas throughout the batter, and resulted in a more even distribution of fat in the finished cake. (11 references.) E. M. J.

Application of the thiobarbituric acid test to cereal and baked products. E. F. Caldwell and B. Grogg (*Food Technol.*, 1955, **9**, 185—186).—The formation of yellow interfering compounds, attributed to the presence of sugar in the digest is the principal difficulty in testing other than pure fats. In tests on extracts of cereal and baked products, a chromatographic procedure is used to separate the yellow components from the residual red colour which is estimated by colorimeter or spectrophotometer. The method described is suitable for following the progress of fatty acid oxidation in samples undergoing normal or accelerated storage tests. E. M. J.

A rapid method for the estimation of sucrose in bagasse. J. A. Shivas and R. W. Bringhurst (*Sugar, N.Y.*, 1955, No. 4, **50**, 48).—Finely milled bagasse (20 g.) is mixed with 200 ml. of water for 1 min. in a Waring Blendor, the bagasse removed by filtering through a Buchner funnel, the solution defecated with basic lead acetate, filtered and polarised in the usual way, the whole operation taking 10 min. When checked by a method employing one-hour digestion on a boiling water-bath, and the results statistically analysed, the rapid method was shown to yield results within 2% of the sugar content by the digestion method. L. G. L. UNSTEAD-JOSS.

Ion exchange: quality of sugar produced by reverse cycle purification of juices. C. A. Fort and B. A. Smith (*Sugar, N.Y.*, 1955, **50**, No. 4, 43—45).—Reverse cycle ion-exchange purification was applied on pilot-plant scale (40 gal. per run) to clarified cane juices, using basic ion-exchanger "S" or IRA-410 and acid exchanger IRC-50 in two columns, and also in three columns using IRC-410 followed by IRC-50 and "S," and in four columns using the same items followed by IRC-50. The effluents were analysed and the batches of sugar crystallised from the treated juices are described. The three-column process gave the highest purity of effluent. Colour removal in the columns was inadequate, but approx. 8·3% increased yield of sugar was obtained by the demineralisation. H. S. R.

Tests for the evaluation of refinability of raw beet sugar. M. P. Devillers (*Sucr. franç.*, 1955, **96**, 1—3).—The qualities of raw sugar which affect refining are reviewed. Two methods of affining are described, one using saturated syrup for washing, the other using 70% alcohol, followed by filtering on a Buchner funnel under vac. A method of analysing the products of the affination is described; products by both methods contain the same impurities. Analysis of eluted fractions after a solution of affined sugar is run on ion-exchange columns indicates that more than 80% of the non-sugars can be determined by this method. Results of analysis of first and second spun beet sugars and third spun cane sugar before and after being affined with syrup and alcohol indicate that affining with two lots of 70% alcohol is equivalent to washing with four lots of syrup. Other methods of evaluating raw sugars are mentioned. SUG. IND. ABSTR. (E. M. J.).

Unfermentable reducing substances in molasses. F. W. Zerban and L. Sattler (*Int. Sugar J.*, 1955, **57**, 71—77).—The origin and nature of the reducing substances in molasses, and problems concerned with their analysis are discussed; these substances are classified as carbohydrates, fragmentation compounds produced by thermal decomposition, reductones which are characterised by enediolic structures, and N-compounds. (60 references.) SUG. IND. ABSTR. (E. M. J.).

Influence of various factors in juice purification on juice colours. W. Dörfeldt (*Z. Zuckerind.*, 1955, **5**, 73—80; cf. J.S.F.A. Abstr. 1955, i, 362).—The effects of nine defecation variants (1, 2, or 3% CaO addition to raw beet juice, each with or without cold or hot pre-liming) on the course of colour changes up to massecuite production were studied. Extinction coeff. were determined at 436, 546, and 579 mμ., solutions being at pH 7. Juices from 14 beet varieties were tested. There was more decrease in colour on increase of lime from 1 to 2% than from 2 to 3%. Cold pre-liming decreased the colour by 20—40% compared with that of juices treated by main liming alone; with cold pre-liming, colours were better in the first saturation and in thin juices, but were the same in thick juices for all three lime additions; with cold pre-liming, the increase in colour from thin juices to massecuite was relatively less than that produced after hot pre-liming. SUG. IND. ABSTR. (E. M. J.).

Superphosphate as an aid to sugar-cane juice clarification. I. J. S. Huja (*Indian Sugar*, 1954, **4**, 435—437, 439, 441—444).—The addition of superphosphate to cane juice before liming is recommended in order to effect the adsorption of colloids on the ppt. and so improve clarification. Tests in the 1951—2 season indicated that an addition of 0·4 lb. of single superphosphate per ton of cane gave a good rise in juice purity especially in juices of low Brix. Tests using triple superphosphate were made in 1952—3 season; 0·1 lb. of triple superphosphate per ton of cane was found to be an optimum amount for juice which had an original phosphate content of 0·06% or 2·41 pt. per 100,000 pt. of juice. The merits of single and triple superphosphate are discussed. SUG. IND. ABSTR. (E. M. J.).

Balance of lead in West Indies sugar factories. H. C. S. de Whalley (*Xth Congr. Int. Ind. Agric.*, 1954, **2**, 1354—1361).—Very low max. limits for the Pb content of refined sugars, syrups, and treacle have been proposed by the Ministry of Food in Great Britain. The Pb content of crusher juices (from canes as received) in factories in Jamaica and Trinidad is generally <1 p.p.m. The proportion may be increased from Pb joints in heaters, but may be eliminated by defecation and in evaporator scale. The final raw sugar and molasses may contain more or less than the original crusher juice, but the amount is low enough to make them suitable for refining and/or syrup or treacle manufacture. The method used for the determination of Pb is given in an appendix. SUG. IND. ABSTR. (E. M. J.).

Analysis of industrial glucose. J. Vollaire-Salva (*Mitt. Lebensm. Hyg., Bern*, 1955, **46**, 58—66).—The method of J. Terrier (*ibid.*,

1952, **43,** 315) is discussed. The mixture of the reducing sugars, glucose and maltose is determined by the Luff-Schoorl reaction, the result being expressed as glucose (**I**); total dextrins are found after saccharification for 3 hr., boiling with a suitable concentration of HCl sp. gr. 1·19, the result being expressed as glucose (**II**), the total dextrins being (**II** − **I**) × 0·9. The proportions of glucose and of maltose can be calculated by the polarimetric deviation of the glucose + maltose mixture. Details of these findings are given for several samples, a typical analysis being: water 14·1, glucose 21·05, maltose 23·3, dextrins 41·45, mineral matter 0·35% respectively. In similar tests the reducing sugars were determined by fermentation with pure *Saccharomyces cerevisiæ* and results were compared. E. M. J.

Paper chromatography of mannose phenylhydrazone. C. P. Natarajan and G. S. Bains (*J. Sci. industr. Res. India*, 1955, **14, C,** 81—82).—Some results are noted from a study of a mannose phenylhydrazone solution in pyridine held for different times (0—120 hr.) at room temp., using ascending and descending paper chromatographic techniques and various solvent systems, *e.g.*, butanol: alcohol: water (4 : 1 : 5); butanol: pyridine: water (3 : 1 : 1·5); and butanol: acetone: water. Spraying agents used were: aniline hydrogen phthalate, resorcinol, benzidine–trichloroacetic acid, and benzidine in alcohol. In solutions over 24 hr. old a new spot appears with R_F close to that of mannose, arabinose, and fructose, due to an as yet unknown substance. G. C. Jones.

Chromatography of fruit sugars: constant presence of small amounts of sugars other than glucose and fructose. L. Genevois, G. Vitte, and C. Guichard (*C. R. Acad. Sci., Paris*, 1955, **240,** 1150—1151).—By paper chromatography of the solution (in anhyd. pyridine) of the dry residue obtained after treatment of fruit juice with basic Pb acetate and removal of Pb with Na_2SO_4 and CO_2, small amounts of xylose, arabinose and mannose (together or separately) have been identified in apples, oranges, cherries, peaches, strawberries, and figs. Glucose and fructose are always present; the sucrose content varies considerably, being nil in ripe cherries (Empress Eugene) but exceeding the content of glucose + fructose in peaches. W. J. Baker.

Vacuum plant for removing excess water from honey. C. R. Paterson and T. Palmer-Jones (*N.Z. J. Sci. Tech.*, **36, A,** 386—400).—In humid areas, the water content of honey frequently exceeds the stipulated upper limit of 17·2% for export honey. Details are given of a vacuum plant which will remove 2% of water from 2·5 tons of honey in 8 hr., while causing no significant darkening in colour and generally improving the flavour. G. Helms.

Discoloration of pectin gels. G. E. Livingston, N. Pandit, M. A. Steinberg, and C. R. Fellers (*Food Technol.*, 1955, **9,** 180—184).—The variables selected to determine their effect on the colour stability of pectin gels were: (*a*) cooking time and temp., (*b*) storage time and temp., (*c*) nature of sugar, (*d*) concentration and nature of carboxylic acid, (*e*) presence of ascorbic acid, and (*f*) nature of headspace gas, the effects being measured by spectrophotometric determination. Changes in the u.v. absorption spectra attributed to 5-hydroxymethyl-2-furaldehyde (HMF) and related sugar degradation products preceded visual discoloration; HMF production and darkening occurred as functions of time or temp. of exposure to heat either in cooking or in storage. These results were increased when fructose was the sole sugar, but little change occurred when glucose was used alone. Ascorbic acid increased the darkening and u.v. absorption of the jelly during heating. Headspace O_2 appeared not to be involved in the thermal degradation processes responsible for these changes. Only minor differences attributable to specific carboxylic acids were found when the original pH values were kept at 3·0. (16 references.) E. M. J.

Factors affecting character grade of frozen strawberries. W. F. Talburt, L. R. Leinbach, J. E. Brekke, and R. O. McHenry (*Food Technol.*, 1955, **9,** 111—113).—Samples of frozen sliced strawberries from raw materials of known maturity and taken from freezing plants using the common ribbon-type mixer, the screw-type mixer, or the two-stage filling operation in which syrup is added to the sliced strawberries in a separate operation, were graded and analysed to find the effects of maturity and type of processing equipment on character, colour, and ratio of constancy of fill of fruit to packing medium. The two-stage filling procedure gave the lowest % of mushy slices and the screw-type mixer gave the highest, the average results being: 3, 13, and 21% respectively. Use of syrup instead of dry sugar did not substantially reduce the % of mushy berries where ribbon-type mixers were used. The constancy of ratio of fruit to packing medium filled into containers was similar for the three types of mixing, and maturity (within the range at which fruit is normally processed) did not affect the % of mushy berries when ribbon-type mixers were used. E. M. J.

Effect of fruit handling methods on juice quality of apples. A. M. Neubert, G. H. Carter, and A. Van Doren (*Food Technol.*, 1955, **9,** 114—118).—Ripening for one week, after harvest of Jonathan, Golden Delicious, and Winesap varieties avoided an undesirably sharp astringent flavour in unblended juices, but ripening for 4 to 6 weeks resulted in poor flavour. There was a decrease in firmness, a loss of starch and acid and an increase in sugars. The advantage to flavour of post-harvest ripening for Jonathan and Winesap was not apparent when juices from these varieties were used in blends with juice from fully-ripened Delicious. Use of unripened fruit of the Jonathan and Winesap permitted inclusion of a considerably higher proportion of Delicious in preparing blends of uniform acid content without appreciable loss of flavour. E. M. J.

Storage of pears and apples in the presence of ripened fruit. F. Gerhardt and H. W. Siegelman (*J. Agric. Food Chem.*, 1955, **3,** 428—433).—The results of studies in which pears and apples of commercial maturity, grown in the Pacific Northwest, were stored at various temp. in fresh air and in measured quantities of ethylenic and non-ethylenic volatile emanations from pre-ripened fruit are presented. There was no significant difference in degree of ripeness at 31°F. The ripening response and storage life of the experimental fruit were evaluated biochemically and organoleptically. At temp. of 31°, 45°, and 65°F. Starking Delicious apples were not affected. The ripening of Anjou pears at 45° and 31° was not hastened by presence of ripened fruit, but at 65°F. the respiratory climax was hastened by about two days. E. M. J.

Activity of starch-splitting enzymes in pears during development and cold storage. G. W. F. M. McArthur-Hespe (*Centr. Inst. Voedingsonder. T.N.O. Publ.* 194, 1955, 70 pp.).—The presence in pears of phosphorylase is demonstrated. Contents of α-amylase decrease during the summer months, but increase during cold storage to 3—15 times (according to the variety) the values found in Sept. Variations in contents of β-amylase are not notable. The investigations led to no important conclusions as to the determination of the optimum time of picking for cold storage. (64 references.) P. S. Arup.

Effect of dilution upon mould counts of raspberries by the Howard method. K. H. Steinkraus, J. M. Copeland, and C. S. Pederson (*Food Technol.*, 1955, **9,** 118—119).—Mould counts were run by the Howard method on samples of raspberries before and after dilution of the pulped berries with 3% pectin solution. Dilution has little effect on highly contaminated samples, but on low mould count samples the count is changed considerably. Berry samples with medium amounts of mould indicated an intermittent behaviour depending on the initial mould count. E. M. J.

Factors influencing the mould count of raspberries. K. H. Steinkraus, J. M. Copeland, and C. S. Pederson (*Food Technol.*, 1955, **9,** 124—125).—Mould counts were run on 473 samples of fresh raspberries during the 1952/3 seasons. In general, environmental factors, *e.g.*, rainfall and humidity during the growing period, are of greater importance than the interval between pickings. The initial mould count and the time interval and temp. before processing were the factors determining the count. E. M. J.

Chemical changes in hot sulphite sultana dipping solutions. A. R. Clarke and E. M. Rossiter (*J. Aust. Inst. agric. Sci.*, 1955, **21,** 21—25).—In laboratory tests carbonation, saponification, and oxidation are the main reactions occurring in hot sulphite sultana-dipping solution (containing Na_2SO_3, KOH, and dipping oil) maintained at 190°F. and mildly aerated. SO_3'' oxidation is inhibited by the grape juice present in the solutions. These results have been correlated with those of field tests. S. C. Jolly.

Pickling Spanish-type green olives. Zdenka Samish (*Food Technol.*, 1955, **9,** 173—176).—The development of the green pickled olive industry from the use of <600 tons of processed fruit in 1948 to about 4000 tons in 1952 has resulted from the introduction of the Spanish method of curing, whereby the olives are pretreated with dilute lye solution which, during its penetration into the fruit, hydrolyses the bitter glucoside oleorupin. The olives are then rinsed and leached until all traces of lye are removed, covered with brine, and permitted to ferment. Blister formation is markedly reduced if NaCl up to 6% is added to the lye. In the removal of lye (after rinsing), neutralisation with lactic acid was tried instead of leaching. Sluggish fermentation of olives from mineral-deficient groves was hastened by the addition of 0·15% of N as NH_4Cl. These modifications of the Spanish method were successful in application to the olive crops in Israel. (18 references.) E. M. J.

Filth test for fruit preserves. G. P. Peeters (*Rev. Ferment. Industr. aliment.*, 1955, **10,** 33).—A report from the 10th International Congress for agricultural and nutritional industries, in which suitable precautions are suggested for the protection of such fruits as are liable to contamination with foreign matter. P. S. Arup.

Algebraic and graphic solutions of mixing problems involving adjustment of total solids and ratio of solids to citric acid in fruit juices. W. Kroehle (*Food Technol.*, 1955, **9**, 159—164).—Recent literature is mentioned; a rapid and complete system of calculating and adjusting batches of orange concentrate, especially for plants with no automatic proportioning equipment is discussed. The following items are covered: the negative sugar concept; proportions of ingredients required to produce (*a*) any desired Brix; (*b*) any desired ratio; (*c*) any desired Brix and ratio simultaneously; four tables relating to sugar values; three nomographs (for ratio of orange or grapefruit juice, ratio of orange concentrate, and for cut-back juice). E. M. J.

Plate type heat-exchanger as a source of bacterial contamination in processing frozen concentrated orange juice. D. I. Murdoch, C. H. Brokaw, and W. E. Allen (*Food Technol.*, 1955, **9**, 187—189).—The plate-type heat-exchanger consisting of a series of stainless steel plates designed to give the maximum amount of heat transfer, is described and figured. A test strain representative of the causal organism of bacterial contamination resembled *Lactobacillus buchneri*. Optimum conditions of growth of the organism in orange juice were in the range of 12°—20° Brix at temp. between 110 and 120°F. E. M. J.

Frozen grapefruit, tangerine, and limeade concentrates. M. K. Veldhuis, W. C. Scott, and F. P. Griffiths (*Food Technol.*, **9**, 198—201).—Laboratory analyses are given for nine samples of grapefruit, three of tangerine, and eight of limeade concentrates. The principal attributes of each type of concentrate are reviewed, recent laboratory work is reported and problems in industrial practice are discussed. Grapefruit concentrate has a greater tendency to clarify and form gels than has orange concentrate. The evaporator feed juice is heated to ~180°F. to reduce pectinesterase activity and provide cloud stability. The cutback juice is not heated. In pink and red grapefruit, pulp is finely dispersed in a colloid mill and added to the concentrate to produce an attractive finished colour. The pectin content of tangerine juice is much less than that of orange and grapefruit juices, resulting in greater stability of tangerine juice. Lime juice because of the high acid content is diluted and sweetened; a concentrate may be diluted to 35 times its volume and sweetened for use. (17 references.) E. M. J.

Scheduling plantings and predicting harvest maturities for processing vegetables. H. L. Seaton (*Food Technol.*, 1955, **9**, 202—209.—The factors influencing an adequate and dependable supply of raw products for successful vegetable canning or freezing operation are reviewed, *e.g.*, labour and equipment, crop control, fluctuations in weather. The "heat summation" or "heat unit" system is considered in regard to formulation of planting schedules, predicting harvest maturities and the merits and limitations of the system. Improvement in long-range weather forecast is needed, and more information on other factors, *e.g.*, optimum and max. temp. at which accumulated temp. are operative, length of day, light intensities, latitude and altitude, and edaphic factors such as soil type, soil moisture levels, soil nutrient levels, etc. (39 references.) E. M. J.

Refrigeration and the peeled tomatoes industry. D. Cagnoni (*Ric. sci.*, 1955, **25**, 519—520).—Tomatoes are cooled rapidly (15—30 sec.) (temp. not stated), and the skins automatically detach themselves. The colour and composition of the fruit is unchanged. T. P. McLaughlin.

Effect of heat processing on tomato juice colour. A. Kramer and W. L. Ogle (*Food Technol.*, 1955, **9**, 177—179).—An equation derived from an earlier study (*ibid.*, 1953, **7**, 400—404) and the nomograph derived from the equation were tested, and found to be valid for calculating colour loss in tomato juice under variable conditions of types and temp. of heating media, container size, and raw stock quality such as may occur in commercial processing. E. M. J.

Effect of processing conditions on the viscosity of tomato juice. D. B. Hand, J. C. Moyer, J. R. Ransford, J. C. Hening, and R. T. Whittenberger (*Food Technol.*, 1955, **9**, 228—235).—A wide range of viscosities can be obtained by adjusting the finishing conditions at any given preheating temp. The viscosity of whole tomato juice (gross viscosity) depends on the viscosity of the serum (tomato juice freed of suspended particles) and on the viscous character of the suspended particles. The rôle of pectin in determining viscosity, and the importance of preheating temp. for the inactivation of the pectic enzymes were emphasised. Serum viscosities ranged approximately from 0·95 to 2·3 centipoises at 30° as measured in a 5-ml. Ostwald viscosity pipette. The apparent gross viscosities varied from 20 to 375 centipoises at 30° as measured in a Brookfield Model LVF viscosimeter. E. M. J.

Problems in the production of tomato juice powder by vacuum. V. F. Kaufman, Francis Wong, D. H. Taylor, and W. F. Talburt (*Food Technol.*, 1955, **9**, 120—123).—Two methods are described for the production by vac. drying of tomato-juice powder which can readily be reconstituted with cold water: (*a*) a direct drying procedure by which high density tomato paste can be converted by one step into a powder and (*b*) a split drying procedure in which the tomato pulp is separated from the serum of single-strength juice, the serum is concentrated and dried separately from the pulp, after which they are recombined in right proportions. The temp. should not exceed 150°F. Addition of small amounts of $NaHSO_3$ raised the max drying temp. to 190°F. (13 references.) E. M. J.

Phosphorus and calcium contents of fifty varieties of beans in Brazil. M. L. B. Bethlem, F. Malouk, H. das Nevea, and M. Taveira (*Rev. Chim. pura appl.*, 1953, **4**, 141—156).—The phosphorus and calcium contents were determined and recorded on 50 varieties of bean (121 samples) using the phosphomolybdate (titrimetric) and oxalate/$KMnO_4$ methods respectively. The phosphorus content was found to vary from 0·0971 P_2O_5 to 3·07% with the bulk of the varieties giving a figure of about 1·0%. The calcium range was from 0·001% to 1·55% as CaO, with a large number of species falling between 0·2 and 0·3%. The absorption and metabolism of phosphorus and calcium in man are discussed, and also the Ca/P ratio and the absorption of phosphorus in organic combination. (33 references.) H. Pritchard.

Rapid moisture determination in frozen lima beans. C. Sterling (*Food Technol.*, 1955, **9**, 190—191).—The application of the Launer–Tomimatsu method of determination of moisture by dichromate oxidation and electrometric titration with ferrous ammonium sulphate, in frozen lima beans is reported. Samples of different varieties and varietal types, of different maturities and from different regions of growth were used. Compared with determination by vac. oven method, the oxidation method gave statistically reliable results. From the titration data the so-called dichromate factor (F) can be calculated; *i.e.*, g. of dry material oxidised per ml. of standard dichromate solution for a given type of material. The average dichromate factor was 0·72%. When this factor is compared with the moisture content via the regression coeff. a statistical relationship is observed between these two values. The sign of the coeff. is negative, indicating that as the moisture content declines with increasing maturity, the dichromate factor increases. E. M. J.

Jojoba bean meal—a potential feed. F. B. Wells (*Cereal Chem.*, 1955, **32**, 157—159).—Beans of the jojoba plant, *Simmondsia californica*, harvested in Arizona in 1942, contain 45·2% oil (extracted with benzene), 16·1% protein, and 14·7% carbohydrate. The ash contains S, Ca, and P. Limited tests with rats and human subjects indicate that the benzene-extracted meal has potential value as a food. The oil and its uses have been previously described (Wells, *J. chem. Educ.*, 1954, **31**, 253—254; J.A.C. Abstr., 1954, ii, 395). N. M. Waller.

Chemical composition of different varieties of ragi (*Eleusine coracana*). S. B. Kadkol and M. Swaminathan (*Bull. centr. Food technol. Res. Inst., Mysore*, 1954, **4**, 12—13).—The Ca, P, Fe, protein, fat, carbohydrate, and thiamine contents of representative samples of eight strains of ragi are determined. All strains are rich in Ca, and only slight variation in chemical composition is observed. N. M. Waller.

Phosphorus components of the white potato. S. Schwimmer, A. Bevenue, and W. J. Weston (*J. Agric. Food Chem.*, 1955, **3**, 257—250).—Data on the changes in composition of acid-extractable phosphate as well as starch phosphate of potatoes resulting from changes in temp. and storage are presented. The centrifuged residue of a potato sample is extracted with trichloroacetic acid, and the Ba salts of the phosphate esters are fractionated; the relative solubilities of the Ba salts are discussed. The dormancy period of the potato is characterised by significant metabolic activity. Phosphate esters separated chromatographically were tentatively identified as nucleotide, phytate, orthophosphate, glycerol phosphate, and phosphates of fructose and glyceric acid. The starch functions not only as a metabolic reservoir for C, but also with phytic acid, for P. Orthophosphate, and not hexose phosphates, may cause non-enzymic browning of processed potatoes. Preliminary removal of polysaccharide is indicated before phosphate fractionation, but other non-polysaccharide material may interfere. (17 references.) E. M. J.

Flavour of Sebago potatoes grown in soil treated with chlordane, heptachlor, Dieldrin, Aldrin, or Endrin. M. E. Kirkpatrick, G. S. Linton, B. M. Mountjoy, and L. C. Albright (*J. Agric. Food Chem.*, 1955, **3**, 409—412).—Tests were made for odour and flavour of cooked potatoes grown experimentally, on three commercial farms in soils treated with chlordane, heptachlor, Dieldrin, Aldrin, or Eldrin, or untreated during three years. Results are tabulated. In the first year's tests no significant amount of off-flavour was

reported in potatoes grown in soil treated with chlordane, heptachlor, or Dieldrin, but with Aldrin, the scoring tended to be lower. In the second year's tests, dosage of 2 lb. per acre of heptachlor resulted in significant off-odour and off-flavour. Wireworm injury in another sample from untreated soil caused off-flavour. In the third year's tests there was no difference in the mean values for flavour or odour of all samples. Location had a significant effect on the scoring. E. M. J.

Method for the comparison of consistency in potato granule samples appraised at different times. E. R. Wood, R. L. Olson, and Marvel-Dare Nutting (*Food Technol.*, 1955, **9**, 164—168).—A system was devised to compare samples judged at different times placing each unknown in one of five categories, by ranking a series of samples in order of rubberiness along with two coded controls which differed in rubberiness, the less rubbery being the high control, and the more rubbery, the low control; samples were assigned to categories according to mean rank (*a*), (*b*), (*c*), (*d*), (*e*). The limitations and merits of the system are discussed. (20 references.) E. M. J.

Factors affecting the texture of rehydrated potato granules. D. E. Severson, A. M. Cooley, and M. Simon (*Food Technol.*, 1955, **9**, 223—227).—Conditions of cooking, mashing, mixing and ageing, and drying affected the amount of cell breakdown during processing, and therefore affected the properties of the reconstituted material. There is also cell damage during reconstitution. Two products (*a*) and (*b*) having approximately the same amount of extracellular starch are described: (*a*) (known to have a very good texture on reconstitution) in the dried state had an average cell size more than double that of (*b*); the cells of (*a*) had been puffed in drying, and in absorbing water the starch swelled inwardly, filling the hollow and driving out the air. In (*b*) the starch swelled outwardly and on stirring the product the intracellular starch fell out of the cell wall. Mechanical stirring of (*a*) had little effect. When water absorption is complete the cells of (*a*) are completely filled with starch whereas those of (*b*) are only partly filled. Glycerol monolaurate (0·25 or 0·5% dry basis) added as a water suspension during the first mix gave the best texture. E. M. J.

Effect of drying conditions on moisture retention and density of dehydrated peas. D. B. Hand, J. C. Moyer, and A. C. Wagenknecht (*Food Technol.*, 1955, **9**, 219—222).—Factors which influence the rate of drying and the final moisture level when peas are dehydrated under various conditions are dealt with. When peas are dried in the conventional air dryer, there is a continuous retardation of the rate and drying stops before the water is completely removed. With adequate air velocity, the drying rate and final moisture content are governed by temp. particle size and R.H. Vac. drying of peas gave a product of low moisture content. There was less shrinkage of the skins and therefore less retarding effect during freeze-drying than during air drying. When the final moisture content was lower the density of the dried product was decreased. E. M. J.

Progress of research in the United Kingdom on fruit and vegetable dehydration. H. R. Barnell, E. G. B. Gooding, and H. G. Wager (*Food Technol.*, 1955, **9**, 168—172).—Recent studies on browning reactions are discussed, and the breakdown of the carotenoid pigments in plant tissues. Practical developments in dehydration are reviewed: (*a*) conventional air drying, and (*b*) vacuum contact plate drying process. Other processing conditions are considered (*a*) scalding, (*b*) sulphiting, (*c*) compression. E. M. J.

Present knowledge regarding fruit wines. R. Lambion (*Rev. Ferment. Industr. aliment.*, 1955, **10**, 25—32).—A review with 79 references, covering Belgian legislation regarding the manufacture of wines from grapes or other fruit, the composition of fruit juices, and the technology of fermentation and preservation. P. S. Arup.

Improvement of quality of wine or sweet musts by refrigeration. W. Saller (*Mitt. Klosterneuberg*, 1955, **5, A**, 101—127).—The advantages of cooling as compared with SO_2-treatment are pointed out, and types of plant are described for cooling in containers, on plate-coolers, or by means of dry-ice. Methods for concentration by refrigeration are described, and occasions for their use are discussed. (22 references.) P. S. Arup.

Evaluation of thresholds and minimum difference concentration for various constituents of wines. II. Sweetness: the effect of ethyl alcohol, organic acids, and tannin. H. W. Berg, F. Filipello, E. Hinreiner, and A. D. Webb (*Food Technol.*, 1955, **9**, 138—140).—A determination of thresholds and min. detectable differences for sucrose at 0, 1, 5, 10 and 15 g. per 100 ml. concentration levels was made using aqueous, ethyl alcohol–water, acid–water and alcohol–acid–water solutions. The effect of tannin was also determined. Alcohol enhances the sweetness of a sugar solution, *e.g.*, a solution containing 8 g./100 ml. of sucrose in 10% alcohol was judged as sweet as an aq. solution containing 10 g./100 ml. sucrose. Acids have a tendency to depress sweetness. The effect of tannin on sugar thresholds and detectable differences was to increase these values significantly over those obtained for sucrose in pure aq. solutions. A significant linear relationship exists between the min. detectable concentration differences and the concentration of sucrose being tested. E. M. J.

Extraction of polyphenolic compounds from wines. J. Amiel, P. Dupuy, and M. Nortz (*C. R. Acad. Sci., Paris*, 1955, **240**, 780—782).—The wine (500 ml.) is evaporated to 150 ml. and then diluted ~700 ml. by addition of 95° EtOH to precipitate salts and pectins. To the filtrate, adjusted to pH 9, are added 100 ml. of 95% ethanol containing 45 g. of neutral Pb acetate, with stirring and keeping the pH constant at ~9. The pptd. lakes are removed by a double centrifugation, mixed with a few drops of EtOH, and then treated dropwise with conc. HCl and ethanol until there is a slight excess of acid. If only anthocyanins are present the solution is bluish-red, if only flavones it is pale-yellow with a ppt. of $PbCl_2$. The clear filtrate is stable and can be used for separating the anthocyanins and flavones by chromatography. Application of this procedure to an alcoholic solution from macerated fruit and leaves of vine yields identical values for anthocyanin content as are obtained for wine by the Ribereau-Gayon procedure. The method also eliminates the interference caused by fluorescence of impurities during u.v. spectroscopy. W. J. Baker.

Tin in relation to wine. E. Kielhöfer and H. Aumann (*Mitt. Klosterneuberg*, 1955, **5, A**, 127—135).—In laboratory experiments, considerable amounts of Sn (~100 mg. per l.) were dissolved by wine, especially in presence of large amounts of SO_2, but a no. of retail samples of wine contained >1 mg. per l. Since even small amounts of Sn can reduce SO_2 to H_2S, and cause pptn. of proteins, the use of tinned surfaces in the manufacture of wine should be abandoned. P. S. Arup.

Alcoholic fermentation in presence of compounds of metals of the platinum group. F. Larèze (*C. R. Acad. Sci., Paris*, 1955, **240**, 919—921).—The inhibitory effect of compounds of Pt, Ir, Rh, and Ru on the consumption of glucose (20 g.) during fermentation in a 10%-yeast medium (100 g.) was determined from the vol. of CO_2 liberated and the wt.-losses of the mixture during several days. Addition of 1% of Na_3IrCl_6 or 0·05—0·1% of Na_2PtCl_6 does not affect fermentation, but 0·5% of the Pt salt has a strong inhibitory effect (only one-half the glucose being consumed). The inhibition is much less effective when the metal is in a complex, *e.g.*, 1—2% of $PtCl_2Py_4$ (Py = pyridine) or $K_2Pt(CN)_4$ having no effect on fermentation. The consumption of glucose is only 5—10 g. when the concn. of added Na_3RhCl_6 or $K_2Ru(NO)Cl_5$ is 1—2%, whereas 1% of $K_4Ru(CN)_6$ has no inhibitory effect. W. J. Baker.

Purification of acrolein-containing spirits by distillation and rectification. G. Vegezzi, P. Haller, and O. Wagner (*Mitt. Lebensm. Hyg., Bern*, 1955, **46**, 41—57).—Processes which are technically practical and economically productive are discussed for the preparation of spirits must, and fine, and extra fine spirits from fermented, and spirits must containing acrolein. A three-boiler distillation process is used and the modified apparatus not only separates the acrolein, but produces spirits of suitable requirement. Three processes are described and a diagram is given of an improved continuous must-distillation apparatus for cider distillation. The limiting contents of traces of acrolein, methanol, or fusel oil in spirits permitted by law are discussed. E. M. J.

Heating water in brewery. A. Juillerat (*Schweiz. Brauerei Rdsch.*, 1955, **66**, 51—53).—The relative advantages and disadvantages of heating by hot water or steam are discussed in relation to circumstances governing power production or availability. P. S. Arup.

Germination release in barley. E. Urion and L. Chapon (*Brasserie*, 1955, **10**, 58—64).—The presence of the husk does not seriously interfere with the germination of normal barley past the dormancy stage, but does definitely interfere in cases where O_2 requirements are abnormally high; such cases include grain which has been stored under moist and anaërobic conditions, or which has been exposed to heat or to HCN treatment. P. S. Arup.

Effect of extracts from spelt on barley enzymes. H. Nolte and A. M. Kirchdorfer (*Brauwissenschaft*, 1955, 66—68).—The extracts reduce the hydrolytic activity of β-amylase (irrespectively of the type of barley from which they are derived), but not (appreciably) that of saccharase. Spelt protein (testinic acid) has no effect on the activity of β-amylase. P. S. Arup.

Significance in metabolism of coenzyme-A and activated acetic acid. W. Heinen (*Brauwissenschaft*, 1955, 72—78).—A review with 36 references. P. S. Arup.

Fermentation carbon dioxide. I. Carbon dioxide production in lagering beer. G. W. A. Brischke (*Brauer u. Mälzer*, 1955, **8**, 10—12).—The results, causes and detection of inadequate attenuation are considered, with examples drawn from practice. P. S. ARUP.

Difficulties in fermentation and ripening of beer, and their avoidance. H. J. Wellhoener (*Brauwelt*, 1955, **B**, 365—367, 397—399).—Advice is given on the choice of malt and yeast, and on fermentation and lager-cellar management. P. S. ARUP.

Fat in kieselguhr for beer filtration. P. Lampl (*Brauwelt*, 1955, **B**, 397).—A sample of kieselguhr was found to contain 1·63% of fatty matter, which rendered it immiscible with water, and totally unsuitable for use. Fat can be detected in kieselguhr by the fluorescence under u.v. radiation of the residue obtained after evaporation of a CCl_4 extract of the sample. This test should be applied as a matter of routine. P. S. ARUP.

Biochemistry of ammonia-producing bacteria. I. Isolation and ammonia-forming capacity. J. P. Voets, R. De Borger, and A. Coolsaet (*Rev. Ferment. Industr. aliment.*, 1955, **10**, 7—16).—A simplified apparatus for enrichment of the NH_3-forming flora in soil is described in which sterile aq. 4% peptone (Difco) is caused to percolate continuously in small amounts through the soil sample, which is contained in an extraction-tube. Plating of three different soils (enriched or unenriched) on mineral + peptone gelose, followed by subculture, has yielded a no. of pure cultures of active NH_3-producers which can hydrolyse the proteins of brewery spent grains down to the NH_3 stage. P. S. ARUP.

Colour determination in wort and beer. E. Schubert (*Brauwissenschaft*, 1955, 50—57; cf. J.S.F.A. Abstr., 1955, i, 370).—The design of the author's colorimeter is based on the use of two photocells, with a third to check the constancy of the source of light. Calibration graphs for different λ are given. The instrument can also be used for turbidity determinations, based on differences between the Tyndall effects observed for beer before and after filtration through a suitable sintered glass filter which does not retain colouring matter. (30 references). P. S. ARUP.

Determination of mercury in beer. A. M. Piette (*Ann. Falsif., Paris*, 1955, **48**, 101—108).—Beer may contain Hg derived from commercial prep. containing org. compounds of Hg, which act as stabilisers, but not as antiseptics. The analytical procedure adopted includes the (slightly modified) method of Andrews and Stringer (cf. Brit. Abstr., C., 1952, 115) for the destruction of org. matter, and the use of dithizone for the colorimetric determination of Hg, either by a direct visual comparative method, sensitive to <60 μg. or by a spectrophotometric method (610 mμ.) sensitive to <25 μg. of Hg. P. S. ARUP.

Foam stability of beer. I. Measurement technique and studies on foam-producing substances in malt. W. Piratzky, H. Beitner, J. Jacker, and B. Nispel (*Brauwissenschaft*, 1955, 42—50).—The method of Ross and Clark is criticised. A method is described in which the foaming capacity is measured by the height of the foam produced after 4 min. when air is passed under standard conditions through a sintered glass filter, and upwards through a mixture of water (400 ml.) and the sample or wort or beer (5 ml.), contained in a glass tube of standard dimensions. Good agreement is obtained between the above values and organoleptic judgements for beer, and also between values for Congress worts and for the respective beers made from the worts. The sole foam-producing substances are readily sol. proteins of high mol. wt. (Lundin's tannin fraction) which, in the malt, are practically confined to the fine grits fraction (not in the proximity of the embryo). Removal (by means of adsorbents) of this fraction (but not of the hop-bitters) from beer destroys the foaming capacity. Removal of foam from fermenting wort causes a reduction in the foaming capacity which is not restored by further fermentation. P. S. ARUP.

Biological survey of bottling-cellar work. G. Stage (*Brauereitechniker*, 1955, **7**, 61—66).—A review covering sources of infection the cleaning of plant and bottles, and the use of disinfectants, with examples showing the value of biological control. P. S. ARUP.

Sterilised or pasteurised milk? II. E. van de Gehuchte (*Conserva*, 1955, **3**, 301—306).—A review covering comparative processing costs, advantages of sterilised milk, homogenisation, physical, chemical, and bacteriological effects of pasteurisation and sterilisation and general observations. (36 references.) P. S. ARUP.

Effect of Menadione (2-methyl-1 : 4-naphthaquinone) on the keeping quality of milk. G. Higginbottom (*J. Dairy Res.*, 1955, **22**, 48—59).—The effect on the keeping quality and bacterial flora due to the direct addition of Menadione to milk held at 37° is studied. The response of individual milk samples varies considerably, some remaining unaffected by as much as 100 μg. Menadione/ml. while others show improved properties in the presence of 10 μg. Menadione/ml. This variance appears to be related to the bacterial flora of the milk. Menadione fed to milking cows (100 mg./day for a three-week period) has no demonstrable effect on the cows, the milk yield, or the keeping quality of the milk. N. M. WALLER.

Milk substitute from coconut. M. N. Moorjani (*Bull. centr. Food technol. Res. Inst., Mysore*, 1954, **4**, 60—61).—To the edible portion of the nut is added twice its wt. of water and the cut-up tissue transferred to a Waring Blendor. The mixture is strained through muslin and about 100 ml. of coconut water is added to it, followed by extra water to give 2 lb. of milk per nut. The liquid so obtained has the % composition: total solids 10·2, protein 0·8, fat 7·1, reducing sugars 0·35, sucrose 1·4, minerals 0·55; Ca 3·2 mg., P 33 mg. This product, because of its low protein and high fat content, breaks up on boiling. This is obviated by addition of 0·8% casein at pH 6·8: steam deodorisation for a few minutes then yields a product which is passable as milk in tea and coffee although not free from coconut flavour. L. G. L. UNSTEAD-JOSS.

Bacterial spoilage of shell eggs. I. Bacteriology. P. C. Trussell. **II. Incidence of spoilage in eggs from ninety-four farms.** P. C. Trussell, C. O. Fulton, and C. J. Cameron. **III. Farm practices promoting spoilage.** P. C. Trussell, R. E. Triggs, and B. A. Greer (*Food Technol.*, 1955, **9**, 126—129, 130—134, 134—137).—I. In tests involving 4821 eggs, 4·9% were found to be potentially spoilable by Gram-negative chiefly of the non-fluorescent types. The greatest amount of infection occurred in wet-washed eggs, followed by those with a visible amount of soiling, and those infected by nesting material.

II. In this extensive survey the farms were selected at random. Eggs infected with spoilage bacteria came from a large proportion of the farms. The average spoilage for the 94 farms was 3·9%; eggs from 29 of the farms had <1% of infection. Non-fluorescent types of bacteria were chiefly responsible. The spoilage of eggs from New Hampshires is statistically less than that of eggs from Leghorns and crossbreeds. The age of the birds, the fertility of the eggs, the frequency with which eggs are collected from the nests apparently have no bearing on the spoilage.

III. The spoilage of unwashed, nest clean eggs (*a*) from 15 farms, (*b*) from roll-away nests from 12 farms, (*c*) from 14 farms when packed in dirty trays, averaged 0·42, 0·69, and 1·60% respectively. These differences in spoilage were not significant at the 5% level. The spoilage of soiled eggs from 14 farms averaged 2·37% and was significantly higher than that of unwashed nest-clean eggs from litter-nests and from roll-away nests. The use of washing machines of the types tested is not recommended. E. M. J.

Effect of inorganic phosphates on animal albumin. V. Effect of pH values on water absorption of coagulated meat. K. Möhler and F. Kiermeier (*Z. LebensmittUntersuch.*, 1955, **100**, 260—266).—A new method of H_2O determination (measuring the dielectric constant) was used to establish the amounts of effused and retained water during the coagulation of salted meat samples (at 70° for 30 min.) with and without addition of phosphates. In either case the loss of H_2O at pH 5·4—6·4 decreased with pH values approaching neutrality. No statistic statements can yet be made about the reduction of H_2O-loss ($\sim$13%) by coagulation with certain phosphates. *E.g.*, from results summarised in tables addition of 0·5% KH_2PO_4 at pH5·2: content of H_2O 81·57%, loss of H_2O 49·69%; addition of 1% Na_2HPO_4 at pH 5·65: content of H_2O 81·62%, loss of H_2O 40·87%, etc. A considerable increase in the effusion of juice was caused by increasing time and temp. of coagulation. (14 references.) L. S.

Discoloration of fresh red meat and its relationship to film oxygen permeability. A. H. Landrock and G. A. Wallace (*Food Technol.*, 1955, **9**, 194—196).—Causes of discoloration are discussed, and the permeability to O_2 of films used in packaging. The colour changes with or without O_2 are represented in figure, *e.g.*, myoglobin, a purple-red compound in which Fe is in the ferrous state as in uncut meats; a freshly cut surface to which O_2 is added forms oxymyoglobin, which is scarlet red, Fe is still in the ferrous state; O_2 supply is cut off and myoglobin is formed again. With low concentrations of O_2 myoglobin is oxidised to metmyoglobin (brown) and the Fe is in the ferric state. Discoloration by bacterial action is discussed. A film suitable for packaging red meat must have a reasonably low water vapour permeability, and must be fairly permeable to O_2; Cellophanes such as MSAT-80 are especially designed for this purpose. (14 references.) E. M. J.

Aureomycin (chlortetracycline) and the control of poultry spoilage. A. R. Kohler, W. H. Miller, and H. P. Broquist (*Food Technol.*, 1955, **9**, 151—154).—When cut-up or whole eviscerated chickens were processed with a solution containing 3, 10, or 30 p.p.m. of Aureomycin, the poultry remained fresh and edible significantly

longer than did untreated controls. In poultry immersed for 2 hr. in a 10 p.p.m. solution of Aureomycin bacteriostatic amounts of antibiotic (about 1 μg. per g.) were found in breast muscle. Aureomycin was destroyed by conventional cooking procedures, even when the meat was dipped in solutions containing Aureomycin in amounts 3—100 times higher than amounts that appeared to be practical for commercial extension of shelf life. Aureomycin was more effective than either oxytetracycline or Achromycin in controlling the no. of micro-organisms growing on the surface of poultry during cold storage. (17 references.) E. M. J.

Starch-acid food products. General Foods Corp. (B.P. 725,981, 24.3.53. U.S., 24.6.52).—The improved starch-acid food product consists of an intimate mixture of maize starch, sugar, flavouring material and adipic or fumaric acid in the ratio of 35 : 50 : 1 : 3, *e.g.*, to be used as lemon pie fillings. L. S.

Dispensing devices for plastic substances [batter for making cakes or biscuits]. Sawa Fabriks A.-B. (Inventor: E. H. Sjoblom) (B.P. 721,020, 6.7.53). H. L. WHITEHEAD.

Extraction of mannitol, laminarin, and alginic acid from seaweed. Nat. Res. Development Corp. (Inventors: W. A. P. Black and E. T. Dewar) (B.P. 727,013, 28.11.49).—Mannitol, laminarin, and alginic acid are extracted from a mixture containing 400 g. of *Laminaria cloustoni* fronds, 4000 ml. of 0·088N-HCl and 4 ml. of 40% formaldehyde. After stirring the mixture for 1 hr. laminarin is deposited by centrifuge and separated. The supernatant, neutralised with NaOH and evaporated under reduced pressure, leaves a viscous residue which, made up to 85% (w/w) concentration with alcohol, precipitates the remainder of laminarin, the fucoidin, and a portion of inorg. salts. The alcoholic filtrate, evaporated to dryness, represents a mixture of mannitol and salts from which water-insoluble triethylidene mannitol is prepared by adding 180 ml. of HCl (sp. gr. 1·19) and 100 ml. of paraldehyde to 174 g. of above mixture and stirring for 15 min. The cryst. mass thus obtained, diluted with 2500 ml. of water, filtered, washed with 2500 ml. of water and dried, yields 89 g. of triethylidene mannitol. The seaweed residue is treated with a basic solution to recover the alginic acid. L. S.

Improved food product [whipped egg white]. General Foods Corp. (B.P. 728,533, 28.1.53. U.S., 7.2.52, 25.6.52).—A composition used for whipped egg white consists of dried egg white, an alkali metal hexametaphosphate, and a pH adjuster. *E.g.*, a mixture of 38 g. of dried egg white (providing a pH 7·5), 1·0 g. of Nahexametaphosphate, and 0·38 g. of tartaric acid is dissolved in 270 ml. of water. Above solution of pH 5·5 can be whipped in 2 min. into a "stiff peaks" whip. L. S.

Production of powdered fish. Soc. Civile d'Étude des Produits de la Mer (B.P. 727,072, 28.10.52. Fr., 30.10.51).—A method of producing powdered fish consists in removing oils and oxidisable fatty substances in three successive stages: (*a*) dehydrating pulped fish with acetone at 50°, (*b*) disintegrating the dehydrated pulp in boiling 90% ethanol for 45 min., and vac. drying the obtained tissues, (*c*) re-extracting the product with boiling alcohol for 30 min., drying with heated air and grinding the dried particles into impalpable powder. L. S.

Machine for the separation of the tissue part from the juices or oils of vegetable products. P.A.S.T. Prodotti Alimentari Sistema Tallarico (B.P. 727,030, 26.2.52. Ital., 26.2.51, 15.12.51).—The machine comprises two grooved cylinders in which vegetable products are disintegrated, two worm conveyors in a slotted casing in which the disintegrated mass is compressed into a tissue cake to yield juices or oils, two major segments of a cylinder in the slotted casing in which the tissue cake is disaggregated by upward and downward rocking, and a number of other devices ensuring satisfactory separation of vegetable solid and liquid parts; 12 figures are given. L. S.

Device for separating shell- and kernel-parts of cocoa beans. J. Toth (B.P. 726,138, 15.6.53. Ger., 12.11.52, 26.2.53).—Shells and kernels of cocoa beans are efficiently separated by an electrostatic contrivance (illustrated), comprising a positively charged conveyor, with shaking and measuring devices and insulated, negatively charged rotating rollers with suction and stripping devices. L. S.

Drying biological substances by distillation. E. Levin (B.P. 727,148, 19.3.52. U.S., 3.4.51).—A new method of drying biological substances, which does not impair their contents of vitamin B_{12} and yields a non-hygroscopic product, consists in a continuous extraction of the dehydrated, defatted, homogenised and emulsified substance, *e.g.*, stick water, fish solubles, beef blood, serum, eggs. The material is first mixed with an organic liquid capable of forming azeotropes with water, *e.g.*, ethylene dichloride at 71°. The fluid emulsion is then introduced into the boiling organic liquid and the continuous extraction process is carried out by simultaneous addition of substance and solvent, removal of azeotrope by distillation at ~60°, collecting the fat-enriched solvent, drying and recovering the dried substance. L. S.

Food packs. John Herbert Johnson, Herbert Kenneth Johnson, and Brian Braithwaite Johnson (B.P. 726,098, 12.3.53).—Pickled vegetables containing a preserving liquid can be pasteurised and marketed in laminated films of flexible, impermeable polythene sheets. L. S.

3.—SANITATION

Ethylene dibromide—a fumigant for the food industry. S. V. Pingale and M. Swaminathan (*Bull. centr. Food technol. Res. Inst., Mysore*, 1954, **4**, 38—40).—A review of the use of ethylene dibromide in prolonging the storage life of fruit, vegetables, and moist grain, and in soil fumigation. (29 references.) N. M. WALLER.

Grain storage studies. XVIII. Mould invasion of wheat stored for sixteen months at moisture contents below 15%. C. M. Christensen (*Cereal Chem.*, 1955, **32**, 107—116).—The invasion of seeds of various wheats by *Aspergillus restrictus* is observed in laboratory and commercially stored samples. The invasion is predominantly in the germ of the seed and is associated with decreased germination and an increasing percentage of discoloured germs. The fungus slowly invades seeds having a moisture content of 13·5% and above. It is considered that the generally accepted "critical" moisture levels for long storage of wheat are too high. N. M. WALLER.

Past and present practices of controlling insect pests in stored grains in India. S. V. Pingale and V. Balu (*Bull. centr. Food technol. Res. Inst., Mysore*, 1954, **4**, 62—65).—A review with 51 references. The possible use of solar energy is mentioned for obtaining heat for heat sterilisation of grains. Other methods described are conventional: their disadvantages and limitations are discussed. L. G. L. UNSTEAD-JOSS.

Estimation of insect fragments in maize. W. D. Raymond, R. G. W. Spickett, and J. B. Ward (*Colon. Plant Anim. Prod.*, 1954, **4**, 334—335).—Difficulties of estimating insect fragments and extraneous matter in heavily infested samples of maize are discussed. Particle size in insect fragment count may be an important factor in causing disagreement in results obtained by different laboratories. An improved A.O.A.C. method for examination of such heavily infested samples is given, in which a preliminary dilution with pure potato starch (free from insect fragments) is effected. Certain other precautions are also noted. S. C. JOLLY.

Household pests and their control. E. J. Udine (*Pa. agric. Exp. Sta.*, 1954, *Circ.* 435, 21 pp.).—A general account. A. H. CORNFIELD.

Suggested improvements in the Weber and Black method for evaluating germicides. T. W. Humphreys and C. K. Johns (*Appl. Microbiol.*, 1954, **2**, 1).—Use of screw-capped jars in place of test tubes, transfer of medication mixture direct by pipette to a Petri dish set at 10° from the bench top, and diminished exposure periods are recommended. E. G. BRICKELL.

Effect of clothing colour on mosquito attack on exposed skin. A. W. A. Brown (*J. econ. Ent.*, 1955, **48**, 130).—There was little significant difference in the total no. of mosquito landings on the face or on the back of persons with unattractive clothing (green and white) and that on persons with attractive clothing (blue and black). Lighter coloured clothing did not increase the attack of mosquitoes on the face but white clothing was less attractive than black. A. A. MARSDEN.

Systemic effect of selected chemicals on the bed bug and lone star tick when administered to rabbits. T. R. Adkins, jun., W. L. Sowell, and F. S. Arant (*J. econ. Ent.*, 1955, **48**, 139—141).—The effects of 13 chemicals given orally to rabbits on the animals and on fifth instar bed bugs, *Cimex lectularius*, and nymphs of the lone star tick, *Amblyomma americanum*, feeding on the treated rabbits are reported. Compound "Bayer L 13/59," administered at the rate of 200 mg./kg., "Bayer 18/178," *O*-[2-(ethylmercapto)methyl] *OO*-dimethyl thiophosphate, at 130 mg./kg., and "Bayer 21/116," *O*-[2-(ethylmercapto)ethyl] *OO*-dimethyl thiophosphate, at 95 mg./kg. all caused 100% kill to bed bugs and ticks feeding on treated rabbits. Hexamethylphosphoramide at 1300 mg./kg., caused 63% kill of bed bugs and 100% kill of ticks, whilst schradan at 20 mg./kg. had no effect on ticks but killed 100% of *C. lectularius* feeding on the rabbits. None of these insecticides caused severe toxic symptoms in the rabbits at these dosages. A. A. MARSDEN.

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