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CONSERVATION OF FRESH AND WILTED GRASS IN AIR-TIGHT METAL CONTAINERS

By N. JACKSON and B. K. ANDERSON

Fresh and wilted timothy–meadow fescue herbage of 19 and 46·6% dry matter content, respectively, was ensiled in rubber-sealed steel cylinders 5 ft deep and 3 ft in diameter. Mean dry matter losses from all silos were about 6%. All silages were well preserved, the wilted material containing less lactic acid and volatile acids and having a higher pH. The dry matter digestibility coefficients from fresh and wilted grass silages were 71·5 and 70·2%, respectively, and were slightly higher than for the grass before ensilage. Ensilage resulted in a fall in metabolisable energy of the wilted grass. Intake of wilted silage by sheep was higher than that of silage from fresh grass.

Introduction

Recent work in this Department[1–4] and elsewhere[5] has been concentrated on making silage from which air has been excluded by means of a completely sealed polythene film. In gas-tight tower silos dry matter losses of 4% have been obtained,[6] while in polythene-sealed silos losses as low as 5% have been obtained with material of almost 50% dry matter. Losses of about 16% have been recorded when grass of low dry matter content has been ensiled in completely sealed polythene.[7]

The experiment reported below was designed to provide data on nutrient losses from normal and very heavily wilted grass ensiled in air-tight metal cylinders.

Experimental

Four metal cylinders were used in this experiment. The cylinders were constructed of ¼ inch steel, with an internal height of 5 feet and an internal diameter of 3 feet. A rubber gasket was fitted between the lid and the top of each metal cylinder. The lid of each silo was fitted with a valve set to release at a pressure of 7 lb/in.^{2} The lids could be screwed down tightly to the silos by means of 32 set screws.

The grass, cut on 20 July 1965, was the second cut of a second year timothy–meadow fescue sward. The grass was cut with a standard semi-mounted mower, and a flail-type forage harvester was used to collect both fresh and wilted material, Freshly-cut grass was put into Silos 1 and 3. After being wilted for approximately 36 hours, material was ensiled into Silos 2 and 4. The weather at the time of cutting and wilting was fine with cloudy periods, the maximum air temperature being 24° and the minimum 9°. The grass was consolidated in the silos by being tramped down. The fresh-grass silos contained a mean weight of 220·2 lb of dry matter while the wilted-grass silos contained a mean weight of 308·2 lb of dry matter.

The silos were opened in February 1966. The weights of the empty silos and of the full silos, immediately after filling and immediately before opening were obtained by placing the cylinders on the weighbridge, and the weight of material at the beginning and end of the ensiling period was obtained by difference. Samples were taken for dry matter determinations and chemical analyses. Chemical analysis was carried out as described by Brown and Kerr.[8] Soluble carbohydrates were determined by the method described by Deriaz.[9] Digestibility measurements on sheep fed at the maintenance level were carried out with the fresh and wilted grass and with the fresh-grass silages and the wilted-grass silages.

Metabolisable energy (ME) for both grasses and silages was calculated from the faecal, urinary and methane energy losses, the methane energy loss being calculated from the following equation:

$$CH_4 \text{ (kcal/100 kcal of gross energy)} = 3{\cdot}64 + 0{\cdot}075D + L\,(1{\cdot}03 - 0{\cdot}028D)$$

where L is the level of feeding as a multiple of the maintenance level and D is the apparent digestible energy (ADE) at the maintenance level of feeding.[10] In addition to the ME determinations for grass and silage at maintenance, ME was also determined by this method for the silage at varying feeding levels. Two wethers were used to obtain mean digestibility data for each type of material and for the ME determinations. The silage gross energy determinations were corrected for the energy content of the volatile acids lost on drying by application of the energy value of acetic acid (209·4 kcal/mole) to this fraction. No corresponding correction was made for the volatile bases.

Results

The chemical composition of the grass ensiled and of the silage removed is given in Table I.

The mean dry matter content of the fresh grass ensiled was 19·0%, while for the corresponding silages the dry matter content was 17·7%. For the wilted grass the mean dry matter content was 46·6% and the corresponding figure for the silage was 43·5%. The crude fibre contents of the grass were about 28%, a figure which is to be expected for the leafy grass used. There was no increase in crude fibre in the silage compared with the grass which suggests that there was no appreciable loss of other nutrients. The protein figures do not give any indication of a difference in the degree of degradation between the fresh and wilted material. The soluble carbohydrate figures are rather low for the grass in all four silos. Wilting caused a lowering in the content of soluble carbohydrates, the value for the wilted grass being approximately 14% lower than that for the fresh grass. However, the data for the silages indicate that the loss of soluble carbohydrate by fermentation processes was greater in the fresh-grass silages than in the wilted-grass silages. This

TABLE I

Composition of grass and silages (percentage of dry matter)

	Fresh-grass silages				Wilted-grass silages			
	Silo 1		Silo 3		Silo 2		Silo 4	
	Grass	Silage*	Grass	Silage*	Grass	Silage*	Grass	Silage*
Ether extract	2·70	2·67	2·67	2·73	2·11	1·94	2·21	2·29
Crude fibre	27·98	27·94	28·27	28·78	28·49	30·76	28·01	28·94
Crude protein	14·80	15·40	15·01	15·27	14·09	15·56	14·69	15·29
Ash	9·69	9·11	9·44	9·53	9·57	9·50	9·73	9·68
N F E	44·83	44·88	44·61	43·69	45·74	42·24	45·36	43·80
Organic matter	90·31	90·89	90·56	90·47	90·43	90·50	90·27	90·32
'True' protein	13·03	11·07	13·17	9·65	12·18	12·22	13·08	10·16
Corrected true protein	—	13·27	—	13·04	—	12·82	—	12·94
Dry matter	19·28	18·32	18·70	17·14	47·72	43·70	45·52	43·44
Soluble carbohydrates	10·9	1·9	10·7	1·8	9·7	2·1	8·9	2·7

* Silage dry matter corrected for volatiles

observation is supported by the organic acid data presented in Table II.

On being opened, the silos were examined for fermentation quality. The fresh-grass silages had an acid smell and were yellow-green. The wilted-grass silages had a pleasant hay-like smell. No inedible waste material was present in any of the silos. The fresh-grass silages had settled about 4 inches from the top of the container. No noticeable settling had occurred in the wilted-grass silages.

The higher pH values for the two wilted-grass silages (Table II) suggest a lower production of organic acids in the wilted material. The volatile acids produced in the silos were about the same on a fresh-material basis but on a dry matter basis the fresh-grass silages had a mean volatile acid content of 4·2%, while for the wilted-grass silages the mean volatile acid content is 1·8%. This low production of organic acids is a characteristic of heavily wilted grass silage.[1,2,11]

On a dry matter basis the lactic acid content of the fresh-grass silage was very high (mean 11·7%) and for the wilted-grass silage it was only 5·8%. The residual acidity of the fresh-grass silages was almost double (mean 11·1%) that for the wilted-grass silages (mean 5·9%). The production of volatile bases was almost identical in both the fresh- and wilted-grass silage dry matters.

TABLE II

pH values, volatile constituents and lactic acid contents of silages

	Fresh-grass silages		Wilted-grass silages	
	Silo 1	Silo 3	Silo 2	Silo 4
pH values	3·90	3·88	4·50	4·42
Volatile acids as acetic acid (%)	0·77	0·72	0·83	0·78
Volatile bases as crude protein (%)	0·23	0·22	0·54	0·53
Amino acids as crude protein (%)	0·88	0·78	1·21	1·36
Residual acidity as lactic acid (%)	2·26	1·70	2·59	2·56
Lactic acid (%)	2·22	1·94	2·71	2·37

In Table III the dry matter filled into and removed from each silo is given. The densities of the silage dry matter in the silos expressed as lb/ft^3 were: Silo 1, 5·72; Silo 2, 8·39; Silo 3, 5·96; Silo 4, 7·93. Thus, although the wilted material was more difficult to consolidate, it was possible to fill more dry matter into the silos than when freshly cut material was ensiled.

The mean dry matter loss from both the fresh- and wilted-grass silos was 6·1%.

The contents of digestible nutrients in the grasses and silages are given in Table IV together with the starch equivalents. The mean content of total digestible nutrients in the fresh-grass silages is 5·4 units higher than for the fresh grass, and for the wilted-grass silages the mean total digestible nutrient content is 0·8 units higher than in the wilted grass. The mean total digestible nutrient contents were 69·4 and 66·9 for the fresh- and wilted-grass silages, respectively, and the corresponding starch-equivalent figures were 50·2 and 47·1.

The percentage losses of individual gross and digestible constituents are given in Table V. In general the greatest percentage loss of crude constituents was in the nitrogen-free extractives (NFE) fraction, although these losses were not high, the mean NFE loss being 7·1% for the fresh-grass silages and 11·4% for the wilted-grass silages. The mean losses of crude fibre were 5·4% and 0·9% from the fresh and wilted-grass silages respectively. The loss of crude protein was low in all silos, the mean loss from the fresh-grass silages being 3·4%, while for the wilted-grass silages a

TABLE III

Weights of dry matter filled into and removed from silos, and dry matter losses

	Fresh-grass silages		Wilted-grass silages	
	Silo 1	Silo 3	Silo 2	Silo 4
Dry matter put in as grass (lb)	210·9	229·6	326·9	289·5
Silage dry matter taken out (lb)—corrected for volatiles	202·3	210·7	296·7	280·4
Loss of dry matter (lb)	8·6	18·9	30·2	9·1
Loss of dry matter (%)	4·1	8·2	9·2	3·1

TABLE IV

Digestible nutrients and starch equivalents of grass and silages

	Fresh-grass silages				Wilted-grass silages			
	Silo 1		Silo 3		Silo 2		Silo 4	
	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage
Ether extract	1·38	1·56	1·36	1·60	0·53	0·97	0·56	1·15
Crude fibre	21·15	22·60	21·37	23·28	23·21	24·35	22·82	22·91
Crude protein	9·50	9·78	9·64	9·70	8·95	9·52	9·34	9·36
Ash	4·93	4·04	4·80	4·22	4·36	4·61	4·44	4·70
N F E	30·21	33·54	30·00	32·72	32·70	30·73	32·59	32·06
Organic matter	62·24	67·48	62·37	67·30	65·39	65·57	65·31	65·48
'True' protein	8·21	5·98	8·30	5·21	7·55	6·89	8·11	5·73
Corrected true protein	—	8·10	—	7·96	—	7·78	—	7·85
Total digestible nutrients	63·96	69·43	64·07	69·30	66·05	66·78	66·01	66·92
Starch equivalent	45·48	50·53	45·37	49·85	47·49	46·40	47·86	47·76

TABLE V

Percentage losses of crude and digestible constituents from the silos

	Fresh-grass silages				Wilted-grass silages			
	Silo 1		Silo 3		Silo 2		Silo 4	
	Crude constituents	Digestible constituents	Crude constituents	Digestible constituents	Crude constituents	Digestible constituents	Crude constituents	Digestible constituents
Dry matter	4·1	+2·1	8·2	2·3	9·2	8·7	3·1	2·5
Ether extract	5·3	+10·3	6·6	+9·7	17·4	+70·6	0·0	+100·0
Crude fibre	4·2	+2·5	6·6	0·0	1·9	4·9	+0·1	2·9
Crude protein	0·0	1·0	6·7	7·7	+0·2	3·7	+0·9	3·0
Ash	9·8	21·1	7·4	19·2	9·9	3·5	3·9	+3·1
N F E	4·0	+6·4	10·1	0·0	16·2	14·7	6·5	4·8
Organic matter	3·5	+4·0	8·3	1·0	9·2	9·0	3·1	2·9
'True' protein	18·5	30·1	32·8	42·4	8·8	17·4	24·8	31·5
Corrected true protein	2·5	5·2	8·9	12·0	4·5	6·5	4·2	6·4
Total digestible nutrients	—	+4·1	—	0·7	—	8·2	—	1·8
Starch equivalent	—	+6·6	—	+0·8	—	11·3	—	3·4

small gain of 0·6% crude protein was found, presumably as a result of error from the balance technique.

With regard to the digestible nutrients, in the two fresh-grass silages the mean difference represented a small gain (1·7%), and for the wilted-grass silages the mean loss was 5·0%.

The mean gross energies of the dry matter of bulked samples of the fresh and wilted grass put into the silos were 4·54 kcal/g and 4·51 kcal/g, respectively.

The ME values for the grasses and silages are presented in Table VI, together with the feeding level, apparently digestible energy, urinary energy and the $\frac{ME}{ADE}$ values.

With increasing feeding level the ADE decreased although this was not reflected in the ME values.

In the case of the fresh material, the energy digestibility rose from 66·2 in the grass to 69·6 in the silage; for the wilted material there was a slight fall from 68·8 in the grass to 66·9 in the silage. The ME in the fresh-grass silage was practically identical with that in the grass, values being 2·51 and 2·47 kcal/g, respectively, while for the wilted material ensiling resulted in a fall of ME from 2·60 kcal/g to 2·39 kcal/g.

The highest level of feeding obtained with the silage was 1·2 times maintenance for the fresh-grass silage and 1·4 times maintenance for the wilted-grass silage. The intake of the wilted material above the maintenance level by the sheep was twice that of the intake above maintenance when the fresh-grass silage was fed. The ME values were 54·9 kcal and 53·9 kcal per 100 kcal of gross energy intake for the wilted- and fresh-grass silage, respectively, at the highest feeding level. These corresponded to values of 2·41 and 2·37 kcal/g of dietary dry matter. The ratio of ME to ADE at the maintenance feeding level, was 80·9% and 79·9% for the wilted- and fresh-grass silages, respectively. These figures are in agreement with results of Clapperton.[12]

Discussion

The results show that dry matter losses were relatively low when both fresh and wilted grass were ensiled in air-tight metal containers. The reduction of dry matter losses by air-tight ensiling has previously been demonstrated in this Department using a complete polythene seal; in the present experiment the seal can be regarded as completely air-tight, while polythene can only be regarded as partly air-tight, some diffusion taking place through it. The fact that the total digestible nutrients content (Table V) of the silages removed from the metal cylinders was greater than that of the grass originally placed in the containers can be attributed, not to

TABLE VI

The mean feeding level, apparently digestible energy, calculated ME and ME/ADE ratios of the two types of grass and silage

Diet	Feeding* level (L)	ADE (kcal/100 kcal food)	ME (kcal/100 kcal food)	ADE (kcal/g)	ME (kcal/g)	Urinary energy (kcal/100 kcal food)	ME/ADE
Fresh-grass silage	0·6	69·9	55·1	3·14	2·47	6·54	78·7
	1·0	69·6	55·6	3·10	2·47	6·04	79·9
	1·2	66·9	53·9	2·94	2·37	5·21	80·6
Wilted-grass silage	0·7	68·4	54·2	3·03	2·42	5·76	79·8
	1·0	66·9	54·2	2·96	2·39	4·96	80·9
	1·4	66·0	54·9	2·90	2·41	3·67	83·1
Fresh grass	1·0	66·2	55·3	3·00	2·51	2·86	83·5
Wilted grass	1·0	68·8	57·6	3·10	2·60	3·13	83·7

* Feeding level expressed as a multiple of maintenance i.e. ME intake/ME for maintenance

any major change in the chemical analysis of the material, but to a higher digestibility of the silage than the grass. The digestibility coefficient of the fresh-grass dry matter was 67·2, and for the fresh-grass silage it was 71·5. In the case of the wilted material the grass had a digestibility coefficient of 69·7 and the silage one of 70·2. This increase is unusual and can probably be attributed to differences in the variability between the animals used for the digestibility determinations, rather than to a real increase in the digestibility of the material with ensiling. The differences were more pronounced in the fresh grass than in the wilted material. In the fresh material, nitrogen-free extractives digestibility increased from 66·8 to 74·6, and the crude fibre digestibility from 75·6 to 80·9, while in the wilted material the NFE digestibility increased from 70·3 to 72·4 and the crude fibre digestibility coefficient fell slightly from 81·5 to 79·2.

When grass is ensiled in the normal way the continuance of aerobic respiration leads to a loss of nitrogen-free extractives,[13] and protein breakdown also occurs.[14] The results for the analysis of grass and silage in Table I indicate that this has not happened very extensively in this method of ensiling. Normally this loss has associated with it a rise in the crude fibre content.

From Table II it appears that the lactic-acid and other acid-producing bacteria have been much more active in the fresh-grass silages, and this has resulted in a much lower pH value in these silages than in those made from the wilted grass. It has been suggested[15] that the osmotic pressure of silage made from wilted grass is a very important limiting factor in the growth of bacteria and in the production of organic acids by bacteria. On the basis that the osmotic effect and the limiting of air effects could be to some extent additive, it might be expected that the dry matter losses would be less from the wilted silages than from the unwilted silages. However, the mean dry matter loss was the same from both the fresh and wilted grass silos. The differences in dry matter losses between silos of similar treatment, and even between silos of different treatments, are of an order which could possibly be attributed to errors inherent in grass conservation experiments.

The dry matter losses are no real improvement on the low losses reported by Larabee & Sprague[5] and Brown and Kerr[1] for silage completely enclosed in a polythene film. It is to be anticipated, especially for the wilted material, that rapid secondary fermentation will occur once the silos are opened.

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VOLUME-REDUCTION TECHNIQUE FOR THE *X*-RAY SPECTROGRAPHIC DETERMINATION OF MICRONUTRIENTS IN PLANT TISSUES

By A. A. THEISEN* and A. PINKERTON**

A volume-reduction technique is described for the *X*-ray spectrographic analysis of the micronutrient elements manganese, iron, and zinc in plant tissues. This simple technique is believed to minimise inter-element effects and utilises dilution by volume instead of by weight, thus avoiding intimate mixing of sample and diluent. The method has particular advantages for the analysis of manganese and iron when they occur in plant tissues in concentrations large enough for inter-element effects to be serious.

Introduction

The main difficulties encountered in *X*-ray spectrographic analysis of micronutrient elements in plant tissues and other biological materials are the lack of appropriate standards, and inter-element effects due to differential absorption and enhancement.

To overcome the former, chemically analysed samples have been used as standards.[1] Whittig *et al.*[2] proposed an addition method for zinc which has since also been applied to the analysis of other elements.[3] Andermann & Kemp[4] proved theoretically that the use of scattered radiation as an internal standard can correct for instrument variables and also, at least partly, for absorption effects.

Whereas the addition technique has proved useful in the analysis of elements occurring in small concentration in plant tissues, shortcomings have been reported for elements occurring in higher concentration, e.g. manganese and iron.[3]

The basic equation for *X*-ray emission spectrography, when absorption effects have to be considered, is given by Liebhafsky *et al.*:[5]

$$I_e^s = \frac{W_e^s \; I_e^e \; a^e}{a^s}$$

where I_e^s = intensity of the analysis line of the element sought in the sample, W_e^s = weight fraction of the element sought in the sample, I_e^e = intensity of the analysis line of the element sought in pure form, a^e or a^s = matrix effects of element and sample respectively. It can be seen that I_e^s is directly dependent on W_e^s and a^s since under any given set of operating conditions I_e^e and a^e are constants. Since a^s is dependent primarily on the mass absorption coefficients of all the elements in the sample, it can change widely from sample to sample. Thus, even if the element sought remains constant, I_e^s can vary widely with changing sample composition. It is possible to minimise variations in a^s by greatly diluting the sample either with intimately mixed-in transparent material (low μ = mass absorption coefficient) or with dense material (high μ)[5].

In this study dilution by intimately mixing diluent and sample was not used. Instead dilution was simulated by a stepwise reduction of sample volume with increasingly larger wedges of material having a mass absorption coefficient close to that of the sample. In this way a standard curve of intensity of the analysis line of the element versus degree of sample volume reduction can be developed, provided a known amount of the element sought has been added to the sample, and sample and volume-reducing wedge are rotated in the *X*-ray beam.

Experimental

Leaf tissue

Leaf samples chosen to provide a wide range of chemical composition were collected from the following crops: bean (*Phaseolus vulgaris*), citrus (mostly *Citrus sinensis* but containing some *C. limonia*), coffee (*Coffea arabica*), grass (*Seteria sphacelata*), lucerne (*Medicago sativa*), maize (*Zea mays*), pineapple (*Ananas comosus*), sisal (*Agave sisalana*), sugar-cane (*Saccharum officinarum*), tea (*Thea sinensis*), and wheat (*Triticum vulgare*).

Preparation of samples, chemical and *X*-ray analysis

All tissue samples were dried at 65° and ground in a stainless-steel hammer mill to pass a sieve with holes 1 mm in diameter. Each sample was thoroughly mixed, and duplicate sub-samples were removed for chemical and *X*-ray spectrographic analysis.

After wet digestion, zinc was determined on an atomic-absorption spectrophotometer,[6] and manganese with periodate.[7] After dry ashing iron was analysed using *o*-phenanthroline.[8]

The plant tissue subsamples (8 g) for *X*-ray analysis were weighed into 2 oz glass bottles previously cleaned with acid. Four ml of a solution containing 100 ppm of manganese and iron, and 30 ppm zinc were added to each bottle. This solution wet about half the plant material; care was taken not to wet the glass. After the tissue and solution mixture was dried at 65°, the samples were ground and mixed with an agate mortar and pestle and loosely poured into circular sample holders provided with ¼ mil Milar windows.

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Peak/background ratios for each element were determined in duplicate at different degrees of volume reduction. Bakelite wedges were used for iron and manganese, and wedges made from stacks of filter paper were used for zinc (Fig. 1). Fig. 2 illustrates the curves for manganese, iron and zinc in maize obtained by progressively replacing $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, $\frac{7}{8}$ and $\frac{15}{16}$ of the volume of the sample with the indicated wedges.

Duplicate peak/background determinations for each element in the plant materials, ground but untreated, permitted calculation of the amount of the element originally present. An appropriate volume reduction wedge must be inserted in the sample holder to obtain peak/background ratios lying on the linear portion of the volume reduction curve.

Results were calculated according to the formula:

$$nX = m(X+a),$$

where X = amount of the element in the plant sample, a = amount of the element added in solution, n = percentage volume reduction of original plant sample and m = percentage volume reduction of sample plus addition read from the curve.

Instrumentation

The equipment used was a Philips vacuum X-ray spectrograph equipped with a tungsten target (1000 watt rating), a pulse-height discriminator, and a scintillation counter. Pulse-height analyser settings were determined on the purest available form of each element. The optimum X-ray generating conditions were found to be 48 kV and 20 mA for each of the elements under examination. The scintillation counter was operated at 930 volts. A lithium fluoride analysing crystal and a fine collimator were used. The goniometer settings are shown in Table I.

TABLE I

Goniometer settings for peak and background
Degrees, 2 θ

	Manganese	Iron	Zinc
Peak	63·01	57·55	41·80
Background	63·90	58·50	41·10

The equipment was allowed to warm up for a minimum period of one hour before use. For each sample 16,000 counts were collected in duplicate at peak and background positions for manganese and iron and 64,000 counts for zinc.

Expression of results

The duplicate counts obtained at the peak and background positions for each element were expressed as a mean 'peak/background ratio' and then related to the concentration of the element in the sample.

Coefficients of variation were calculated for each method of analysis. The results obtained from X-ray analysis were compared with those from standard chemical procedures by correlation analysis.

Results

Agreement between the proposed X-ray spectrographic method and wet analysis was good at all concentrations for all three elements (Table II).

Concentrations exceeding 3000 ppm of manganese and iron in tea and lucerne respectively could be estimated satisfactorily, as well as a zinc concentration as low as 9 ppm in coffee.

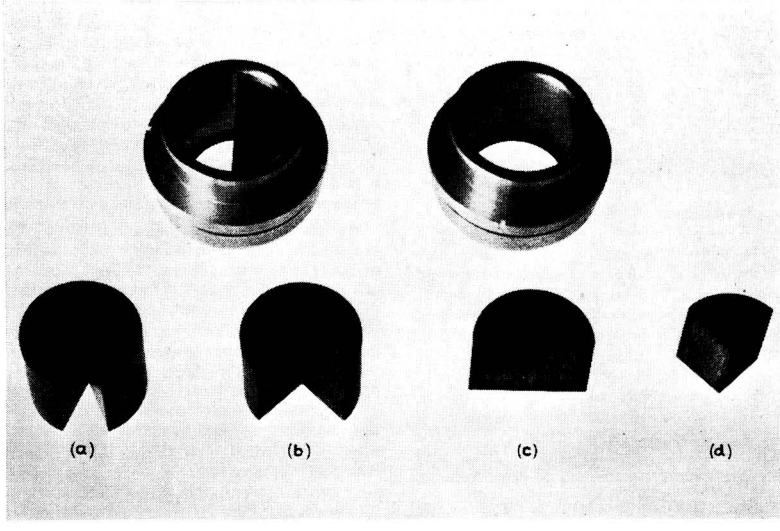

FIG. 1. *Bakelite dilution wedges and sample holders*
% Plant tissue (a) 12·5, (b) 25, (c) 50, (d) 75

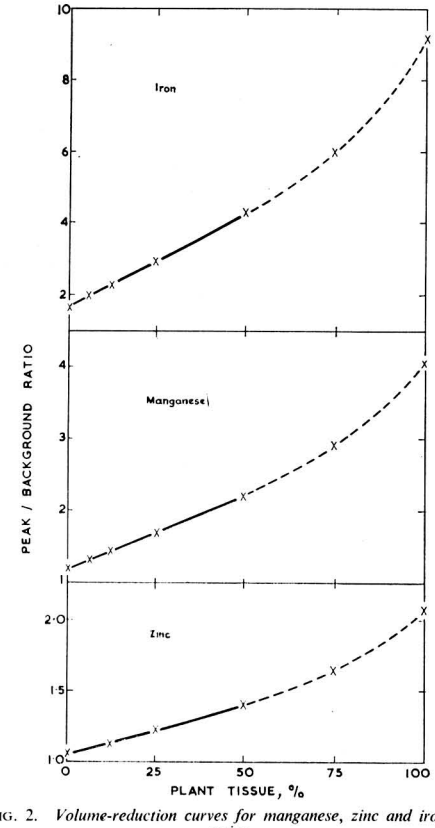

FIG. 2. *Volume-reduction curves for manganese, zinc and iron in maize*

Coefficients of variation for the volume reduction method were, however, not as low as those for the wet determinations. The correlation coefficients between the results of the *X*-ray and the wet analyses exceeded 0·99 for all three elements.

In addition to bakelite as wedge material for the analysis of iron and manganese, filter paper wedges of similar dimensions were tried for a number of plant tissues (Table III).

No difference was obtained between bakelite and filter paper as the volume-reducing wedge.

Discussion

From earlier considerations, one may assume that advantages can be gained by using a diluent wedge with a mass absorption coefficient close to that of the plant material. Mass absorption coefficients for a wavelength equal to 2·0 Å (Fe Kα = 1·938 Å, Mn Kα = 2·104 Å) are given in Table IV.

These values were calculated from analytical results for the material.[9,10] The method of calculation was found to be valid by comparison with a direct determination of the mass absorption coefficient employing the relationship:

$I = I_0 e^{-\mu\varrho x}$, which for filter paper gave 14·3.

When the mass absorption coefficients of diluent and plant material are close, volume reduction need not be extensive to minimise inter-element effects. If the discrepancy between mass absorption coefficients is high, however, volume reduction must be very considerable before the mass absorption coefficient of the sample becomes essentially that of the diluent.* The minimisation of inter-element effects becomes apparent when adjusted intensities (peak/background × volume reduction factor) are plotted against degree of volume reduction for the elements iron and manganese in several plant materials (Fig. 3). In each instance, it will be noted that the slope of the curve gradually falls with increasing wedge size. For a wedge in excess of 50% of volume, the curves are approximately horizontal, which indicates that the

* When tin wedges (μ = 495 for λ = 2 Å) were used in the analysis of manganese and iron in wheat, curvilinear relationships between peak/background ratio and extent of volume reduction were obtained

TABLE II

Manganese, iron and zinc analyses by the chemical and the *X*-ray volume-reduction method
(mean ppm)

Plant tissue	Manganese		Iron		Zinc	
	Chemical	*X*-ray Bakelite wedges	Chemical	*X*-ray Bakelite wedges	Chemical	*X*-ray Filter paper wedges
Bean	218	212	579	567	33·2	35·2
Citrus	46	45	281	257	19·4	17·7
Coffee	192	203	161	150	9·0	8·2
Grass	294	300	148	132	31·4	31·7
Lucerne	107	108	3119	3450	68·6	66·8
Maize	90	92	490	525	47·1	46·9
Pineapple	54	61	174	178	56·8	53·3
Sisal	32	33	70	64	18·8	17·0
Sugar-cane	191	180	111	96	17·4	16·4
Tea	3190	3109	207	217	16·4	15·6
Wheat	99	92*	794	770	62·4	63·8
Coefficient of variation %	1·20	2·48	1·72	2·47	2·56	4·12
Correlation coefficient with chemical analysis		0·997		0·997		0·997

* The wedges here consisted of filter paper

TABLE III

Manganese and iron analyses using Bakelite and filter paper wedges
(mean ppm)

Plant tissue	Manganese			Iron		
	Chemical	*X*-ray		Chemical	*X*-ray	
		Bakelite wedges	Filter paper wedges		Bakelite wedges	Filter paper wedges
Bean	218	212	212	579	567	567
Citrus	46	45	46	281	257	—
Sugar-cane	191	180	186	111	96	110

TABLE IV

Mass absorption coefficients

Material	$\lambda = 2$ Å μ
Cellulose	16·4
Bakelite	13·5
Wheat	18·8
Maize	17·0
Grass	13·1
Coffee	12·5

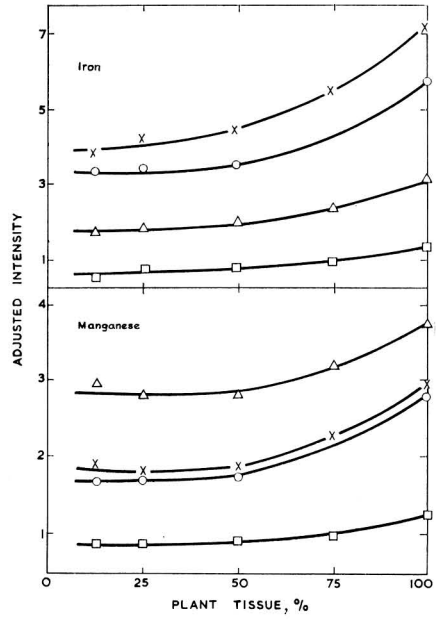

FIG. 3. *Adjusted intensity (peak-background ratio × volume-reduction factor) vs. volume reduction for iron and manganese in four different plant materials*
× Maize ○ Beans △ Copper □ Sisal

effect of other elements has been minimised. From this as well as from an inspection of Fig. 2, an unknown can be expressed in terms of the known only when it falls on the linear portion of the curve. By suitable reduction of volume any unknown, regardless of content, can be made to fall on the linear portion of the standard curve. The maximum size of the volume-reducing wedges is defined by the sensitivity of the equipment.

The wedge must be free from the element being determined. Bakelite was found to contain traces of zinc and hence was unsuitable for the analysis of this element. Moreover, it is of advantage to have a diluent that can be machined easily to the shapes desired; other diluents in addition to the ones used here should prove suitable.

To further demonstrate the usefulness of the volume reduction technique it was compared with the straight addition technique for the elements manganese and iron. The results are listed in Table V.†

Manganese and iron contents were consistently overestimated with the addition method; moreover, the differences between the results obtained thus and the results from wet analysis were statistically significant. It is inferred that, particularly at higher levels of manganese and iron in the plant materials analysed, inter-element effects become noticeable.

† A range of additions of up to 400 ppm of manganese and iron was made

TABLE V

Manganese and iron analyses by the *X*-ray addition method
(mean ppm)

Plant tissue	Manganese	Iron
Bean	250	—
Citrus	52	—
Coffee	192	222
Lucerne	120	—
Maize	92	1075
Pineapple	62	—
Sisal	36	79
Sugar-cane	215	—
Tea	not possible	267
Wheat	113	—
Coefficient of Variation %	5·36	4·50
Correlation Coefficient	0·994*	0·990*

* Determinations by the addition method are significantly higher than by the chemical method ($0{\cdot}05 > P > 0{\cdot}01$), see Table II

The coefficients of variation for the volume reduction method were considerably better than those for the addition method, but not as low as those for the wet determinations. The coefficients of variation for the addition method (Table V) were of the same order of magnitude as those of previously published results.[3]

With the addition method the zinc concentration in all plant materials was determined as satisfactorily as with the dilution method. Zinc apparently occurs in the plant tissues analysed in concentrations that are not high enough for inter-element effects to become serious.*

* The same is true for the concentration of copper which in these plant tissues (varying in Cu from 4·2 to 73·0 ppm) was determined by the *X*-ray spectrographic addition method with a coefficient of variation (%) of 3·57 versus 2·63 for the chemical method

Conclusion

For the determination of manganese and iron concentrations in plant tissues, volume reduction with appropriate wedges offers decided advantages not only because of its simplicity but also because of its greater accuracy in that it reduces inter-element effects. For determining zinc concentrations the main advantage of the wedge technique is to be found in its simplicity.

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AMINO ACID COMPOSITION OF SOUTH AFRICAN AND AUSTRALIAN WHEAT VARIETIES AS A FUNCTION OF THEIR NITROGEN CONTENT

By D. W. ROBINSON and R. SAGEMAN

Samples of six varieties of South African and Australian wheats sown on 4 dates were found to range in total nitrogen content from 2·42 to 3·86%.

Analyses of these samples indicated that no major change in the proportions of the amino acids occurred between samples of varying nitrogen content, and an inverse relationship between lysine and nitrogen content (reported in the literature for wheats of low protein content) could not be demonstrated.

Introduction

It is well known that cultural practice,[1] nitrogen status in the soil,[2] and variety[3] will influence the crude protein content of cereal grains. The relationship between the crude protein content of the grain and the relative proportions of amino acids in the protein has been the subject of several investigations (for example by Block & Mandl,[4] Price,[5] and McDermott & Pace[6]) in which particular attention has been given to lysine as the main limiting essential amino-acids in cereal-based diets.

However, few of these studies have investigated these relationships at very high crude protein levels. A recent study by Beech & Norman[7] on the interaction between sowing date and wheat variety produced grains varying from 15·1 to 24·2% crude protein, and the present study concerns the amino acid composition of the whole grains from this experiment.

Experimental

Grain samples

A 6×4 factorial experiment with three replications was carried out under irrigation in the Ord River Valley in north-western Australia. A summary of the treatments is shown in Table I. Details of cultural operations, yield attributes and their significance have been fully discussed by Beech & Norman.[7] Samples of grain from all treatments shown in Table I except Gala and Wit Spitkop at the fourth sowing date were obtained, and the grains were prepared for analysis by being ground to a fine flour.

TABLE I

Summary of experimental treatments

Varieties		Sowing dates
South African varieties Gondveld Penkop Wit Spitkop Australian varieties Gabo Gamenya Gala	×	24 April (1) 30 May (2) 4 July (3) 6 August (4)

Hydrolysis

0·455 g dry material were hydrolysed for 21 hours with 600 ml 6 N-HCl. The hydrolysate was diluted to 1000 ml with distilled water, and an aliquot of 30 ml was mixed with 2 ml of standard norleucine in hydrochloric acid. This was vacuum-dried and washed with distilled water three times, and finally taken up in 4 ml 10% sucrose solution in 0·1% HCl.

Amino acid analysis

From this solution 1 ml was applied to the column of a Technicon amino acid auto-analyser and the chromatogram was run for 21 hours. Colour development was by the use of ninhydrin reagent prepared in the following manner: 10 g B.D.H. ninhydrin reagent was combined with 0·75 g hydratin and 325 ml methyl cellulose. Nitrogen was bubbled through for 30 minutes, 175 ml of sodium acetate (pH 5·5) were added with further 30 minutes of nitrogen bubbling and 1500 ml of 1 : 1 methyl cellulose–distilled water were added. Nitrogen was bubbled through for 20 minutes. The preparation was then ready for use. This investigation does not include the determination of tryptophan, which is completely decomposed by the acid hydrolysis.

Nitrogen determination

The determination of nitrogen in the samples was carried out by two methods which were found to agree exactly. One was by micro-Kjeldhal digestion followed by Markham distillation and titration with 0·1 N-HCl after collection of ammonia in boric acid solution. The second was digestion of 0·300 g material in a 100 ml Erlenmeyer flask containing 5 ml concentrated H_2SO_4, 500 mg K_2SO_4 and 5 mg Se at 370° on a temperature-controlled hotplate, followed by dilution with water and analysis for ammonium content by means of the Technicon auto-analyser.

Results and Discussion

Results of amino acid analyses carried out on the six wheat samples at four sowing dates are shown in Table II. The results are expressed as amino acid nitrogen as a percentage of the crude protein for comparison of samples differing in their nitrogen content.

Trends between wheat protein fractions and certain amino acids were shown to exist in the Australian wheats studied by Simmonds.[8] Proteins of the albumin–globulin group contained a higher proportion of lysine (the main limiting amino acids for the human and pigs) and arginine (the main limiting amino acid for poultry), while the gluten group contained a greater proportion of the non-essential glutamic acid. Pence *et al.*[9] demonstrated that the gluten prepared from flours of widely differing protein content and from different varieties had an almost constant amino acid composition. Bell & Simmonds[10] have shown that flours of low total nitrogen content have a high proportion of proteins of the albumin–globulin group, and since those of high total nitrogen content have a greater amount of the gluten group of proteins,[11]

TABLE II

Amino acid composition of grain of four sowings of South African and Australian wheats
(g amino acid N/16 g N)

		Gondveld				Penkop				Wit Spitkop				Gabo				Gamenya				Gala			
Sowing date		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Nitrogen content (%) (dry matter basis)		2·96	3·15	3·28	3·80	2·83	2·95	3·24	3·62	2·86	3·22	3·38		2·59	2·65	3·25	3·86	2·42	2·51	3·12	3·74	2·81	2·93	3·28	
Aspartic acid	(0·03)*	5·82	5·81	6·48	6·57	5·30	5·68	6·02	5·96	5·14	5·12	4·78		4·01	4·47	5·13	5·28	4·93	3·74	4·67	5·20	4·81	3·81	4·67	
Threonine	(0·01)	3·17	3·25	3·35	3·29	3·06	2·89	3·14	3·14	3·00	2·88	2·58		2·97	2·59	2·79	3·07	2·80	2·11	2·93	2·90	2·63	3·28	2·91	
Serine	(0·02)	4·63	4·67	4·63	4·62	4·47	4·71	4·98	5·11	4·21	3·91	3·91		3·49	3·89	4·21	4·11	4·18	3·06	3·91	4·10	3·83	3·20	3·85	
Glutamic acid	(0·02)	32·8	32·2	32·9	34·3	35·1	35·9	35·0	34·7	35·2	35·2	36·2		35·0	33·8	33·9	36·6	35·5	27·7	32·8	34·8	33·0	35·4	32·7	
Glycine	(0·02)	4·47	4·40	4·51	4·47	4·34	4·65	4·75	4·50	3·62	3·57	3·56		3·25	3·34	3·56	3·97	4·09	3·07	4·03	3·99	3·84	3·53	3·70	
Alanine	(0·02)	3·73	3·84	4·21	3·94	3·07	3·63	3·92	4·02	3·06	3·10	3·03		2·62	2·90	3·15	3·31	3·27	2·49	1·86	3·28	3·28	2·43	2·88	
Valine	(0·02)	4·72	4·73	5·07	5·13	4·51	4·61	4·62	4·31	4·82	4·72	4·06		3·30	4·30	3·90	4·68	4·14	3·26	4·05	4·23	3·85	3·04	3·49	
Cystine	(0·01)	2·83	2·55	3·55	3·47	2·20	2·39	2·30	2·17	2·26	2·13	2·24		1·99	2·07	2·23	2·47	2·53	2·51	2·38	2·83	2·39	2·36	2·13	
Methionine	(0·01)	1·49	1·55	1·65	1·82	1·61	1·75	1·68	1·59	1·41	1·64	1·39		1·17	1·18	1·32	1·36	1·39	1·11	1·44	1·60	1·29	1·25	1·15	
Isoleucine	(0·02)	3·95	3·92	4·28	4·27	3·91	4·04	3·98	3·78	3·62	3·37	3·34		2·77	3·06	3·17	3·48	3·43	2·56	3·45	3·49	3·28	2·50	3·03	
Leucine	(0·03)	7·46	6·79	7·50	7·25	7·23	7·88	7·59	7·14	6·83	6·41	6·76		5·25	5·51	6·05	6·33	6·53	4·85	6·62	6·73	6·08	5·88	5·40	
Tyrosine	(0·02)	3·72	3·56	3·67	3·50	3·62	3·79	3·67	3·88	3·33	3·10	2·32		2·70	2·84	3·12	3·29	2·99	2·49	3·30	3·16	2·95	3·99	4·10	
Phenylalanine	(0·02)	5·43	5·27	5·11	5·94	5·24	5·30	5·56	4·59	5·37	4·33	4·59		3·65	3·78	4·36	4·48	5·60	3·43	4·50	4·67	4·54	6·09	6·32	
Lysine	(0·01)	2·93	2·90	3·18	3·09	2·59	2·79	2·70	2·71	2·37	2·29	2·20		2·17	2·23	2·55	2·48	2·56	1·97	2·46	2·47	2·31	2·95	2·20	
Histidine	(0·01)	2·71	2·71	2·77	2·74	2·61	2·72	2·66	2·56	2·53	2·34	2·18		1·97	2·00	2·28	2·30	2·36	1·73	2·27	2·33	1·95	1·33	2·40	
Arginine	(0·02)	5·36	5·41	5·84	5·70	5·12	5·62	5·50	5·27	4·72	4·66	4·40		4·03	4·23	4·77	3·09	4·80	3·66	4·96	5·64	4·30	3·51	4·06	

* Figures in parentheses are regression coefficients of amino acid with crude protein content

variations in the amino acid composition of whole grains can be accounted for by variations in the proportions of these protein fractions.

The inverse relationship between the total crude protein and lysine content of whole grains which has been reported by several workers[5,6,12] is presumably based on the higher gluten content in high-protein samples. In a study of the amino acid composition of Swedish wheat proteins, Silhbom[13] obtained a negative correlation between the lysine content in the protein (y) and the total crude protein (x) in the sample, which was described by the following equation:

$$y = -0{\cdot}13x + 4{\cdot}53$$

This was in close agreement with that of Lawrence *et al.*[14] who found the correlation to be:

$$y + -0{\cdot}13x + 4{\cdot}20$$

Studies demonstrating this inverse relationship have been carried out on samples generally low in crude protein content, and according to Lawrence *et al.*[14] the upper limit of the range of crude protein levels within which this relationship is valid is 13·5%. In the present study the lowest crude protein value of 15·2% was higher than the upper levels in most of the previous studies cited. When the lysine content is represented as a function of the crude protein content (Fig. 1) no correlation was found to exist in the present study.

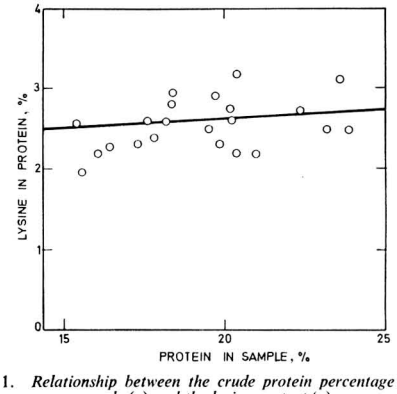

FIG. 1. *Relationship between the crude protein percentage in the sample* (x) *and the lysine content* (y)
$y = 0{\cdot}0047x + 2{\cdot}44$

Histidine and arginine, which are regarded as being dependent on the crude protein in the same way as lysine (Hepburn *et al.*[15]) gave low regression coefficients and showed no tendency to rise or fall with the crude protein level. This is in agreement with the work of Silhbom[13] who was unable to confirm the dependence of these amino-acids on the crude protein content of the sample, and obtained regression coefficients ranging from 0 to −0·11 for the other amino acids.

The results from samples of widely different protein content within a variety were similar when expressed as a percentage of the protein. There were no changes in the amino acid patterns which would create limitations from a nutritional point of view, since minor decreases in the level of an amino acid due to inverse relationships with protein would be completely masked when expressed on a dry matter basis, rather than a crude protein basis.

The use of high-protein cereal grains in compounded rations for livestock may be biologically efficient[16] where supplementation with synthetic amino-acids is contemplated, but the high cost of amino acids may preclude it commercially. High-protein cereals would provide a greater proportion of the amino-acids, the level of supplementation to meet standard requirements would be reduced. For example, from the fourth sowing date if Gabo would provide 90% of the total diet of a growing pig it would also supply 50% of its lysine requirement compared with 20% provided by the soft wheats of 10–12% crude protein conventionally used in compounded diets.

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GRASS TETANY—EFFECTS OF ENVIRONMENT AND FERTILISERS ON PASTURE HISTAMINE LEVELS

By M. O'SULLIVAN

The food consumed by animals suffering from grass tetany had as well as a low magnesium content, a high histamine content, and experiments were carried out to explain these high histamine values. Levels of ammonium sulphate and potassium chloride up to 8 and 6 cwt/ac, respectively, did not affect pasture histamine content which varied considerably, during the spring tetany period. Herbage histamine levels rose four to seven days after the start of a dry period, especially if temperatures were low during this period. The accumulation of histamine in the herbage appeared to be connected with the phenomenon of guttation. Suggestions are advanced to show how climate and pasture histamine content may contribute to the occurrence of grass tetany.

Introduction

The occurrence of grass tetany in cattle and sheep has been known for many years. The condition is always accompanied by a low serum magnesium level, values below 1 mg/100 ml being common. These low levels of serum magnesium do not necessarily arise from a deficiency of magnesium in the diet, but rather from a disturbance in the magnesium metabolism of the animal.[1] Evidence from the literature[2-5] suggests that hypomagnesaemia is not the sole cause of grass tetany but is rather a constant and typical prodromal symptom.

The different forms of tetany are simply the external symptoms of increased neuromuscular excitability. This excitability is a function of balance between mineral elements. Over fifty years ago, Leob (cited by Voisin[1]) studied the effect of potassium, sodium, calcium and magnesium ions on the neuromuscular junctions. He expressed the effect of this ionic balance by the formula:

$$\text{Neuromuscular excitability} = \frac{(K+)(Na+)}{(Ca++)(Mg++)}$$

In other words, the elements of the numerator increase neuromuscular excitability, while those of the denominator decrease it. This is noteworthy from the point of view of grass tetany because the herbage that causes tetany is usually high in potassium and low in magnesium and calcium. However, certain organic compounds[6,7] can accentuate the effect of potassium on neuromuscular excitability. Such compounds are known as 'sensitisers to potassium', and Goffart[7] has stated that these sensitisers, above all else, contribute to the action of potassium on the neuromuscular system.

One of these sensitisers is histamine.[6,7] Hypomagnesaemia causes the release of histamine by the mast cells.[8,9] In addition, histamine gives rise to the release of adrenaline from the adrenal medulla in humans,[10] and it has been stated[11] that adrenaline can be a factor triggering tetany. Histamine has also been found in grasses and clovers,[12] and Fowler[13] advocated a wider investigation into the relationships between magnesium deficiency and histamine metabolism.

Climate appears to play an important rôle in the incidence of grass tetany. Kemp[14] claimed that tetany was unknown when the daily temperature remained above 14° for six consecutive days. Moreover, the lower the temperature was before it began to rise, the greater the increase in the number of tetany cases. Seekles[15] found that there was a regular interval of about five days between the variation in temperature and the appearance of tetany.

It was decided, therefore, to investigate the effect of histamine levels and climate on the incidence of grass tetany. The investigation, which was started in spring 1964, was divided into the following parts: analysis for magnesium and histamine of herbage and foodstuffs from farms where grass tetany had occurred; examination of the histamine content of herbage throughout the months of March and April; examination of the effect of N and K fertilisers on the histamine levels in herbage; investigation of the possible variation of herbage histamine levels with climate and a possible connexion with the phenomenon of guttation.

Experimental and Results

Estimation of histamine and magnesium

Histamine was estimated by the fluorometric method of Shore *et al.*[16] with modifications as described by Kremzner *et al.*,[17] Noah *et al.*,[18] and von Redlich *et al.*[19] Estimation was carried out on a Locarte single-sided fluorimeter MK4 using LF/2 and LF/3 as primary and secondary filters, respectively. All chemicals were Analar grade and the n-butanol and n-heptane used were redistilled twice before use. All aqueous solutions were prepared in triple glass-distilled water. It was important that all water used was stored in glass containers, as the use of plastic containers for this purpose increased the fluorescence in the final determination. Furthermore, the increase in fluorescence was dependent on the length of time the water had been standing in the plastic containers. Magnesium was estimated by the method of Kame Anderson.[20]

Herbage sampling on farms where tetany had occurred

Samples of herbage and other foodstuffs were taken from farms where outbreaks of grass tetany had occurred. Samples were taken 1–2 days after an animal had contracted the disease. Immediately after being cut, herbage samples were placed in plastic bags, and transported to the laboratory in an ice-cooled Thermos flask. All estimations were made within 1 hour of cutting.

A list of cases examined is shown in Table I together with the magnesium and histamine contents of the various food-

TABLE I

Magnesium and histamine contents of foodstuffs consumed by animals suffering from grass tetany

	% Mg (dry matter)	μg/g Histamine (dry matter)
15-year-old cow, wintered out and had very little grass. Calved 10 days before attack on 2 March, 1965. Revived on injection of Mg and Ca		
Crushed oats and barley meal	0·18	1·2
Cow meal	0·24	8·5
Turnips	0·15	1·6
Grass (very poor)	0·16	2·6
6-year-old cow, calved 14 days before attack on 20 March, 1965. Revived on injection of Mg and Ca		
Hay	0·25	2·8
Beet pulp	0·21	9·5
Barley meal	0·19	1·3
Grass	0·17	6·9
5-year-old cow calved 7 days before attack on 3 April, 1965. Revived on injection of Mg and Ca		
Grass	0·18	4·4
Grass	0·18	4·0
2-year-old store, wintered out. Contracted grass tetany on 10 April, 1965. Revived on injection of Mg and Ca		
Grass	0·19	6·1
Grass	0·20	5·0
7-year-old cow, calved 21 days before attack on 13 April, 1965. Revived on injection of Mg and Ca		
Grass	0·19	5·5
Grass	0·18	4·9
Nine cases of grass tetany on one farm within two weeks in 5- to 8-year-old cows. Four of the nine cows died. All animals grazed on poor marshland and pasture until a week before calving, and were then transferred to a field near farmhouse. Cases of tetany occurred 1–6 days after calving. Marshland herbage very poor and highly poached. Cases occurred between 8 and 21 January, 1966		
Grass (Marshland)	0·12	14·3
Grass (near house)	0·18	20·3
Three cases of tetany on one farm in the same week. All animals grazed on two fields with good grass. Cases of tetany occurred 4–12 days after calving. Cases occurred between 6 and 11 March, 1966. One cow died, two revived on injection of Mg and Ca		
Grass Field 1	0·16	6·5
Grass Field 2	0·14	6·8
Grass Field 2	0·14	8·4
Grass Field 2	0·13	7·9

stuffs. Only a limited number of cases have been examined and consequently no definite conclusions can be drawn. However, in all cases, two facts were consistent—low magnesium and high histamine contents.

Herbage histamine content in March and April

To measure any variation in the histamine content of herbage throughout the spring tetany period, quadruplicate samples of herbage were taken from a field at intervals throughout March and April, and histamine content was estimated as described. The solid line in Fig. 1 (b) shows the plot of the mean daily histamine values of these samples. It can be seen that the level of histamine remained constant for approximately 26 days, after which it rose abruptly to give a fourteen-fold increase. The broken line shows the rainfall during the period of the experiment. As can be seen a dry period of four days preceeded the sudden rise in histamine content.

Fig. 1(a) shows the minimum grass temperature throughout the experiment. While nothing conclusive can be drawn from this, the greater variation in temperature immediately preceding the rise in histamine may be noted. Kemp[14] has shown that a variation in temperature is closely related to the occurrence of grass tetany.

Effects of N and K fertilisers on herbage histamine content

It has often been reported that heavy dressings of nitrogenous and potassic fertilisers decrease the magnesium content of herbage.[21–23] An experiment was laid down to examine the effect of different levels of these fertilisers on the histamine concentration of herbage. Treatments consisted of factorial combinations of three levels of nitrogen (0, 3 and 8 cwt sulphate of ammonia/ac) and three levels of potassium (0, 3 and 6 cwt potassium chloride/ac). All plots received a basal dressing of 4 cwt superphosphate/ac and were sown with perennial ryegrass on 3 May. Treatments were replicated four times on randomised plots of 2 yards square. Representative samples were taken from all plots on the following dates: 6, 12, 17, 18, 19, 20, and 22 August. The grass at this time was at the three- to four-leaf stage and six to nine inches high.

Nitrogen and potassium at the levels applied did not affect the histamine content of the grass which, however, varied considerably from day to day. The full line in Fig. 2 repre-

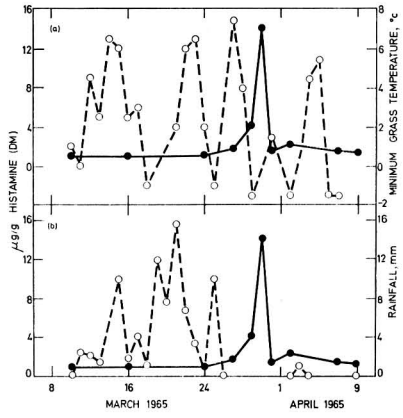

FIG. 1(a). *Influence of temperature on the histamine concentration in herbage in spring*

●——● histamine content ○— — —○ minimum grass temperature

FIG. 1(b). *Histamine content in herbage as a function of rainfall in spring*

●——● histamine content ○— —○ rainfall

sents the variation in the histamine content of perennial ryegrass grown on a plot receiving 3 cwt sulphate of ammonia and 3 cwt potassium chloride/ac. The eight other treatments gave similar patterns. As in Fig. 1 the histamine level increased some days after the start of a dry period. Comparison of the levels of histamine in Figs 1 and 2 shows that spring herbage contains far more histamine than autumn herbage. This may be significant in relation to the frequency with which grass tetany occurs in spring compared with autumn.

Pasture histamine content and guttation

It appears that the accumulation of histamine during a dry period may be, in part at least, connected with the phenomenon of guttation. Samples of guttate taken from pasture in the early morning were found to contain 0·1–0·4 μg histamine/ml. In an effort to provide further evidence for the presence of histamine in the guttate, the following experiment was performed.

Timothy was grown in liquid culture. When the grass was 1–1½ inches high and at the two-leaf stage, histidine was added to the nutrient solution to give a final concentration of 0·2%. After a further three days, guttation was induced by hermetically sealing the pots in plastic bags. Both histidine and histamine were detected in the guttation liquid by chromatography on Whatmann No. 1 filter paper eluted for 16 hours with a solvent mixture of t-butanol-methyl-ethyl ketone-water-ammonium hydroxide (4:3:2:1).

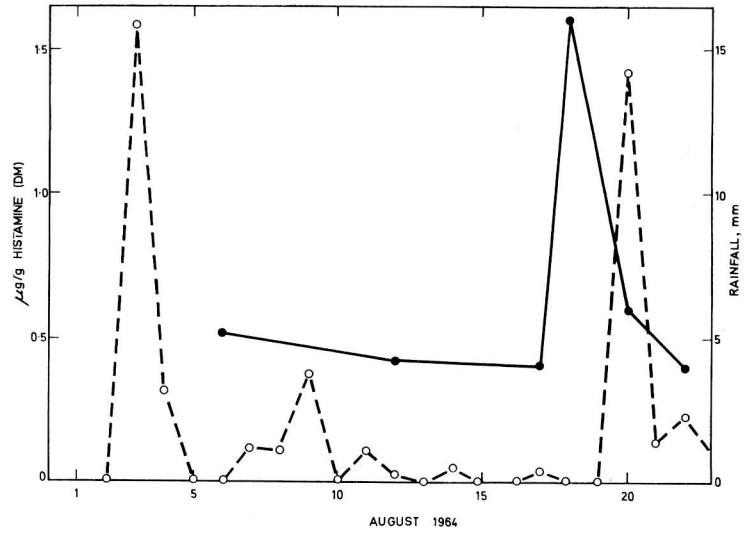

FIG. 2. *Influence of rainfall in autumn on the histamine concentration in herbage treated with 3 cwt sulphate of ammonia and 3 cwt potassium chloride/ac*

●——● histamine content ○— —○ rainfall

Discussion

Initially it was thought that the accumulated histamine in an animal suffering from grass tetany was of endogenous origin. In 1962, however, Fowler[12] reported its presence in grass. This showed that a significant amount of histamine may in fact be ingested. The magnesium and histamine levels in tetanigenic herbage are shown in Table I, and it seems significant that, in the case in Table I where nine cattle (11% of herd) contracted grass tetany on one farm, the magnesium content was low and histamine concentration very high. This may be explained by the stimulating effect of histamine on the activation of the neuromuscular system by potassium. The low magnesium contents of the foodstuffs will also magnify this effect as is shown by the Loeb formula. However, it cannot definitely be stated that histamine is directly concerned with the occurrence of grass tetany until histamine is fed to animals on diets of varying magnesium content and a rigidly controlled ionic balance. Sjaastad & Stormorken[24] and McDonald *et al.*[25] have fed histamine to adult sheep without any adverse effect. In these experiments, however, no account has been given of the magnesium content of the diet. In both cases, as the effect of histamine on the diet of the animal was being studied, one must assume that the magnesium contents were normal. Because magnesium[26] and calcium[27] can act as anti-histamines, it is reasonable to assume that any effect from histamine would occur only when these elements were low in the diet. Many other substances, e.g. guanidine, adenosine, uracil, urea, thiocyanate, etc. can act as sensitisers of potassium,[6,7] but the level of these substances in herbage has not been studied in the present investigation.

It appears that the accumulation of histamine during a dry period is, in part at least, connected with the phenomenon of guttation. The explanation of this accumulation may be that the liquid guttated by the plant during a rainy period contains histamine and thus regulates its concentration in the plant. During a dry period, when guttation is either drastically reduced or completely stopped, a rise in herbage histamine results. It is worthy of note that the high level of histamine is not maintained in Figs 1 and 2. However, recent work in this laboratory showed that the histamine concentration of perennial ryegrass increased with decreasing temperature. The rapid increase in herbage histamine may then be a function of both rainfall and temperature. Thus, in spring, when the magnesium content of herbage is low, a cold dry spell can increase the histamine intake to an animal which, because of the low temperature, already has a greater requirement for magnesium, and so lead to the combination of low magnesium and high histamine levels which accompanies grass tetany. The results thus indicate that a dry period, especially if accompanied by low temperature, is especially conducive to the occurrence of grass tetany. This is in agreement with the work of MacKeller[28] and Kemp.[14]

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2-AZIDO-4-ALKYLAMINO-6-ALKYLAMINO-s-TRIAZINES: A NEW GROUP OF HERBICIDAL TRIAZINES*

By P. A. GABBOTT** and G. E. BARNSLEY†

Phytotoxicity to 7 crop species, and degree of inhibition of the Hill reaction were assessed for several 2-azido-4-alkylamino-6-alkylamino-s-triazines. Biological activity increased with increased alkylation of the amino groups, with increasing chain length of the alkyl substituents up to 5 to 6 C atoms, and with increased degree of branching of the alkyl chain. For several of the compounds tested there was good agreement between phytotoxicity tests and potency against the Hill reaction. The most active compound was 2-azido-4-ethylamino-6-t-butylamino-s-triazine (WL 9385), which was particularly effective in controlling seedling annual grasses when applied post-emergence.

Introduction

Despite the discovery a decade ago[1] of the herbicidal properties of 4-alkylamino-6-alkylamino-s-triazines with 2-halo, 2-alkoxy and 2-alkylthio- groups few s-triazine compounds with other substituents in the 2 position have been reported or so far developed. Extensive research in these laboratories has involved the systematic examination of some 60 series of s-triazines and led to the discovery of novel 2-azido-4-alkylamino-6-alkylamino-s-triazines, a new group of herbicidal triazines.

Experimental

Phytotoxicity on whole plants was determined in glasshouse tests with compounds of >95% purity using seven crop species treated at two stages of development in separate pre- and post-emergence tests. In these tests pre- and post-emergence phytotoxicity can be related to uptake of compound by the seed and roots and to combined uptake via roots and aerial parts of the shoot respectively.

The species used were maize (*Zea mays*), oats (*Avena sativa*), ryegrass (*Lolium perenne*), pea (*Pisum sativum*), linseed (*Linum usitatissimum*), mustard (*Sinapis alba*), and sugar beet (*Beta vulgaris*). The test plants were propagated and treated in experimental glasshouses (temperature range 20–25°) under natural light, in John Innes No. 1 potting medium in plastic 3 in. pots or 13 × 38 × 6 cm dishes. In the pre-emergence test, species were sown in bands 0·5–1·0 cm deep in a single dish, and the soil surface was covered with flint grit, watered, and sprayed within a few hours of cessation of free drainage. Seed dishes and pots containing the individual test species as young seedlings (1- to 2-true-leaf stage) were sprayed on a belt moving under a stationary nozzle (Teejet, 80015-E), at a dosage rate of 1 kg/ha active ingredient in 630 litres 1 : 1 aqueous acetone/ha; the spray solution contained 0·6% w/v Triton X 100 as wetting agent and 5% v/v glycerol as humecant. The dishes and pots were surface-watered as required, and phytotoxicity was scored visually on a 0–9 scale (0, no effect; 9, complete kill) 7 and 11 days after post- and pre-emergence treatment, respectively.

Photosynthesis is probably the physiological system most sensitive to the action of the phytotoxic s-triazines,[2] and all available evidence suggests that the site of action is located in the photo-electron transport system associated with the short wavelength-absorbing pigment system and the reactions involved in oxygen evolution.[3] Preliminary studies on the inhibition of the Hill reaction by 2-azido-s-triazines have been undertaken using so-called 'broken' chloroplasts isolated from young pea seedlings.

Chloroplasts were prepared from young pea seedlings (cv. Alaska) by the method described by Whatley & Arnon.[4] The suspending medium was an ice-cold isotonic salt solution consisting of 0·35 M-NaCl in 0·02 M Tris–HCl buffer, pH 7·2. Total chlorophyll was determined by the method of Arnon[5] (K = 36·0 at 652 mμ). Photochemical activity was assayed by measurement of the rate of reduction of ferricyanide; maximum reaction rates were obtained by uncoupling of photophosphorylation with methylamine.[6] The assay was performed in 25 ml conical flasks with a light intensity at flask level of 1800 lumen/ft² and the temperature held constant at 20°. The reaction mixture (final volume 5·0 ml) contained, in μmole: Tris–HCl (pH 7·2) 96; NaCl, 280; $K_3Fe(CN)_6$, 2·5; methylamine HCl, 60 and once-washed chloroplasts containing 10–20 μg chlorophyll per ml. At the end of the assay (8 min) 10% trichloroacetic acid (1 ml) was added to each flask, and the contents were centrifuged. Ferricyanide reduction was measured photometrically at 420 mμ. Compounds were prepared at different concentrations by serial dilution in 50% v/v acetone–water and added to the standard reaction mixture in a volume of 0·2 ml. Inhibition of ferricyanide reduction was expressed as a percentage of the control activity, and several points on the concentration/inhibition curve were established for each compound. All determinations were made in duplicate with

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chloroplasts obtained from at least two different extractions. Inhibitor potency was expressed as the mean pI_{50} value over replicate determinations, with a standard error of the mean calculated from the pooled variation between replicates (pI_{50} value is $\log_{10}$ of the reciprocal of the molar concentration causing 50% reduction of photochemical activity).

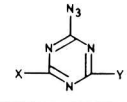
Results and Discussion

Communications on the gross structure/activity relationships within the herbicidal s-triazines at both the whole plant and chloroplast levels have already appeared.[6–8] Thus Gysin & Knüsli[7] describe some twelve classes of s-triazines, giving the herbicidal properties of each and Exer, B. (unpublished work), Good,[6] and Moreland & Hill[8] have studied the potency of selected s-triazines as inhibitors of the Hill reaction. Data in the present report for the 2-azido-4,6-diamino (and alkylated amino)-s-triazines confirm and extend the conclusions of the earlier studies. The results in Table I show that biological activity increased with alkylation of one (**II**) or both amine groups (**III**, **IV** and **V**); the parent 4,6-diamino-s-triazine (**I**) was so low in activity that it was virtually non-phytotoxic. Monoalkylation conferred greater activity than dialkyl substitution, the highest activity occurring with the 4,6-bis(ethylamino)-s-triazine (**III**). Compound **IV**, containing both an ethylamino and diethylamino substituent, was intermediate in herbicidal activity between compound **III** and the 4,6-bis(diethylamino)-s-triazine (**II**), but almost inactive in the Hill reaction test. Similar results were found in the 2-chloro-s-triazine series by Gysin & Knüsli;[7] thus trietrazine (2-chloro-4-ethylamino-6-diethylamino-s-triazine) and the 2-chloro analogue of compound **II** were intermediate in herbicidal activity between chlorazine (2-chloro-4,6-bis-(diethylamino)-s-triazine) and simazine (2-chloro-4,6-bis-(ethylamino)-s-triazine).

The influence of increasing the length of the alkyl substituents, and branching of the alkyl chain was studied for selected series of 4-alkylamino-6-alkylamino-s-triazines (Tables II and III). In each series, activity first increased with increasing

TABLE I

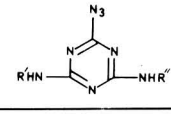
Phytotoxicity of a series of 2-azido-4,6-diamino- (and alkylated amino-) s-triazines



Compound No.	X	Y	No. of alkyl C atoms	Mean Phytotoxicity Index for 7 species: Pre-emergence	Mean Phytotoxicity Index for 7 species: Post-emergence	Hill reaction inhibition
I	NH_2	NH_2	0	0·0	0·3	<3·00
II	$NHCH_2CH_3$	NH_2	2	0·0	3·5	5·13 (±0·18)
III	$NHCH_2CH_3$	$NHCH_2CH_3$	4	1·6	5·3	5·99 (±0·12)
IV	$NHCH_2CH_3$	$N(CH_2CH_3)_2$	6	2·0	3·7	3·24 (±0·25)
V	$N(CH_2CH_3)_2$	$N(CH_2CH_3)_2$	8	0·4	0·9	4·40 (±0·25)

TABLE II

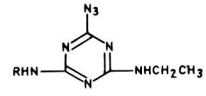
Phytotoxicity of a series of 2-azido-4,6-bis(alkylamino)-s-triazines



Compound No.	R′	R″	No. of alkyl C atoms	Mean Phytotoxicity Index for 7 species: Pre-emergence	Mean Phytotoxicity Index for 7 species: Post-emergence	Hill reaction inhibition
VI	$-CH_3$	$-CH_2CH_3$	3	0·6	3·0	5·26 (±0·12)
VII	$-CH_3$	$-(CH_2)_2CH_3$	4	1·3	4·6	5·50 (±0·25)
VIII	$-CH_3$	$-CH(CH_3)_2$	4	2·3	5·9	5·82 (±0·14)
IX	$-CH_3$	$-(CH_2)_3CH_3$	5	0·3	5·9	6·14 (±0·25)
X	$-CH_3$	$-CH_2CH(CH_3)_2$	5	1·0	4·6	6·11 (±0·18)
XI	$-CH_3$	$-C(CH_3)_3$	5	2·9	6·6	6·75 (±0·14)
XII	$-CH_3$	$-C(CH_3)_2C_2H_5$	6	2·1	5·1	6·70 (±0·14)
XIII	$-CH(CH_3)_2$	$-CH(CH_3)_2$	6	1·6	5·7	6·87 (±0·18)
XIV	$-CH(CH_3)_2$	$-C(CH_3)_3$	7	3·1	5·9	6·85 (±0·14)

TABLE III

Phytotoxicity of a series of 2-azido-4-ethylamino-6-alkylamino-s-triazines



Compound No.	R	No. of alkyl C atoms	Phytotoxicity Index; pre- and post-emergence: Maize pre	Maize post	Oats pre	Oats post	Ryegrass pre	Ryegrass post	Pea pre	Pea post	Linseed pre	Linseed post	Mustard pre	Mustard post	Sugar-beet pre	Sugar-beet post	Mean Phytotoxicity Index for 7 species pre	Mean Phytotoxicity Index for 7 species post	Hill reaction inhibition
VI	$-CH_3$	3	0	0	0	5	0	0	0	0	0	7	2	6	2	3	0·6	3·0	5·26 (±0·12)
III	$-CH_2CH_3$	4	0	0	1	7	0	4	0	3	1	7	7	8	2	8	1·6	5·3	5·99 (±0·12)
XV	$-(CH_2)_2CH_3$	5	0	0	2	7	1	2	0	1	2	8	8	9	8	8	3·0	5·0	6·13 (±0·18)
XVI	$-CH(CH_3)_2$	5	0	0	0	7	0	6	0	5	7	8	8	9	7	9	3·1	6·3	6·54 (±0·18)
XVII	$-(CH_2)_3CH_3$	6	0	2	0	7	0	5	0	3	0	7	3	9	1	7	0·6	5·7	6·80 (±0·25)
XVIII	$-CH_2CH(CH_3)_2$	6	0	1	0	8	0	5	0	4	0	9	5	8	0	8	0·7	6·1	6·64 (±0·14)
XIX	$-C(CH_3)_3$	6	0	2	1	8	2	6	0	6	8	9	9	9	4	7	3·4	6·7	7·46 (±0·12)
XX	$-C(CH_3)_2C_2H_5$	7	0	1	0	7	0	3	0	5	3	9	7	9	8	8	2·6	6·0	6·77 (±0·14)

chain length and then levelled off or decreased. Optimum activity was attained with asymmetric compounds having a total of 5 or 6 alkyl carbon atoms (**XI, XII, XVI** and **XIX**). Again similar results had been found in the 2-chloro-4-methylamino and 2-chloro-4-ethylamino series by Gysin & Knüsli, in that compounds having an additional amino group containing 2–4 carbon atoms in the 6-position of the triazine ring were most active herbicidally.[7]

Within the 2-azido-s-triazines so far investigated, branching of the side chain also has an important effect on biological activity. Thus in both the methylamino and ethylamino series the isopropylamino compounds (**VIII, XVI**) were more active than the n-propylamino compounds (**VII, XV**) and the t-butylamino compounds (**XI, XIX**) more active than the n-butylamino (**IX, XVII**) or isobutylamino analogues (**X, XVIII**). This latter effect was particularly evident from the pre-emergence phytotoxicity data. At the chloroplast level, the methylamino and ethylamino compounds having s-butylamino substituents were intermediate in activity between the corresponding n-butylamino (or isobutylamino) and t-butyl amino compounds, having pI_{50} values of $6{\cdot}49 \pm 0{\cdot}07$ and $7{\cdot}04 \pm 0{\cdot}07$, respectively. Previously, in the 2-chloro-s-triazine series, asymmetric compounds having methylamino or ethylamino substituents with n-propyl or n-butyl substituents on the 6-amino radical had shown some herbicidal promise[7] but were not developed. It is perhaps significant therefore that the first development compounds in the asymmetrically-substituted 4-alkylamino-6-alkylamino-s-triazine series were the branch-chain isopropylamino compounds, atrazine, atratone and ametryne (2-chloro-, 3-methoxy, and 2-methylthio-4-ethylamino-6-isopropylamino-s-triazines respectively).

Fig. 1 shows the relationship between the relative potency of the 2-azido-4-alkylamino-6-alkylamino-s-triazines against the Hill reaction, and their glasshouse phytotoxicity. In general there is good agreement between inhibitor potency and herbicidal activity, with the exception of the n-butylamino (**IX** and **XVII**) and isobutylamino (**X** and **XVIII**) compounds which have very low pre-emergence phytotoxicity. In the 2-chloro-s-triazines also, there were certain parallels between inhibition of the Hill reaction and herbicidal activity.[7] The relationship was not always clear, however; in this respect it may be noted that 2-chloro-4-ethylamino-6-n-butylamino-s-triazine was cited as a compound of high inhibitory activity

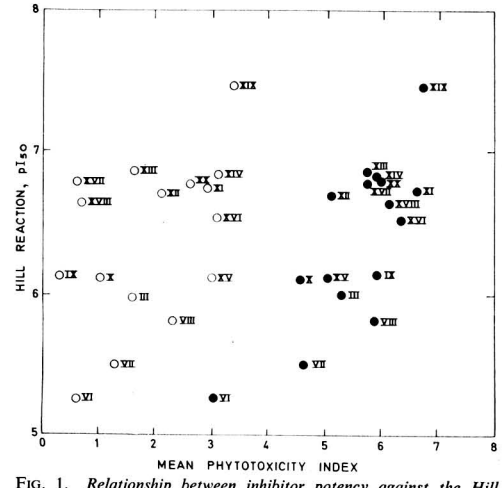


FIG. 1. *Relationship between inhibitor potency against the Hill reaction and glasshouse phytotoxicity for selected 2-azido-4-alkylamino-6-alkylamino-s-triazines*

○ pre-emergence
● post-emergence

in the Hill reaction but poor herbicidal activity.[7] The 2-chloro-s-triazines are essentially pre-emergence herbicides and it is evident that other factors, such as those determining availability in the soil, penetration into roots and translocation within the plant, are important for high activity.[7,9] Similarly, in the 2-azido series, the somewhat improved correlation with post-emergence phytotoxicity probably relates to the more direct entry of the compounds from the external leaf surface to their site of action in the chloroplasts.

These results confirm the view that at least one NH group and preferably two are required for high activity in the s-triazines[6,7] and that the alkylamino substituents have a

critical chain length.[7,8] In addition biological activity is markedly enhanced by branching of the alkyl chain. Similar conclusions have been reported, in part, for another class of photosynthesis inhibitors, the substituted phenylamides.[10] Using the general extrathermodynamic approach developed by Hansch *et al.*[11,12] a preliminary separation of partition, electronic and seric substituent effects in the Hill reaction studies has shown that the major substituent effects for compounds in Tables II and III are probably associated with the hydrophobic bonding capacity of the alkyl side-chains and electron release by the alkyl substituents to the NH groups (Gabbott, P. A., in press).

The phytotoxicity ratings in Table III indicate outstanding tolerance by maize to both pre- and post-emergence treatment. Oats, ryegrass and pea were tolerant of pre-emergence but not post-emergence treatment. Glasshouse and laboratory studies have shown that species selectivity, at least in the case of cereals, is dependent on environmental factors such as stage of development, growth rate and seed placement, rather than on specific biochemical detoxification mechanisms in tolerant species.[13] The main phytotoxic effect of the 2-azido-4-alkylamino-6-alkylamino-s-triazines is on the growth of young plants. When the herbicides are applied to the soil, the toxicity symptoms are similar to those of the 2-chloro-s-triazines and occur some time after treatment. With post-emergence application, however, the symptoms appear much more rapidly.

The most active compound of this new class of s-triazines is 2-azido-4-ethylamino-6-t-butylamino-s-triazine (**XIX**), coded WL 9385. Typically WL 9385 exhibits broad-spectrum pre- and post-emergence herbicidal activity and is particularly effective in controlling seedling annual grasses post-emergence, but not established annual and perennial species. In contrast to the earlier 2-substituted-s-triazines, WL 9385 has a short predictable half-life in the soil and is virtually non-leaching; decomposition is not bio-dependent but requires adequate soil moisture, very little decomposition occurring on air-dry soils (Osgerby, J. M., unpublished work). In the combination of short soil persistence, broad spectrum weed control and a range of exploitable selectivity (notably in cereals) this compound presents some new opportunities for crop management in agriculture and horticulture, particularly in intensive mixed-cropping systems which require herbicides of limited and predictable duration.[14]

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MILLING OF SOLVENT-EXTRACTED WHEAT, SEMOLINA AND FLOUR.

I.—Effect on endosperm fragmentation and protein shifting

By N. L. KENT and A. D. EVERS

The degree of endosperm fragmentation in milled flour and degree of protein shifting in air-classified fractions were slightly augmented by pre-milling treatment of Manitoba wheat and milled products with acetone; they were more markedly affected by treatment with methanol if the residual solvent was promptly removed after treatment. In these circumstances, gluten could be recovered from the flour. Methanol, acting upon wheat, semolina or flour for extended periods of time, had a deleterious effect on the protein, severely reducing endosperm fragmentation and preventing the recovery of recognisable gluten from the milled flour; aqueous butanol had a similar effect.

Introduction

As part of a project concerned with the effect of conditioning and pre-milling treatments of wheat on the breakdown of endosperm during the milling process—and particularly its breakdown to particles below 35 microns in size, suitable for air-classification and protein shifting—a study has been made of the effect of solvent extraction of wheat upon its subsequent milling behaviour.

Electron-microscope studies of starch granules by Hess[1] indicated that a fibrillar layer of protein ('haft' protein) attached to the starch granule surface is separated from the wedge (or 'zwickel') protein lamellae by a layer of lipid and lipoprotein. Buttrose[2] studied the development of starch granules of cereal endosperm with the electron microscope and found that each granule is enveloped by the double membrane of the proplastid in which it develops. It was considered possible that removal of lipid or lipoprotein from whole wheat grains by solvent extraction might promote the breakdown of endosperm, and particularly the separation of protein from starch granules. Solvent extraction was therefore investigated as a pre-milling treatment.

As there was some doubt whether the solvents used actually reached the interior of endosperm cells of the intact wheat grains (although lipid was undoubtedly removed), solvent extraction experiments were also carried out with: I Break grind (i.e., wheat that had been passed through the first grinding stage of the roller-milling process), which, after treatment, was subsequently milled to completion on the roller-mills; semolina, which was roller-milled to produce flour; and flour, which was subsequently pin-milled. The flours milled from solvent-extracted materials were air-classified and the degree of protein shifting was calculated from yields and protein contents of the air-classified fractions.

Experimental

Materials

One hard wheat and one soft wheat were extracted. The hard wheat was a Manitoba No. 2 wheat, having 13·0% moisture content (m.c.) when received, and 13·85% protein content (14% m.c. basis). The soft one was a U.S. Soft White wheat, 13·5% m.c. as received, 8·8% protein content (14% m.c. basis). Both samples were obtained from commercial sources.

The I Break grinds for solvent treatment were prepared in the laboratory from the untreated Manitoba wheat, by passing the wheat through the normal I Break rolls.

The semolina, a commercial sample of 'A'-roll feed, milled from a mixed grist (55% Manitoba No. 2, 27·5% Plate, 17·5% English wheats), was obtained from a British flour mill; it contained 14·9% moisture as received, and when analysed gave 0·55% ash, 11·6% protein, 0·9% fat by petrol extraction and 1·7% fat by acid hydrolysis (14% m.c. basis).

The flours for solvent treatment were milled in the laboratory from untreated portions of the Manitoba and Soft White wheats.

Methods

Solvent extraction

Whole grain wheat, I Break grind, and flour were extracted with petrol (b.p. below 40°), or with petrol and acetone (Analar grade) in succession, or with petrol, acetone and methanol (general purpose reagent) in succession. Manitoba flour was also extracted with water-saturated n-butanol (general purpose reagent). Semolina was extracted with petrol, or with aqueous butanol, or with methanol, and in the latter case the methanol either was or was not washed off with acetone and petrol, used successively. All extractions were carried out at room temperature by steeping, or by percolation, or by sedimentation.

Steeping

Wheat was extracted by covering it with the solvent, in a jar, and renewing the solvent periodically. Thus, in one trial, Manitoba wheat was steeped in petrol for 99 days, which reduced the petrol extractable fat content from 1·5% to 0·9%; after this, a portion of the petrol-extracted wheat was further extracted with acetone for 103 days (to 0·8% fat

content), and a portion of the petrol+acetone-extracted wheat was further extracted with methanol for 37 days (to 0·7% fat content). In another trial, a sample of the same original wheat was extracted with petrol for 18 days, then with acetone for 259 days, then with methanol for 150 days, by which time the petrol-extractable fat content was again reduced to 0·7%. Residual solvent was removed by aeration or, in the case of the 150 day methanol extraction, by washing with acetone and petrol in succession, followed by aeration, and the wheats were cold-tempered for milling. I Break grind was extracted by a similar procedure.

Percolation

Flour was extracted by the slow percolation of solvent down a narrow column of flour contained in a glass tube. In later trials with methanol, the solvent was washed off with acetone and petrol in succession.

Sedimentation

Following reports by Mecham & Mohammad[3] that aqueous butanol could be used as an extractant for flour without adverse effects, an attempt was made to use this solvent. Percolation through a column of flour was unsuccessful, because a degree of doughing occurred. In the technique finally adopted a large cylinder was filled with aqueous butanol and the flour was introduced at the top gradually through a sieve. The flour dispersed in the solvent and eventually settled on the bottom of the cylinder. Most of the solvent was then decanted, with as little disturbance of the sediment as possible, and the cylinder was filled up with acetone. The suspension was then stirred, and after the flour had settled, the acetone was decanted, and the washing process was repeated with petrol. The flour recovered showed no signs of 'doughing' or clumping. However, it carried a strong odour of residual butanol, removal of which was attempted by vacuum drying. The flour was humidified to 17% m.c. and then dried in a vacuum-oven for 2 days at room temperature and 0·5 in. Hg in the presence of P_2O_5. The humidifying and drying cycle was repeated four times. Even after this treatment, a noticeable odour of butanol persisted. Others have reported difficulty in removing the last traces of butanol from flour.[4] The flour samples in this series extracted with other solvents were similarly treated in a vacuum-oven; none retained any aroma of solvent after one cycle.

Semolina was extracted with petrol or with methanol by the column percolation method, and with aqueous butanol by the cylinder sedimentation method. The extracted samples in one series were dried in a vacuum-oven, the butanol-treated sample retaining an odour of butanol after four cycles of humidifying and drying.

In each trial a portion of the starting material was retained in the unextracted state, and was processed otherwise in exactly the same way as the extracted samples. All the samples within any one series were brought to the same moisture content, as far as possible, before being milled, by being humidified, or dried at $>40°$.

Milling

Wheat was milled on a MIAG laboratory roller mill, using a standard 4 breaks and 8 reductions system, as described elsewhere.[5] Semolina was milled on the reduction section of the same system. Portions of the semolina were also milled on a Minikek pinned disc mill. Flour yields were calculated on products of milling, and are expressed as parts of fat-free flour yielded per 100 parts of fat-free products of milling.

Extracted flours, and also flours milled from the extracted wheat or semolina, were pin-milled on a Minikek laboratory pinned disc mill operated at 19,740 rev/min, as described elsewhere.[6] Feed rate to the pin mill was about 24 g/min for flour, 40–45 g/min for semolina.

Air-classification

Flours, both roller-milled and reground by pin mill, were air-classified on a laboratory-scale Bahco Centrifugal Air Elutriator, as described elsewhere.[6] Cuts were made successively at 17 and 35 μ, dividing the flour into three fractions: fine (0–17 μ) high-protein fraction, intermediate (17–35 μ) low-protein fraction, and coarse (>35 μ) medium-protein fraction. Flour was fed to the Bahco machine at about 2 g/min. Weights of the fractions were recorded, and moisture and protein contents of the parent flours and of the fractions were determined; yields of the fractions, on a dry matter basis, are expressed as percentages of the total dry matter recovered (generally about 98%).

The sum of the yields of fine and intermediate fractions was used as an indication of the extent of endosperm fragmentation, and the weighted average protein content of the fine and intermediate fractions as the protein content of the fragmented endosperm. Degree of endosperm fragmentation during regrinding of flour on the pin mill was calculated as $100(a-b)/a$, where a and b are percentages of coarse fraction in the roller-milled and the pin-mill reground flours, respectively. Total protein spread (an indication of the degree of protein shifting) was calculated as: $\frac{X(x-p)+Y(p-y)+Z(z-p)}{p}$, where p is the protein content of the parent flour, x, y and z the protein contents (at 14% m.c.) of the fine, intermediate and coarse fractions, respectively, and X, Y and Z the corresponding percentage yields.

Analytical methods

Moisture content was determined as the loss, at 120° during 4 h (wheat) or 1·5 h (semolina, flour), or by the Marconi electrical moisture meter. Protein was determined as N by Kjeldahl or micro-Kjeldahl methods, and expressed as N × 5·7. Fat contents were determined by petroleum extraction[7] and by acid hydrolysis[8] methods. All analytical results are expressed on 14% m.c. basis.

Gluten quality

Flour–water doughs were made from all the flours used in this work, and an attempt was made to collect a ball of gluten by washing out the starch under running water. In some cases, rheological properties of the dough were further characterised by testing on the Research extensometer.[9]

Results

Analytical data on the extracted and unextracted wheats and I Break grinds, and on the products of roller-milling are shown in Table I. Tables II and III show the results of air-classification of the roller-milled flours of Table I, and of the same flours after pin-mill regrinding, respectively. Tables IV and V show similar results for the extracted and unextracted flours, and Tables VI, VII and VIII the results for semolinas.

Extracted wheat

Flour yield (Table I) was consistently increased by solvent extraction of whole grain wheat before milling, a maximum of 4% additional flour yield (fat-free basis) resulting from extraction with petrol and acetone.

Flour yield was considerably depressed when I Break grind was extracted with methanol (following petrol and acetone extractions), although treatment with petrol alone augmented the flour yield.

Extraction of the soft wheat considerably improved the sieving properties of the ground stocks, which appeared quite free-flowing and gritty, resembling stocks from hard wheats.

An adverse feature in the milling of solvent-extracted wheat was the tendency for the stock to adhere to the smooth reduction rolls, causing pasting or 'ringing'. The effect was most troublesome when petrol or petrol+acetone had been used.

Yield of bran and fine offals from the milling of wheat and I Break grind decreased as flour yield increased following progressive solvent treatments. The petrol-extractable fat content of the milling offals likewise decreased following petrol and petrol+acetone treatment of wheat and I Break grind, and petrol+acetone+methanol treatment of I Break grind, but the fat content of the bran from petrol+acetone+methanol treated wheat was consistently higher than that from wheat extracted only with petrol+acetone; this suggests that during the methanol extraction there was some movement of fat from the interior of the grain towards the bran, where it was apparently deposited. The increased fat content of the bran from methanol-treated wheat is not explainable on the basis of yield differences.

Degree of fragmentation of the endosperm in the flour roller-milled from Manitoba wheat was unaffected by petrol extraction, but was slightly increased by petrol+acetone extraction. After petrol + acetone + methanol extraction, however, fragmentation markedly increased, from 10% (in the unextracted sample) to 18% (see Table II). A repetition of the experiment, carried out 21 months later on a fresh sample of Manitoba wheat, gave almost identical results (fragmentation increased from 9% to 17%). In the second trial, the methanol had been washed off with acetone and petrol. Total protein spread in the air-classified fractions of the roller-milled flours was augmented, from 4% in the unextracted sample to 9% or 11% after petrol+acetone+methanol extraction; a difference of 3% in total protein spread, between treatment means, was significant ($P=0.05$). Similar treatment of the soft wheat, however, led to progressive decrease in fragmentation. It is noteworthy, in the case of Manitoba wheat extracted with petrol+acetone+methanol, that the protein content of the intermediate (low-protein) air-classified fraction of the roller-milled flour was decreased by more than 1% in comparison with the unextracted control (parent flour protein contents 13·3% and 13·1%, respectively). From the weighted average protein

TABLE I

Analytical data on solvent-extracted and unextracted wheats and I Break grinds, and on the products of milling

Series	Previous treatment of wheat	Extraction treatment: Solvent	Duration, days	Wheat: m.c.,* %	Wheat: fat P-extd.,[1] %	Wheat: fat ac. hyd.,*[1] %	Flour yield: as milled, %	Flour yield: fat-free,[2] %	Roller-milled flour: m.c., %	Roller-milled flour: fat P-extd.,[1] %	Roller-milled flour: fat ac. hyd.,[1] %	Roller-milled flour: protein,[1] %	Bran: Yield, %	Bran: fat P-extd.,[1] %	Fine offals: Yield, %	Fine offals: fat P-extd.,[1] %
Manitoba wheat (protein 13·85%)																
I	None	Unextracted		15·7	1·5	2·6	69·5	70·2	14·7	0·8	1·5	13·3	17·1	3·9	13·4	3·9
	None	Petrol	18	15·9	1·1		70·6	71·0	14·6	0·7	1·4	13·2	18·0	3·0	11·4	2·0
	None	Petrol	99	15·7	0·9	1·9	72·8	73·1	15·0	0·6	1·2	13·4	16·9	2·4	10·3	1·6
	*P-extd.	Acetone	103	15·7	0·8	1·9	74·0	74·2	14·4	0·6	1·2	13·3	16·3	2·2	9·7	1·2
	P, A-extd.	Methanol	37	15·3	0·7		71·9	72·3	13·9	0·5	1·1	13·1	15·4	2·9	12·7	1·3
III	None	Unextracted		16·4	1·5	2·6	71·0	71·6	15·2	0·7	1·5	13·5	16·8	3·6	12·2	3·9
	P-extd.	Acetone	259	16·4	0·7	1·9	72·2	72·4	14·8	0·5	1·3	13·4	16·2	2·1	11·6	1·1
	P, A-extd.	Methanol[3]	150	16·1	0·7	1·8	73·2	73·5	13·8	0·4	1·3	13·3	16·0	2·6	10·8	1·1
Soft White wheat (protein 8·8%)																
	None	Unextracted		14·9	1·7	2·5	70·8	71·4	14·3	0·8	1·2	7·5	17·1	2·9	12·1	4·6
	None	Petrol	74	15·0	1·1	2·0	71·9	72·2	14·3	0·6	1·15	7·4	16·9	2·5	11·2	2·1
	P-extd.	Acetone	66	14·5	1·0	1·9	73·2	73·5	13·0	0·5	1·0	7·8	15·8	2·5	11·0	1·4
	P, A-extd.	Methanol	28	14·6	0·8	1·6	72·8	73·1	12·5	0·4	0·9	7·7	14·9	2·6	12·3	1·1
Manitoba I Break grind (protein 13·85%)																
	None	Unextracted		15·4[4]	1·5	2·6	70·9	71·6	14·3	0·8	1·4	13·2	16·9	3·6	12·2	4·6
	None	Petrol	16	15·0[5]	0·4		73·3	73·5	15·0	0·2	0·9	13·3	14·1	1·8	12·6	0·7
	P-extd.	Acetone	70	16·2[6]	0·19		71·5	71·6	12·1	0·1	0·6	13·4	12·4	0·6	16·1	0·3
	P,A-extd.	Methanol	29	15·6[7]	0·17		64·4	64·6	13·0	0·1	0·5	13·6	10·3	0·4	25·2	0·3

*P = Petrol, A = acetone
*m.c. = moisture content
*ac. hyd. = acid hydrolysis
[1]14% moisture basis
[2]Parts of fat-free flour as percentage of total fat-free products of milling
[3]Washed with acetone and petrol in succession
[4]Mill feed 16·2% m.c. I Bk. grind dried to 15·4% m.c.
[5]Mill feed 16·0% m.c. I Bk. grind 15% m.c. after P treatment
[6]Mill feed 16·0% m.c. I Bk. grind 12·1% m.c. after P and Ac treatments; humidified to 16·2% m.c. for milling
[7]Mill feed 16·0% m.c. I Bk. grind 11·2% m.c. after P, A and Methanol treatments; humidified to 15·6% for milling

contents of fine plus intermediate fractions (Table II), it appears that the additional endosperm that was being fragmented in the wheats solvent-extracted to progressively greater extents was of lower protein content than that fragmented in the unextracted wheat.

Endosperm in the roller-milled flour from petrol+acetone +methanol extracted I Break grind (Table II) showed decreased fragmentation and reduced protein spread in comparison with the unextracted control.

After pin-mill regrinding of the Table II flours, and air-classification, an augmented effect of solvent extraction on endosperm fragmentation was evident (Table III). Endosperm fragmentation increased from 52% in the unextracted control to 61% and 71% with petrol+acetone and petrol+acetone+methanol extraction, respectively (or from 53% to 56% and 72%, in the second trial). Breakdown of endosperm on the pin mill increased from 46–48% to 65–66%, and total protein spread increased from 19–20% to 32%, after petrol+acetone+methanol extraction (sign. diff. at P =0·05, 4%). It is noticeable that protein content of the total fragmented endosperm (fine plus intermediate fractions) showed little variation, whereas the protein content of the fine (high-protein) air-classified fraction was 1% higher, and that of the intermediate (low-protein) fraction 0·7–1·1% lower in the flour from petrol+acetone+methanol extracted wheat than from the control, indicating an increase in protein shifting due to improved separation of starch granules from surrounding protein. Typical fields from microscopical mounts of the fine and intermediate air-classified fractions of the pin-mill reground flours, roller-milled from unextracted and from petrol+acetone+methanol extracted wheats, observed at a magnification of ×320, are shown in Fig. 1. Flour from the extracted wheat had (in comparison with flour from the unextracted wheat) a larger proportion of single free starch granules, a smaller proportion of protein/starch 'clusters', and a greater degree of stripping of starch granules from fragments of protein.

Effects of extraction with petrol+acetone in the pin-milled flours were generally similar to those of petrol+acetone+methanol extraction, but less marked. The effects of all solvents on endosperm fragmentation in the soft wheat were small (Table III), although there was an increase in total protein spread. The effects of petrol, and of petrol+acetone, on I Break grind of Manitoba wheat were negligible, while additional extraction with methanol reduced endosperm fragmentation on the pin mill, and decreased protein spread in the pin-mill reground flours.

Extracted flour

Extraction of Manitoba flour with petrol or with petrol+acetone reduced the flour fat content, as shown in Table IV, but did not affect protein content or air-classification performance of the unreground flours. Additional extraction with methanol reduced flour protein content by nearly 1%; protein contents of all air-classified fractions were low, but yields were not much altered. Extraction of flour with butanol did not reduce protein content, but had a marked aggregating effect on the flour particles. Both flour particle aggregation and reduction of flour protein content followed extraction of the soft wheat flour with petrol+acetone+methanol.

Air-classification of the pin-mill reground extracted Manitoba flours (Table V) showed that petrol alone had little

TABLE II

Air-classification of roller-milled flours from solvent-extracted and unextracted wheats and I Break grinds

Series	Treatment	Yield of air-classified fractions			Protein content[1]						
						Air-classified fractions					Total protein spread[3] %
		Fine %	Inter-mediate %	Coarse %	Whole flour %	Fine %	Inter-mediate %	Coarse %	Weighted average[2] %	Fine+Inter.[3] %	
Manitoba wheat											
I	Unextracted	1	9	90	13·3	16·1	10·4	13·6	13·3	11·0	4
	*P-extd. (18 days)	1	9	90	13·2	16·4	10·0	13·6	13·3	10·6	5
	P-extd. (99 days)	1	9	90	13·4	15·9	10·2	13·7	13·4	10·9	4
	P+A-extd.	1	12	87	13·3	16·1	9·8	13·6	13·2	10·5	6
	P+A+M-extd.	2	16	82	13·1	15·1	9·1	13·8	13·1	9·8	9
III	Unextracted	1	8	91	13·5	17·5	9·9	13·6	13·4	10·7	4
	P+A-extd.	1	9	90	13·4	14·7	9·7	13·6	13·3	10·3	4
	P+A+M-extd.	2	15	83	13·3	16·2	8·6	14·1	13·3	9·6	11
Soft White wheat											
	Unextracted	7	44	49	7·5	14·4	5·2	8·7	7·6	6·5	28
	P-extd.	8	39	53	7·4	14·2	4·9	8·4	7·4	6·4	27
	P+A-extd.	8	39	53	7·8	13·9	5·0	9·1	7·9	6·5	29
	P+A+M-extd.	6	36	58	7·7	11·8	5·2	9·3	8·0	6·1	27
Manitoba I Break grind											
	Unextracted	1	11	88	13·2	16·9	10·0	13·1	12·9	10·8	5
	P-extd.	1	10	89	13·3	16·4	10·7	13·6	13·3	11·4	4
	P+A-extd.	1	9	90	13·4	16·0	10·7	13·7	13·4	11·3	4
	P+A+M-extd.	1	6	93	13·6	15·3	11·0	13·6	13·5	11·5	2

*P = petrol, A = acetone, M = methanol
[1]14% moisture basis
[2]Weighted average of fine, intermediate and coarse fractions
[3]See text

TABLE III

Air-classification of pin-mill reground flours from solvent-extracted and unextracted wheats and I Break grinds

Series	Treatment	Flour m.c.* to pin mill %	Yield of air-classified fractions			Protein content[1]						Total protein spread[3] %	Decrease in coarse fraction[3] %
							Air-classified fractions						
			Fine %	Intermediate %	Coarse %	Whole flour %	Fine %	Intermediate %	Coarse %	Weighted average[2] %	Fine+Inter.[3] %		
Manitoba wheat													
I	Unextracted	14·7	10	42	48	13·3	17·8	9·8	15·0	13·1	11·4	20	46
	*P-extd. (18 days)	14·6	11	39	50	13·3	17·8	9·7	15·3	13·3	11·4	22	45
	P-extd. (99 days)	15·0	12	41	47	13·3	18·2	9·9	15·1	13·3	11·7	21	48
	P+A-extd.	14·4	14	47	39	13·1	18·2	9·6	15·3	13·1	11·6	25	55
	P+A+M-extd.	13·9	17	54	29	13·1	18·9	9·1	16·7	13·0	11·5	32	65
II[4]	Unextracted	11·3	12	45	43	13·3	18·9	10·1	15·0	13·5	12·0	22	52
	P-extd. (99 days)	11·2	12	44	44	13·3	18·5	10·0	15·1	13·3	11·9	22	52
	P+A+M-extd.	11·5	18	56	26	13·2	19·4	9·3	17·1	13·2	11·8	33	68
III	Unextracted	13·5	10	43	47	13·3	18·9	10·2	14·5	13·1	11·9	19	48
	P+A-extd.	13·6	13	43	44	13·3	18·9	9·7	14·8	13·1	11·8	22	51
	P+A+M-extd.	13·2	17	55	28	13·2	19·8	9·1	16·2	12·9	11·7	32	66
Soft White wheat													
	Unextracted	14·3	19	71	10	7·4	15·0	4·9	11·5	7·4	7·0	48	79
	P-extd.	14·3	21	67	12	7·4	14·6	4·1	10·4	7·0	6·6	55	78
	P+A-extd.	13·0	22	65	13	7·6	15·3	4·2	12·1	7·7	7·1	59	76
	P+A+M-extd.	12·5	23	66	11	7·8	15·4	4·6	10·9	7·8	7·4	54	82
Manitoba I Break grind													
	Unextracted	14·3	11	42	47	13·2	18·4	9·9	15·1	13·3	11·7	22	47
	P-extd.	15·0	12	41	47	13·2	17·9	9·8	15·2	13·3	11·6	22	47
	P+A-extd.	12·1	12	43	45	13·2	17·8	10·1	15·4	13·4	11·7	22	50
	P+A+M-extd.	13·0	7	30	63	13·6	16·6	10·6	15·1	13·8	11·7	15	32

*P = petrol, A = acetone, M = methanol
*m.c. = moisture content
[1]14% moisture basis
[2]Weighted average of fine, intermediate and coarse fractions
[3]See text
[4]Series II was a fresh pin milling of three flours of Series I

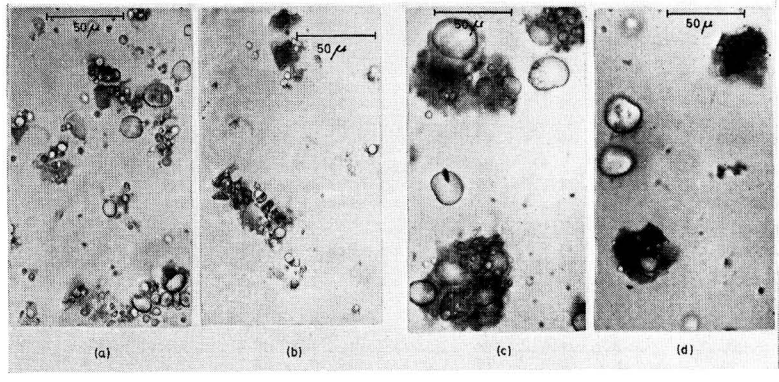

FIG. 1. *Photomicrographs of fine* (a, b) *and intermediate* (c, d) *air-classified fractions of flour milled from unextracted Manitoba wheat* (a, c) *and from the same after extraction with petrol, acetone and methanol successively* (b, d)

a and c show protein/starch 'clusters' with abundant small starch granules. In b and d, starch granules have been detached from the clusters to a greater extent than in a and c. Scale indicates 50 μ

TABLE IV

Characteristics and air-classification data on solvent-extracted and unextracted flours

Series[1]	Treatment of flour	Treated flour				Air-classification of treated, but not reground, flours							
						Yield of air-classified fractions			Protein content of air-classified fractions[2]				
		Moisture %	Fat P-extd.,[2] %	Fat ac.hyd.,*[2] %	Protein[2] %	Fine %	Intermediate %	Coarse %	Fine %	Intermediate %	Coarse %	Weighted average[3] %	Total protein spread[4] %
Manitoba flour													
IV	Unextracted	12·9	0·82		13·1	1	9	90	15·6	10·0	13·4	13·1	4
	*P-extd.	13·2	0·13		13·1	1	8	91	15·7	10·1	13·4	13·2	4
V	Unextracted	13·2	0·75	1·39	13·6	1	7	92	15·1	9·8	13·8	13·6	4
	P-extd.	13·2	0·06	0·78	13·7	1	7	92	14·9	10·2	13·8	13·6	3
	P+A-extd.	12·5	0·04	0·61	13·8	1	7	92	14·8	10·5	14·0	13·8	3
	P+A+M-extd.	12·5	0·05	0·45	12·7	1	6	93	12·6	9·5	12·7	12·5	3
VI	Unextracted	13·5	0·28	1·05	13·3	1	10	89	15·6	9·9	13·6	13·3	5
	P-extd.	12·6	0·04	0·74	13·3	1	8	91	16·1	9·9	13·7	13·4	5
	P+A-extd.	12·7	0·02	0·59	13·4	1	8	91	16·3	10·0	13·9	13·6	6
	P+A+M-extd.[5]	12·1	0·04	0·52	12·5	2	10	88	13·8	8·7	13·0	12·6	6
	B-extd.[5]	12·6	0·02	0·51	13·7	0	4	96	13·7	9·8	13·9	13·7	3
Soft White wheat													
	Unextracted	14·3	0·79	1·26	7·5	7	44	49	14·4	5·2	8·7	7·6	28
	P-extd.	13·8	0·55	0·70	7·5	9	43	48	14·1	5·0	8·3	7·5	28
	P+A-extd.	13·1	0·30	0·52	7·6	9	44	47	13·7	5·1	8·7	7·9	29
	P+A+M-extd.	12·0	0·20	0·45	7·1	3	31	66	9·7	5·2	7·8	7·0	16

*P = petrol, A = acetone, M = methanol, B = butanol
*ac.hyd. = acid hydrolysis
[1]Series IV, V and VI were prepared from three different stocks of Manitoba flour. Series VI samples were vacuum oven treated
[2]14% moisture basis
[3]Weighted average of fine, intermediate and coarse fractions
[4]See text
[5]Washed with acetone and petrol successively

effect; additional extraction with acetone increased endosperm fragmentation in two cases, while further additional extraction with methanol severely reduced endosperm fragmentation and protein shifting when residual solvent remained on the flour, but increased both fragmentation and protein shifting if the methanol was quickly washed off with acetone and petrol. Effects of solvent extraction of the soft wheat flour on fragmentation of endosperm on the pin mill were small, but generally in line with those on Manitoba flour. Butanol extraction of Manitoba flour had a protein-binding effect, despite the subsequent washing and vacuum-oven treatments.

Extraction of semolina

Petrol and butanol extraction of semolina at room temperature reduced the petrol-extractable fat content to negligible proportions and decreased acid hydrolysable fat content from 1·7% to 1·0% and 0·6%, respectively (Table VI). Methanol extraction, without subsequent washing, did not remove as much fat, but when followed by acetone and petrol washing, fat removal was similar to that by butanol. Treatment with butanol reduced protein content of the semolina by 0·1%; methanol treatment reduced it by 0·2%.

Degree of fragmentation of the roller-milled flours (Table VII) was slightly reduced by petrol extraction, by butanol extraction, and by extraction with methanol if the methanol was not rapidly washed off. Extraction with methanol, if this was quickly washed off, had little effect. Butanol treatment of the semolina markedly decreased degree of fragmentation of the endosperm in flour made by grinding the semolina on the pin mill.

Fragmentation of endosperm in the pin mill, when the primary (roller-milled) flours were reground, was slightly increased by treatment of the semolina with petrol or with methanol (if washed off), but was markedly reduced by butanol, and slightly reduced by methanol (not washed off)—see Table VIII.

It may be noted that treatment with butanol markedly reduced the protein content of the fine (high-protein) air-classified fractions of roller-milled and pin-milled primary flours (Table VII), and of pin-mill reground flour (Table VIII), whilst protein contents of the other air-classified fractions were not much altered.

Gluten quality

Normal recognisable gluten could be recovered from all flours milled from materials that had been treated with petrol or acetone only, and from flours similarly treated, but gluten could not be collected from any of the materials that had been treated with methanol or butanol if attempts had not been made to remove these solvents. When methanol or butanol-treated material was washed with acetone and petrol, or was vacuum-oven treated, recovery of gluten depended on the solvent, the material treated, and the method of removal, as shown in Table IX. Gluten obtained from methanol or butanol-treated material was invariably abnormal being short and lacking in cohesion.

Extensometer curves on 12-month-old flours milled from extracted or unextracted Manitoba wheat are shown in Fig. 2, and similar curves on freshly milled flours from two of the wheats in Fig. 3. The generally greater height (resistance to

TABLE V

Air-classification of pin-mill reground solvent-extracted and unextracted flours

Series[1]	Treatment of flour	Yield of air-classified fractions			Protein content[2]					Total protein spread[4] %	Decrease in coarse fraction[1] %
						Air-classified fractions					
		Fine %	Inter-mediate %	Coarse %	Whole flour %	Fine %	Inter-mediate %	Coarse %	Weighted average[3] %	Fine+Inter.[4] %	
Manitoba flour											
IV	Unextracted	11	41	48	13·1	18·3	9·8	14·4	13·0	11·7	20	47
	P-extd.	11	40	49	13·1	18·2	9·5	14·8	13·1	11·4	23	46
	P+A-extd.	13	45	42	13·1	18·0	9·8	15·2	13·1	11·6	23	54
	P+A+M-extd.	4	23	73	13·3	15·9	9·9	14·3	13·3	10·8	12	20
V	Unextracted	11	37	52	13·6	17·5	10·2	15·0	13·5	11·9	18	44
	P-extd.	12	37	51	13·5	17·7	10·2	15·3	13·7	12·0	19	45
	P+A-extd.	13	39	48	13·7	18·0	10·2	15·2	13·6	12·1	20	47
	P+A+M-extd.	9	29	62	12·7	14·4	9·1	14·6	12·9	10·3	17	34
VI	Unextracted	12	42	46	13·0	18·3	9·5	14·4	12·8	11·5	21	48
	P-extd.	12	41	47	13·0	18·4	9·3	14·5	12·9	11·5	22	48
	P+A-extd.	9	35	56	13·3	18·5	9·8	14·8	13·3	11·5	19	40
	P+A+M-extd.[5]	13	45	42	12·3	17·8	8·5	14·6	12·3	10·6	28	53
	B-extd.[5]	7	32	61	13·6	16·5	10·0	15·3	13·7	11·1	18	37
Soft White wheat												
	Unextracted	19	71	10	7·4	15·0	4·9	11·5	7·4	7·0	48	79
	P-extd.	22	67	11	7·3	15·1	3·7	10·2	7·0	6·6	61	78
	P+A-extd.	21	68	11	7·3	14·8	4·1	11·2	7·2	6·7	58	77
	P+A+M-extd.	18	65	17	7·1	12·4	4·6	10·3	7·0	6·3	44	75

*P = petrol, A = acetone, M = methanol, B = butanol
[1]Series IV, V and VI were prepared from three different stocks of Manitoba flour. Series VI samples were vacuum oven treated
[2]14% moisture basis
[3]Weighted average of fine, intermediate and coarse fractions
[4]See text
[5]Washed with acetone and petrol successively

TABLE VI

Characteristics of solvent-extracted and unextracted semolina, and of flours milled therefrom

Series[1]	Treatment of semolina	Treated semolina				Yield of flour %	Milled flour			
		Fat, P-extd.,[2] %	Fat, ac.hyd.,*[2] %	Protein,[2] %	Moisture (when milled) %		Moisture %	Fat, P-extd.,[2] %	Fat, ac. hyd.,[2] %	Protein,[2] %
Roller-milled										
VII	Unextracted	0·91	1·67	11·6	12·4	77·3	12·3	0·71	1·46	11·1
	P-extd.	0·07	0·98	11·6	11·5	82·9	11·8	0·13	0·88	11·4
	M-extd.[4]	0·35	1·06	11·4	11·2	81·7	11·2	0·22	0·85	11·0
	B-extd.[3,4]	0·03	0·62	11·5	11·9	78·9	11·9	0·08	0·52	11·1
VIII	Unextracted	0·91	1·67	11·6	14·0	85·7	13·6	0·51	1·18	11·3
	M-extd.	0·18	0·80	11·5	14·1	83·8	13·5	0·24	0·78	11·0
	M-extd.[3]	0·07	0·65	11·4	14·2	84·2	13·4	0·11	0·74	11·0
Pin-milled										
VII	Unextracted	0·91	1·67	11·6	12·4	87·4	11·5			11·7
	P-extd.	0·07	0·98	11·6	11·8	88·0	11·2			11·5
	M-extd.[4]	0·35	1·06	11·4	11·0	88·0	10·8			11·5
	B-extd.[3,4]	0·03	0·62	11·5	11·8	87·4	11·1			11·5

*P = petrol, M = methanol, B = butanol
*ac. hyd. = acid hydrolysis
[1]Series VII and VIII were milled at different moisture contents, as shown
[2]14% moisture basis
[3]Washed with acetone and petrol successively
[4]Vacuum-oven treated

TABLE VII

Air-classification of flours milled from solvent-extracted and unextracted semolina

Series[1]	Treatment of semolina	Air-classified fractions: Yield Fine %	Yield Intermediate %	Yield Coarse %	Protein[2] Fine %	Protein[2] Intermediate %	Protein[2] Coarse %	Total protein spread %
Roller-milled								
VII	Unextracted	2	14	84	15·3	7·6	11·6	9
	P-extd.	2	11	87	14·9	8·1	11·8	7
	M-extd.[4]	2	10	88	13·2	7·7	11·3	6
	B-extd.[3,4]	1	8	91	11·3	8·3	11·3	3
VIII	Unextracted	2	11	87	15·1	7·7	11·7	7
	M-extd.	1	9	90	12·6	7·7	11·3	5
	M-extd.[3]	2	12	86	12·6	8·5	11·4	6
Pin-milled								
VII	Unextracted	9	42	49	17·7	8·9	12·6	19
	P-extd.	12	37	51	16·8	8·0	12·5	21
	M-extd.[4]	10	38	52	15·7	8·2	12·8	20
	B-extd.[3,4]	6	25	69	13·0	8·0	12·4	14

*P = petrol, M = methanol, B = butanol
[1]Series VII and VIII were milled at different moisture contents, as shown
[2]14% moisture basis
[3]Washed with acetone and petrol successively
[4]Vacuum-oven treated

TABLE VIII

Air-classification of pin-mill reground flours milled from solvent-extracted and unextracted semolina

Series[1]	Treatment of semolina	Moisture content of flour to pin mill %	Air-classified fractions: Yield Fine %	Yield Intermediate %	Yield Coarse %	Protein[2] Fine %	Protein[2] Intermediate %	Protein[2] Coarse %	Protein[2] Weighted average[5] %	Protein[2] Fine+Inter. %	Total protein spread %	Decrease in coarse fraction on pin-milling %
Primary grinding on roller mill												
VII	Unextracted	12·9	12	48	40	17·1	8·2	12·3	10·9	10·0	23	52
	P-extd.	12·9	15	45	40	17·2	7·8	12·4	11·0	10·1	26	54
	M-extd.[4]	12·0	12	45	43	15·0	7·9	12·5	10·7	9·4	23	51
	B-extd.[3,4]	12·1	10	36	54	13·7	8·1	12·6	11·1	9·3	20	40
VIII	Unextracted	13·6	12	47	41	17·0	8·1	12·3	10·9	9·9	24	52
	M-extd.	13·5	11	43	46	14·7	7·7	12·6	10·7	9·1	24	48
	M-extd.[3]	13·4	13	49	38	15·3	7·9	13·0	10·8	9·5	26	56
Primary grinding on pin mill												
VII	Unextracted	12·3	16	55	29	17·5	8·7	12·8	11·3	10·6	25	40
	P-extd.	12·2	17	52	31	17·8	8·2	13·0	11·3	10·6	28	40
	M-extd.[4]	12·2	14	53	33	16·7	8·5	13·2	11·2	10·2	25	37
	B-extd.[3,4]	12·2	10	37	53	13·9	8·5	13·2	11·5	9·6	20	23

*P = petrol, M = methanol, B = butanol
[1]Series VII and VIII were milled at different moisture contents, as shown
[2]14% moisture basis
[3]Washed with acetone and petrol successively
[4]Vacuum-oven treated
[5]Weighted average of fine, intermediate and course fractions

stretching) of the Fig. 2 curves in comparison with those of Fig. 3 is a consequence of the long time interval between milling and testing. The extensometer results confirm that petrol and acetone had relatively small effects on physical characters of the gluten, and that methanol had a marked shortening effect.

Discussion

Two effects of solvents on wheat endosperm are relevant to the study of endosperm fragmentation: a direct effect of solvent on the protein; and removal of lipid or lipoprotein, which may have an indirect effect on physical characteristics of the protein.

TABLE IX

Summary of effects of solvent extraction on endosperm fragmentation and gluten recovery

Solvents used*	Solvent removal method		Materials extracted									
			Whole grain (Manitoba)		I. Bk. grind (Manitoba)		Flour (Manitoba)		Semolina (Mixed grist)		Whole grain (Soft White)		Flour (Soft White)	
P			o	G	o	G	o	G	o	G	–	G	o	G
P+A			+	G	o	G	+	G			–	G	o	G
P+A+M			++	X	– –	X	– – –	X			– –	X	– –	X
P+A+M	A+P washing		++	g										
P+A+M	A+P washing	Vacuum-oven					+	g						
M									–	X				
M	A+P washing								+	X				
M		Vacuum-oven							–	g				
B								X						
B	A+P washing	Vacuum-oven					– – –	g	– – –	X				

*P = petrol, A = acetone, M = methanol, B = butanol

Key to symbols:

Endosperm fragmentation:
o: no effect on fragmentation
+, ++: degrees of increased fragmentation
–, – –, – – –: degrees of decreased fragmentation

Gluten recovery:
G: normal gluten recovered
g: short non-cohesive gluten recovered
X: no gluten recoverable

Solvent effect on protein

As judged by their ability to yield recognisable gluten by washing the starch out of a dough, the materials treated with petrol or acetone contained protein that had been unaffected by the solvents.

Both methanol and aqueous butanol had deleterious effects on the protein to such an extent that recognisable gluten could not be recovered from materials treated with either of these solvents if steps (other than aeration) had not been taken to remove the solvent. Muller *et al.*[4] also found that addition of methanol to dough modified the physical properties of the dough, and that flour was irrevocably damaged when extracted with butanol. They considered that the effect of alcohols was due to protein denaturation. Grosskreutz[10] on the other hand believed that the effect of butanol on gluten was primarily due to removal of lipids.

When attempts were made to remove residual solvent, the deleterious effects of methanol and butanol on the proteins were mitigated, and appeared to be related to the length of time during which the solvents were allowed to act on the materials; this, in turn, depended on the relative ease of removal of the solvents from the treated material, which varied according to the solvent, the particle size of the material, and the method of removal employed. Removal of butanol was more difficult than removal of methanol; material of coarse particle size, e.g., semolina, was freed from

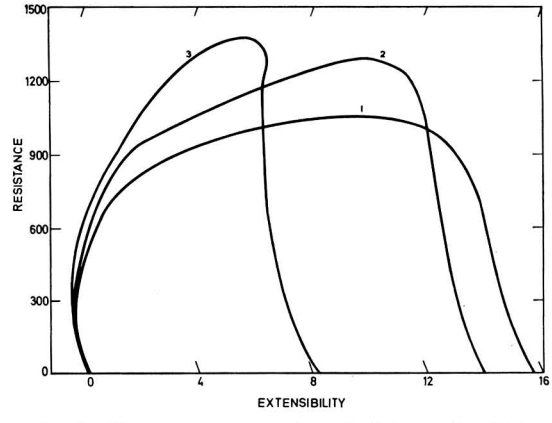

FIG. 2. *Extensometer curves on* 12*-month-old flours roller-milled from unextracted Manitoba wheat* (1) *and from the same after successive extractions with petrol and acetone* (2), *or with petrol, acetone and methanol* (3)

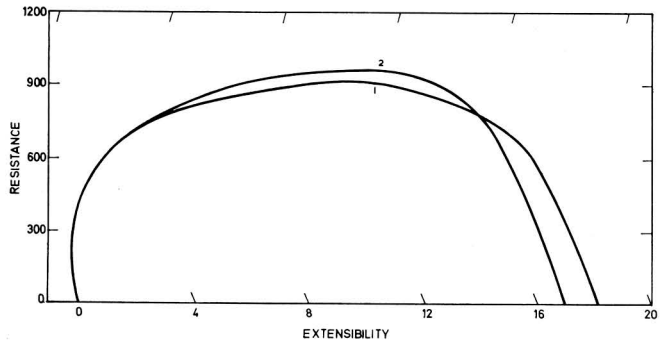

FIG. 3. *Extensometer curves on flours freshly milled from unextracted Manitoba wheat* (1) *and from the same after successive extractions with petrol and acetone* (2)

traces of solvent less easily than material of finer particle size, e.g., flour. Thus, traces of methanol and butanol were removed from flour fairly well by washing it with acetone and petrol, followed by vacuum-oven treatment. Thereafter, gluten could be collected from the dough in a recognisable form. Mecham & Mohammad[3] found that butanol removed more fat from flour than any other solvent, that after such treatment gluten could still be washed out, and that the gluten was elastic but less extensible than the control. In the present work, however, the gluten collected from methanol- or butanol-treated flour was not elastic. It was extremely short, and tore into fragments when extended.

When semolina was treated with the same solvents, washing with acetone and petrol was ineffective in removing either methanol or butanol; vacuum-oven treatment successfully removed methanol, but even this treatment did not remove butanol sufficiently well to prevent the deleterious effect of residual butanol on the protein, which made it impossible to collect recognisable gluten from the milled flour. As regards endosperm fragmentation, the direct effects of methanol and butanol on the proteins were revealed as an apparent hardening of the endosperm, leading to markedly reduced fragmentation during grinding, and reduced protein shifting during air-classification.

Solvent extraction of lipids

Successive treatment with petrol and acetone removed lipid from wheat endosperm without adversely affecting the protein. As treatment with petrol+acetone led to some increase in endosperm fragmentation, it may be concluded that the effect on fragmentation was the consequence of lipid removal rather than of the direct effect of the solvent on endosperm protein.

Additional treatment with methanol removed more lipid, the endosperm of Manitoba wheat extracted with petrol+acetone+methanol broke down more readily, and the milled flour—both roller-milled and pin-mill reground—showed a markedly increased degree of fragmentation. These effects were similar to those of petrol+acetone extraction, but greater in degree, and appear to be the consequence of removal of lipid rather than of a direct effect of methanol on the protein; in any case, the effect of methanol on the protein was not sufficiently severe to prevent the washed-out gluten cohering as a recoverable ball.

When flour was treated with methanol, which was rapidly removed by being washed off with acetone and petrol and by vacuum-oven treatment, again lipid was removed, endosperm breakdown was increased, and cohering gluten could be recovered. The trials in which treatment with methanol resulted in decreased endosperm fragmentation and inability to recover gluten were those in which residual methanol was not removed from the material, and in which, presumably, the deleterious effect of residual methanol on the protein, acting over a considerable period of time, obscured the protein-loosening effect of lipid removal.

Location of lipid in endosperm

If removal of lipid weakens the mechanical structure of endosperm, the precise effect produced should vary according to the location of the lipid material removed. Location might be in the wedge protein, or in the adherent protein, or at the starch/protein interface, i.e., surrounding each starch granule. Location in the protein itself might be expected to lead to increased fragmentation of larger endosperm particles without necessarily alteration of the character of the finer fragments, whereas location at the starch/protein interface might lead to an increased degree of separation of protein from starch, shown, e.g., by particles of protein having fewer starch granules attached, and by starch granules having less adhering protein.

Hess[11] reported lecithin contents of haft (adherent) protein and zwickel (wedge) protein as 45·0% and 3·24% (protein basis), respectively. Rohrlich & Niederauer[12] found 13·1% of lipoprotein in adhering protein, 2·5% in wedge protein separated from flour that had been extracted with methanol.

Traub[13] stated that wheat protein appears to be intimately bound with a portion of the flour fat; Traub *et al.*[14] concluded, from the results of *X*-ray studies of wheat endosperm, that the protein fibres in endosperm are crosslinked by layers of phospholipid, with the fat molecules roughly perpendicular to the protein fibres. Hess[15] found that the haft protein appeared under the electron microscope to consist of a network of fibrils about 100 Å thick. Comparison of electron micrographs of specimens of haft protein treated with mild fat solvents and untreated specimens indicated the presence of a layer of extractable fat covering the fibre network. Thickness of the fat layer around the starch granules was reported to be 380 Å. Chiang *et al.*[16] presented results which supported the hypothesis that an adhesive protein or lipoprotein layer exists on the surface of intact starch granules. Seckinger & Wolf (personal communication), from electron microscopy studies, concluded that free lipids were distributed throughout the matrix protein, and that bound lipids were present in matrix protein as randomly distributed inclusions extending up to the surface of the starch granules. They found no separate lipid-rich fibrous protein.

There is, thus, evidence that lipid in wheat endosperm is located partly in the protein and partly at the starch/protein interface. Evidence from endosperm fragmentation in the present study is consistent with this view, as both increased fragmentation of large particules and increased separation of protein from starch were observed in, e.g., the case of flour milled from Manitoba wheat that had been treated with petrol+acetone+methanol.

In the present work, it was found that the effect of butanol treatment of semolina in reducing the degree of endosperm fragmentation was much more marked when the semolina was milled on the pin mill than when milled on the roller mill. This suggests that the effect is more akin to a reduction in brittleness, i.e., an effect on the protein, than to a decrease in plasticity,[17] a possible consequence of lipid removal if the lipid present in the protein functions as a lubricant to the movement of the protein molecules relative to one another.

Stringfellow & Pfeifer[18] prepared a low-protein (8·1%) air-classified fraction of flour from Hard Red Winter wheat and extracted this with hexane or with aqueous butanol. The treated flours were then pin-milled and air-classified. Treatment with hexane did not improve fractionation response, whilst treatment with butanol was extremely deleterious and reduced protein shifting from 41·5% (in the untreated controls) to 14·7%. These results are similar to those reported here for the effects of petrol and of butanol on flour.

The effect of methanol treatment of whole-grain Manitoba wheat (markedly increasing endosperm fragmentation) contrasts sharply with the effect of similar treatment of Manitoba I Break grind and flour, mixed grist semolina, and Soft White whole-grain wheat and flour (hardening of the endosperm and a decrease in fragmentation). The difference is probably associated with varying degrees of accessibility of endosperm protein to the solvent: endosperm in I Break grind, semolina and flour is exposed and, furthermore, a proportion of the cells comprising the endosperm is to some extent damaged during preparation of these materials, and the cell contents are made accessible to the solvent; in the case of the Soft White whole grain wheat, the endosperm, although intact, may still be accessible to the solvent on account of its open mealy texture. On the other hand, the close vitreous texture of the endosperm of Manitoba whole grain wheat may hinder accessibility of solvent to the endosperm protein.

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Herts.

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5α-ANDROST-16-ENE-3-ONE:—COMPOUND RESPONSIBLE FOR TAINT IN BOAR FAT

By R. L. S. PATTERSON

The volatile compounds stripped from boar, hog and gilt fats under conditions of high vacuum and elevated temperature have been subjected to gas chromatographic and olfactory analysis. The compound primarily responsible for the taint of boar fat has been isolated and identified by mass spectrometry as 5α-androst-16-ene-3-one. It has an unpleasant odour of perspiration and is detectable in most boars of 200 lb liveweight and over but not in hogs or gilts.

Introduction

The undesirable odour of heated fat from an entire adult male pig (boar) has been a problem facing the pig industry for many years and has been the subject of a number of reports in the literature.[1-4] Chemical analyses have been unsuccessful and the compounds (or compound) responsible have not been identified or even located by the analytical procedures used. Williams & Pearson[5] reported that gas chromatographic analysis of the non-saponifiable residue from boar fat, in which the taint or sex-odour could be detected subjectively quite easily by smell, showed no differences from barrow (hog) fat treated in the same manner in which no taint was detectable.

A further attempt has been made to identify the odorous compounds responsible, and the results reported in this paper have been obtained by a re-examination of the complex mixture of volatile compounds which can be isolated directly from heated pork fat under high-vacuum conditions.

Experimental

Materials

The present quest into the nature of boar odour has been directed from the standpoint of food quality rather than from other aspects of the problem related to the physiology of the pig. Consequently, fat used for examination was cut from parts of the carcass which would normally be used for human consumption in the form of bacon or pork. Fat samples from the shoulder, back or rump areas of a boar seemed to differ little from one another in the intensity of the odour produced when they were tested by being boiled in water or warmed slowly in the dry state. Similarly, kidney fat was found to possess the same odour or taint although this was frequently accompanied by other odours. Whenever possible, at least three people, but frequently more, were called upon to evaluate the odour of a sample. This was necessary because there appears to be a considerable variation in the capacity of individuals to detect boar taint. Furthermore, since much of the analytical procedure depended upon olfactory detection, multiple judgements were desirable to compensate for day-to-day fluctuations of olfactory sensitivity.

The taint, which has been variously described as being like urine, perspiration or onions in nature,[1,6] was detectable in a number of boars of 200 lb liveweight and, on occasions, in younger animals. Older boars exhibited the taint to a more marked degree, and fat samples from animals of 2 years and upwards were tested as potential sources of strong taint. Six were selected, of which two were exceptionally odorous and were used for the purpose of isolation and identification of the odorous compound. They were obtained from Large White boars, one 3 years old, liveweight approximately 650 lb and the other 3½ years old, liveweight approximately 520 lb. Three hogs and two gilts were used as untainted controls, and fat samples from these animals were examined in the same manner as those from the boars.

Kidney, rump and back fat, freed from skin and other connective tissue as far as possible, was cut into strips and chilled to −10°. The strips were minced while still frozen, and approximately 250 g of the comminuted fat was melted slowly by being warmed in a flask to a temperature of 55°. No particular precautions were taken to exclude atmospheric oxygen because it was intended that the experiment should parallel normal domestic handling conditions as far as possible, since the effect of oxygen on the production of the odour was not known.

To remove connective tissue, the melted fat was filtered through layers of butter muslin supported in a warmed Büchner funnel. The fat was cloudy after filtration, and anhydrous sodium sulphate was added to remove water. A second filtration under a water-pump vacuum through a clean warmed Büchner funnel containing two Whatman No. 541 filter papers removed the sodium sulphate and yielded a clear colourless oil. A second treatment with anhydrous sodium sulphate was necessary if the fat sample was particularly wet. The volume of fat (150 ml) required for use in the fat stripper was stored at −10° until required.

Isolation of fat volatiles

The fat stripper, shown in Fig. 1, was based on a design by C. Weurman (personal communication). It consisted of a separating funnel (A), with an extended stem, fitted with a high-vacuum greaseless diaphragm valve (B) and a pressure equalising side-arm and stopcock (C), leading down through a still-head terminating 1 cm above the top coil of a 30 cm coil condenser erected in a vertical position (D). A round-bottomed flask was fitted to the lower end of the condenser, and the assembly was supported on a cork ring mounted on a lab-jack which permitted vertical adjustment of the stripper to facilitate alignment with the cold trap and high-vacuum system.

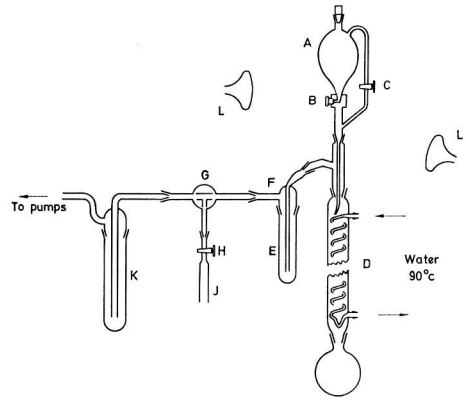


FIG. 1. *Fat stripper*

The small liquid-nitrogen cold trap (E) was made by suitably extending the inlet tube of a receiving adapter to fit a test-tube used as the trap-body and by replacing the standard vacuum connexion with a B19 socket (F). This socket connected the stripper to the high-vacuum system by means of the 3-way stopcock (G) which also permitted controlled re-admission of the atmosphere through stopcock (H) and the calcium chloride guard tube (J). The high-vacuum unit consisted of an oil diffusion pump backed by a rotary oil pump, capable of reducing the pressure in the system to less than 10^{-4} mm Hg. A second larger liquid-nitrogen cold trap (K) was positioned between the pumps and the 3-way stopcock to prevent back diffusion of pump oil into the stripper.

With stopcocks (B), (C) and (H) closed to the atmosphere, the system was evacuated and checked for air leaks. The 150 ml of liquefied fat was poured into the reservoir (A), a stopper was inserted and stopcock (C) in the pressure equalising side-arm was opened slowly to evacuate the reservoir and de-gas the fat. The fat was prevented from solidifying in the reservoir, diaphragm valve and stem by heat from two infra-red lamps (L). Hot water at 90° was circulated through the condenser coil by a small pump fitted to a thermostatically controlled water bath.

The diaphragm valve (B) was opened slowly until a steady flow of fat was obtained. The rate of flow was controlled by the valve so that one drop of liquefied fat impinged on the top of the hot coil every 8 to 10 seconds. Each drop, heated immediately to 90°, spread out in a thin film and spiralled down the coil, finally dropping into the flask at the bottom. A faster drip-rate resulted in merging of the drops and flooding of the coil.

Components in the fat which were volatile under these conditions were drawn off through the side arm of the still-head and trapped in the liquid-nitrogen cold trap (E). The volatiles were removed from the trap with the minimum quantity (3 ml) of purified diethyl ether (carbonyl- and peroxide-free), and the solution was dried with anhydrous sodium sulphate before concentration to approximately 0·25 ml prior to gas chromatographic analysis.

Gas chromatography

A Pye Series 104 gas chromatograph was employed to separate the mixture into its components which were detected by a flame ionisation detector. The odour of each compound (or coincident compounds) producing a chromatographic peak was evaluated as it was eluted from the column by extinguishing the flame of the detector after the apex of the peak had been recorded and smelling the effluent. Continuous olfactory examination of the effluent of the chromatograph during duplicate analyses provided an important supplementary means of detecting odorous compounds which were not present in sufficient quantity to produce a response in the flame detector and hence a peak on the chromatogram. The odour recognised in the fat as boar taint was first located in the effluent of the gas chromatograph in this way although no peak was observed on the recorder chart.

A number of columns containing packings of different properties were tested at various temperatures in an attempt to achieve maximum resolution of the numerous components and to produce an observable peak for boar taint on the chromatogram. Suitable stationary phases were 3% QF-1 on Aeropak-30, 5% silicone elastomer '30' (SE-30) on Chromosorb G (acid-washed and dimethyldichlorosilane-treated) and 5% phenyldiethanolamine succinate (PDEAS) on Chromosorb G. The initial work directed towards locating the odour and its corresponding peak was carried out on a 3 ft × 4 mm i.d. glass column packed with 3% QF-1 operated at 151° and flow rate of 60 ml/min argon. The peaks from 20 μl injections of the concentrated volatiles stripped from boar fat were compared with those from the samples of hog and gilt fats prepared in an identical manner. There was a pronounced 'taint' odour accompanying the peak indicated by the letter T in Fig. 2, which was completely absent from the peak with slightly greater retention recorded in the chromatograms of the volatiles stripped from both hog and gilt fats. The marked presence of the taint odour in boar fat samples and the complete absence of the odour in the corresponding part of the chromatogram for hog and gilt fats was checked by extinguishing the flame of the detector and smelling the effluent continuously over a period of time encompassing the elution of these peaks.

Column packings consisting of 5% SE-30 and 5% SE-30 plus 5% PDEAS were developed to give improved resolution of the mixtures and to facilitate trapping of the peak responsible for taint. The column which proved to be most satisfactory was a 3 ft × 4 mm i.d. glass column packed in two sections containing 5% SE-30 on 80/100 mesh Chromosorb G and 5% PDEAS on 80/100 mesh Chromosorb G in the ratio of 5:2 by length. The column was operated at a temperature of approximately 175° with an argon carrier gas flow of approximately 60 ml/min. Under these conditions, the component responsible for the characteristic odour was eluted as a single compound of high molecular weight with a retention time of ~40 min.

Gas chromatography trapping procedure

It was apparent that the quantity of material involved was very small and that the trapping of the peak concerned could be carried out effectively on a modified 'analytical-scale' g.l.c. instrument. The modified instrument was a Pye Argon chromatograph converted to take U-shaped columns by removal of the aluminium plugs, normally inserted in the central tube which is surrounded by the heating element.

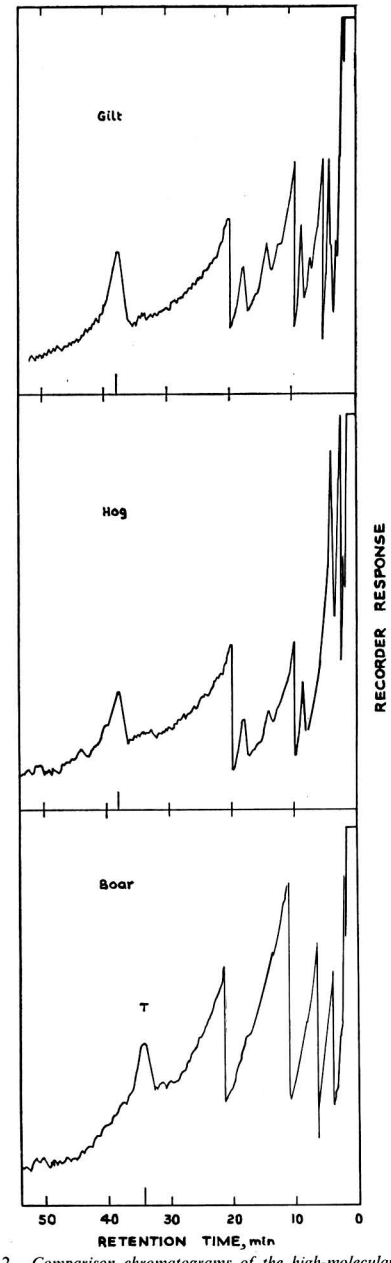


FIG. 2. *Comparison chromatograms of the high-molecular-weight components stripped from gilt, hog and boar fats*

The peak marked T possessed the odour of boar taint. Chromatographic conditions: 3 ft. column of 3% QF-1 on Aeropak-30 at 151°C.

The injection system fitted was the standard type supplied for the Pye Series 104 instruments. A flame detector was mounted on the top of the unit and coupled to the tapered end of the column by a short length of 0·107 in. i.d. Teflon tubing which allowed quick removal of the detector and easy connexion of a trap. A splitter was not used to enable the detector to be operated while trapping was in progress as the concentration of compound in the effluent was so low that a considerable proportion of the materials would have been required to pass through the detector to produce a response, consequently reducing the quantity available for trapping.

The 3 ft × 4 mm i.d. U-shaped column was filled sectionally in the ratio of 5:2 with the 5% SE-30 and 5% PDEAS packings and operated at 180° with an argon gas-flow of approximately 60 ml/min. The use of a 4 mm. i.d. column rather than a 'preparative-scale' column of larger diameter was an advantage because no loss of column efficiency was incurred. Under these conditions, the peak for boar taint was eluted with a retention time similar to that on the 104 instrument.

The type of trap used consisted of a 6·5 cm length of 2·5 mm i.d. Pyrex glass tubing, tapered slightly at one end to fit 0·107 in. i.d. Teflon tubing and partly filled with 0·177 mm diameter glass beads. The beads were retained by small plugs of glass wool. A 25 mm length of tubing filled with beads was found to be sufficient to give a trapping efficiency of approximately 95% without the pneumatic resistance of the beads reducing the carrier gas flow by more than 1·5 ml/min.

When the desired peak emerged from the column, the detector was removed and the trap was fitted to the end of the column by the Teflon tubing. During the 5 min that it was in position, no cooling was applied other than a cold air-stream from a hand-held electric blower directed at the base of the trap. The beads 'filtered' the column effluent very efficiently, virtually removing all odour. By this method, the major problem of loss by fog formation during trapping, resulting from the rapid cooling of small amounts of vapour in a moving gas-stream, was avoided.

Replicate injections (20 μl) of the concentrated solution of volatiles were made until, after six or more injections the intensity of odour breaking through at the outlet of the trap indicated that the trap had become saturated. The trapped material was washed out of the trap by introduction of ether which was allowed to percolate down through the beads and glass wool into a small tapered collection tube (capacity 0·5 ml). Approximately 0·3 ml purified ether was required to remove the odorous compound from the trap. Control traps were attached to the outlet of the column for a period equivalent to the time that the sample trap was in position, in order to determine the contribution of stationary phase bleed from the gas chromatography column to the mass spectrum recorded for the trapped compound.

Mass spectrometry

The mass spectrum was obtained by use of an A.E.I. M.S.9 instrument. The ether solution of the trapped compound was concentrated to 100 μl, transferred to the tip of the direct-insert probe and, after the ether had evaporated, the low resolution spectrum was recorded. The high resolution spectrum of the molecular ion peak was recorded on another sample which enabled calculation of the exact mass number of the molecular ion and hence the exact molecular weight and formula of the compound.

Results and Discussion

Fig. 2* shows chromatograms obtained on the QF-1 column at 151° comparing the high-molecular-weight components stripped from gilt, boar and hog fats. The peak in the boar fat volatiles eluted consistently earlier than the associated peak in the hog and gilt samples and possessed the sharp odour of perspiration associated with boar fat, whereas neither of the other peaks had a detectable odour.

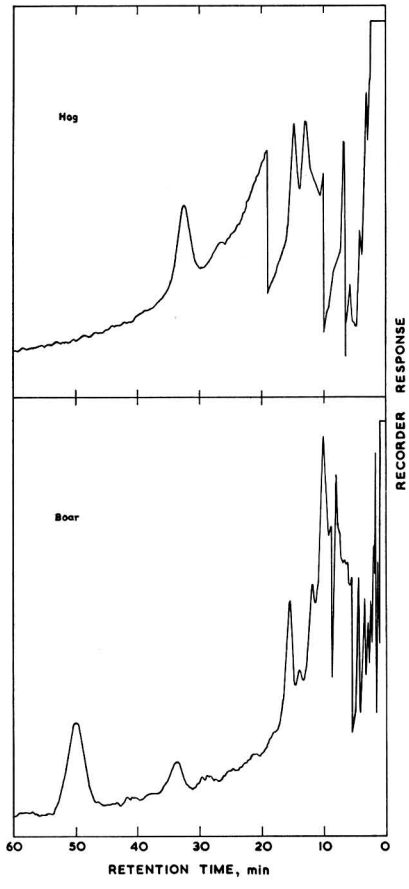


FIG. 3. *Comparison chromatograms of the high-molecular-weight components stripped from hog and boar fats showing the additional peak for boar taint at 50 min retention*

Chromatographic conditions: 3 ft sectional column of 5% SE-30 and 5% PDEAS on Chromosorb G at 174°C.

Fig. 3 shows the pattern for boar and hog fat when the volatiles were chromatographed on the 5:2 SE-30:PDEAS column at 174°. The last major peak in the boar chromatogram is the taint compound which is absent in the hog sample.

The chromatograms in Fig. 4 demonstrate the similarity of the volatiles stripped from equal volumes (150 ml) of fat from two parts of the same boar, namely back fat surrounding the eye muscle and kidney flare fat. The size of the peak for the taint compound is approximately the same in both samples and is considerably larger than that normally obtained. This particular boar possessed the greatest degree of taint found and these samples were used for the isolation and identification of the odorous compound.

A temperature-programmed run (Fig. 5) on the same kidney fat sample shows the complexity of the complete mixture of volatiles with at least 60 components resolved. It is quite

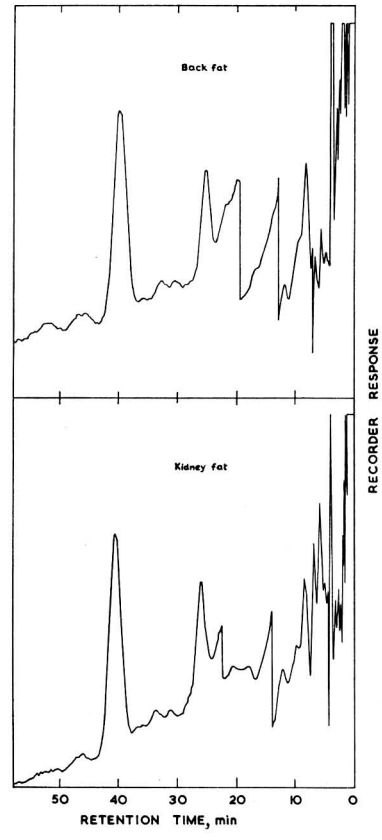


FIG. 4. *Comparison chromatograms showing the similarity of the high-molecular-weight components stripped from the back fat and kidney fat of the same boar*

Peak for boar taint at 40 min retention. Chromatographic conditions: 3 ft sectional column of 5% SE-30 and 5% PDEAS on Chromosorb G at 178°C

* It should be noted that the *vertical* lines, forming the right hand boundaries of *apparently* tailing gas chromatographic peaks, are due to alterations in the attenuation of the amplifier and are *not* genuine responses of the flame detector to changes in the composition of the carrier gas

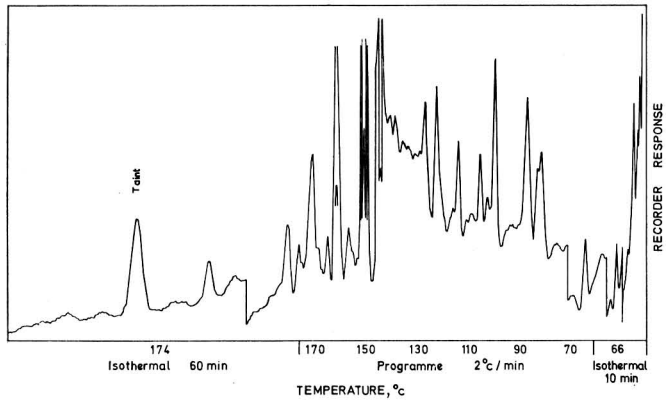


FIG. 5. *Temperature-programmed chromatogram of volatile compounds stripped from boar fat showing relative position of taint compound*

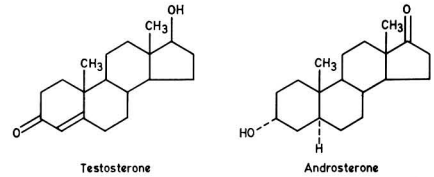
Chromatographic conditions: 3 ft sectional column of 5% SE-30 and 5% PDEAS on Chromosorb G. Temperature programme as shown.

probable that some of the apparently single peaks are composite but the slow temperature programme rate of 2°c/min from 66° to 174° did not alter the symmetrical shape of the taint peak recorded during the faster isothermal runs. The peak was therefore assumed to be homogeneous.

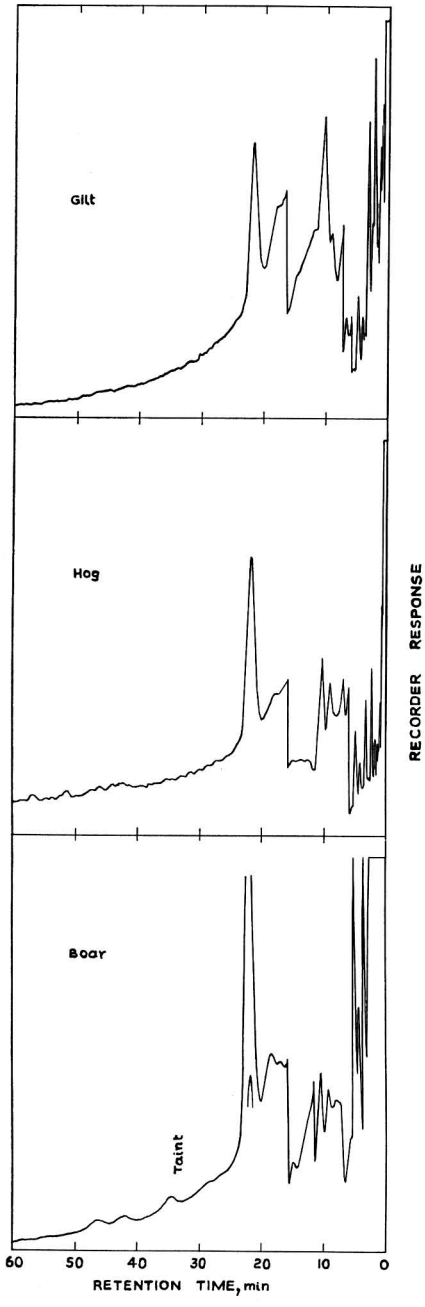
The relative position of the taint component on the chromatogram may be considered in relation to the other volatiles. The normally low concentration and long retention, resulting in it being among the last of the compounds to be eluted, explains why the compound has eluded chromatographic detection until the present time. Furthermore, probably complete reliance was placed upon conventional gas chromatographic detectors as the means of detection without recourse to complementary olfactory analysis of the effluent gas-stream. The fact that the odour can be detected in steam rising from boiling water containing pieces of boar fat tends to lead to the conclusion that the component is 'quite volatile', a description which cannot be applied with accuracy to a compound exhibiting these retention characteristics. This situation has arisen because of the very low threshold of detection of the compound of which relatively few molecules are required for stimulation of the olfactory receptor mechanism. As an example, Fig. 6, shows comparison chromatograms of fats from litter-mate gilt, hog and boar pigs slaughtered at 200 lb liveweight; the very small peak or undulation on the base-line was the only response produced by the flame detector for the taint component, but the accompanying odour in the effluent carrier gas was of considerable intensity. There was neither a detector response nor a detectable odour at the corresponding parts of the hog and gilt chromatograms. It must therefore be stressed that although no response is obtained from a flame detector for the taint compound, considerable odour may still be detectable in the effluent gas-stream.

The mass spectrum of the isolated compound is shown in Fig. 7a. The spectrum was more complex when first recorded but subtraction of the background spectrum of the mass spectrometer and the spectrum of the stationary phase bleed from the SE-30–PDEAS column, resulted in the above spectrum (Fig. 7a). The molecular ion peak M^+, at *m/e* 272 was the base peak of the spectrum. The intense peak at *m/e* 257 showed that elimination of a methyl group from the molecule (M-15) was a predominant reaction. Exact mass measurement of the parent ion peak gave a value of 272·2137, agreeing well with 272·2140 for the formula $C_{19}H_{28}O$. The taint compound therefore possessed a molecular weight of 272 and the formula $C_{19}H_{28}O$.

The elimination of taint by castration of male pigs implies that production of the odour is related to the presence of male sex hormones in the body. The formula for the taint compound, $C_{19}H_{28}O$, differs only from the formulae for testosterone, $C_{19}H_{28}O_2$, by a single oxygen atom and for androsterone, $C_{19}H_{30}O_2$, by the elements of a molecule of water. The remaining single oxygen atom in the taint molecule was shown to be present as a carbonyl group by reaction of the compound with 2,4-dinitrophenylhydrazine which completely eliminated the characteristic odour. The structures of testosterone and androsterone are shown below:



Some ketosteroids of the androstane series are described in the literature as possessing urine-like odours.[7] These compounds include 5α-androstan-3-one, 5α-androst-2-ene-17--one and/or 5α-androst-3-ene-17-one (especially when hot), 5α-androst-16-ene-3-one and 5β-androst-16-ene-3-one. The last compound is reported as having a distinct urine-like odour but which is weaker than that of the 5α-epimer which is described as intense.



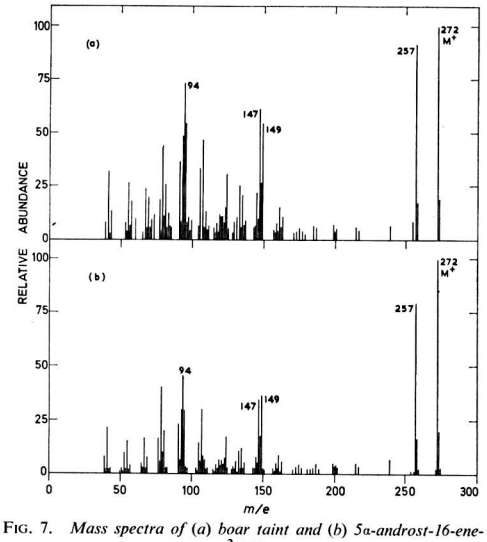


FIG. 7. *Mass spectra of (a) boar taint and (b) 5α-androst-16-ene-3-one*

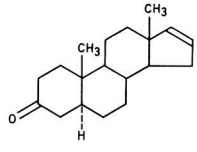
Olfactory examination of a number of monoketoandrostanes and -androstenes confirmed that several possessed the same characteristic smell which was identical in quality but not necessarily in intensity to the odour of the experimental material isolated from the boar fat. For example, the odour of 5α-androstan-3-one was strong whilst that of the 5β-epimer was weak; androst-4-ene-3-one possessed a strong odour; androst-4,16-diene-3-one had a strong odour whilst that of androst-3,5-diene-17-one was weak. Odourless compounds included 5α-androstan-17-one, 5α-androst-2-ene-17-one and 5α-androstan-11-one. It therefore appeared that the strongest odours were associated with compounds containing a carbonyl group at C_3 and the hydrogen atom at C_5, if present, in the α-orientation.

The formula established for the experimental compound, $C_{19}H_{28}O$, requires the presence of one double bond and one carbonyl group, probably at C_3, in the androstane molecule. Compounds with more than one double bond can be deleted as their molecular weight is less than 272. The simplest derivative of testosterone to conform to these requirements and possess the characteristic odour is androst-4-ene-3-one. The mass spectrum of this compound has been published[8] but differs considerably from that of the isolated compound and exhibits principal peaks in the high mass range at *m/e* 230, 187, a single dominant ion at *m/e* 149 and *m/e* 124 as the base peak. This method of finger-printing compounds is very precise and the taint compound was clearly not androst-4-ene-3-one.

FIG. 6. (*left*) *Comparison chromatogram of the high-molecular-weight components stripped from the fats of 200 lb liveweight gilt, hog and boar littermate pigs*

Development of taint in the boar is evident. Chromatographic conditions: 3 ft sectional column of 5% SE-30 and 5% PDEAS on Chromosorb G at 180°C.

The description of 'intense, urine-like odour' applied to 5α-androst-16-ene-3-one prompted investigation of this compound. As far as could be ascertained, it was not available commercially or from the Steroid Reference Collection nor was there mass spectrum information in the literature. The compound was prepared by 8N chromic acid oxidation in acetone[9] of the corresponding secondary alcohol, 3β-hydroxy-5α-androst-16-ene. The reaction proceeded rapidly to apparently 100% completion as no residual alcohol or other high-molecular-weight products were detected by gas chromatography. The odour of the ketone and its retention on the gas chromatograph agreed exactly with the taint compound isolated from the boar fat. The prepared compound was passed through the gas chromatograph and trapped in the manner already described prior to mass spectral analysis. The spectrum is shown in Fig. 7b and although the peaks are less intense, the fragmentation pattern is the same as that obtained for the taint compound (Fig. 7a). The use of the fragmentation reactions induced by electron bombardment of samples in a mass spectrometer is well established as a technique for the identification of organic compounds.[10,11] Minor variations can be expected in the relative abundances of the fragment ions in spectra of the same compound recorded at different times as a result of the variation of a number of factors intimately associated with the spectrometer, for example, the temperature of the source, ionisation chamber, etc.[10] (D. H. Williams, personal communication.) Also uneven volatilisation from the probe or decrease of sample concentration results in a greater abundance of ions at the start of a scan than at the finish. The fragmentation pattern of 5α-androst-16-ene-3-one (Fig. 7b) exhibits all the major ions present in the spectrum of the isolated taint compound (Fig. 7a); this confirms that 5α-androst-16-ene-3-one is the compound responsible for the taint of boar fat. Its structure is shown below:



5α-androst-16-ene-3-one

The terms boar odour, boar taint and sex odour which are used synonymously, appear however to be applied on occasions to more than one odour associated with a mature boar. In some quarters, the terms are used to refer to the carcass odour of a freshly gutted animal. This odour, which is suggestive of curry, could conceivably be described as urine-like, but it does not appear to merit the terms 'perspiration-like' or 'onion-like'. It becomes distinctive in the carcasses of older boars (approximately 2 years and upwards), but it is not usually detectable in the carcasses of young boars slaughtered at 200 lb liveweight.

The terms are also applied to a strong 'pig' odour which can be detected coming from the live animal, especially in hot weather. This smell is only one of a number of odours which can be expected to emanate from a live boar. The preputial diverticulum frequently contains odorous secretions which, under alkaline conditions, smell strongly of ammonia and phenolic compounds,[12,13] whereas, under acid conditions, the predominant smell is that of acetic, propionic, butyric and valeric acids (R. L. S. Patterson, unpublished results). These compounds would be expected to permeate the fatty tissue surrounding the prepuce and to impart a characteristic odour to it, but one which would not be found elsewhere in the carcass. Dutt *et al.*[14] reported that heated fatty tissue from the prepuce gave the greatest concentration of boar odour in comparison with fat from other parts of the animal. These odours are indeed boar odours, but it is believed that they are not primarily responsible for the general taint of boar fat.

Conclusion

The compound 5α-androst-16-ene-3-one, which has been isolated from boar fat but which has not been detected in either hog or gilt fat, is responsible for the odour, generally considered to be offensive, of heated shoulder, back and rump fat of mature boars. However, it is recognised that there are other odours associated with a mature boar and that these may also influence the general odour quality of heated boar flesh.

The molecular structure of the identified compound points unequivocally to the male sex hormones as the precursors for its formation although the exact metabolic pathway is not yet clear.

Acknowledgments

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ACIDIC COMPONENTS OF BOAR PREPUTIAL FLUID

By R. L. S. PATTERSON

Several acids present in the acidic fraction of boar preputial fluid have been identified by gas chromatography. They are acetic, propionic, n-butyric, isobutyric, n-valeric, isovaleric, α- and β-methylvaleric, caproic, n-nonanoic and n-undecanoic acids. Unsaturated aliphatic acids include pent-4-enoic, pent-2-enoic, hept-2-enoic and oct-2-enoic acids. Benzoic, phenylacetic and β-phenylpropionic acids were also identified. Several peaks remained unidentified.

Quantitative data for the major components (aromatic acids and acetic acid) are presented and also for the C_3 to C_5 saturated aliphatic acids which are known to have unpleasant odours and low thresholds of detection.

The contribution of these compounds to the odour of a boar is discussed, but it is concluded that they contribute little to the taint of heated boar fat.

Introduction

The work described in this paper represents the second stage of an examination of the odorous constituents of boar preputial fluid. The results of the first part, detailing the qualitative and quantitative analysis of the phenolic fraction of preputial fluid, have been reported in a previous paper.[1] The project is part of a programme of investigation into the nature and source of boar odour. This taint or off-odour, which has been reported[2,3] as being strongest in the fatty tissue surrounding the penis and prepuce, is responsible for the rejection of boar carcasses as a source of bacon and pork.

The analyses were carried out to determine whether the preputial fluid contained the compound or compounds which are responsible for the taint frequently observed when boar fat is cooked. Samples of fluid were chemically separated into acidic and phenolic fractions. The phenolic compounds identified did not appear to contribute significantly to the taint of boar fat, with the possible exception of the fatty tissue immediately surrounding the penis and prepuce. These conclusions were in accordance with the results of a more recent paper[4] in which the isolation of 5α-androst-16-ene-3-one from the fat of mature boars was reported. This compound is believed to be almost entirely responsible for the taint of heated boar fat. 5α-androst-16-ene-3-one has since been detected in small quantity in preputial fluid, but unpublished results indicate that the prepuce is not the site of production.

However, the odorous phenols and acids found in the preputial fluid contribute significantly to the odour of the live boar and his environment. They could also be responsible for contamination of a boar carcass if preputial fluid was spilled during slaughter.

This paper presents the results of a qualitative and quantitative analysis of the acids, detected by gas chromatography, in extracts of boar preputial fluid.

Experimental

The preputial diverticulum, situated near the orifice of the penis, is a sac-like structure which can become filled with fluid. About 10 to 15% of mature boars produce fluid regularly and it can be obtained by 'milking' the pouch behind the penis. The volume of fluid varies from a few drops to about 150 ml. It is an opaque dark brown liquid with a strong odour, and is composed of secretions and residues of urine and semen.

The analysis of the acids present in the preputial fluid was carried out on the same three samples of fluid which were used for the analysis of the phenolic components.[1] Two samples of fluid were obtained from the same boar and the third from a litter-mate. The boars were Large Whites, and the samples of fluid were obtained at liveweights of 166, 193 and 195 lb, respectively.

After filtration to remove pieces of straw, grit and other solid material, each sample of fluid was extracted batchwise at its original alkaline pH (8·5 to 9·5) with diethyl ether (carbonyl- and peroxide-free), until no further odour was extracted by the ether. This was judged by dipping a glass rod into each batch of ether and smelling it after the ether had evaporated. The pH of the residual fluid was then reduced to 4·5, by addition of 1N hydrochloric acid, and extraction with fresh ether continued until no further odour was removed. The aqueous fluid was almost completely odourless after extraction. The two ether extracts were concentrated separately by slow fractional distillation at 38°, to volumes of approximately 100 ml, before each was repeatedly extracted

with 5% sodium bicarbonate, followed by similar extraction with IN sodium hydroxide. Acidification of these four alkaline extracts with IN hydrochloric acid, followed by individual extraction with ether, produced two pairs of ethereal solutions, one pair consisting of a concentrated acid and dilute phenol solution and the other pair, a dilute acid and a concentrated phenol solution. The main acidic and phenolic solutions had quite different odours, but both were recognisably associated with pigsties. The concentrated and dilute acid solutions were combined and analysed by gas–liquid chromatography after concentration by slow fractional distillation to 5 ml standard volume.

The compounds present in the acidic fractions of the three samples of preputial fluid were subjected to qualitative and quantitative analysis on a Pye '104 Series' chromatograph equipped with a flame ionisation detector. The column used was a 5 ft × 4 mm glass column packed with 10% diethyleneglycol adipate + 2% phosphoric acid supported on 100–120 mesh celite, operated isothermally at temperatures of 110°, 130° and 160°. Argon carrier gas was used at a flow rate of approximately 60 ml/min. In this way, three samples were found to be qualitatively similar but to differ quantitatively.

Qualitative results

In Table I(a), relative retention values, corrected to three significant figures, are shown for authentic samples of the low-molecular-weight fatty acids related to caproic acid (n-hexanoic acid) as an internal standard at a column temperature of 110°. The values are compared with similar ones for the first twelve peaks detected in the experimental material. Chromatographic agreement is good for all the compounds listed, with perhaps the possible exception of peak No. 9; this indicates that all the straight-chain acids from C_2 to C_6 are present in the preputial fluid, as well as isobutyric acid, isovaleric acid, the α- and β- methylvaleric acids and the pent-2-enoic and pent-4-enoic acids. Whenever possible, the identity of a peak was checked by extinguishing the detector flame and comparing the odour of the effluent compound with that of an authentic sample.

Relative retention values with respect to n-nonanoic acid were obtained for less volatile acids at a column temperature of 130°; they are shown in Table I(b). Few were identified. Similarly, values based on phenylacetic acid as an internal standard at 160° are shown in Table I(c). The choice of the compounds used as internal standards was dictated by their retention time and relatively isolated position on the chromatogram. At the two lower temperatures, retention agreement was reproducibly good between the selected internal standard and the appropriate peak in the acid extract. At the higher temperature, the characteristic odour of phenylacetic acid was recognisable in the chromatographic effluent which, together with good retention agreement with an authentic sample of the acid, confirmed the presence of the compound in the experimental solution.

Several peaks in the acid extract were not identified although a considerable number of additional acids were chromatographed. These included straight-chain, branched-chain and unsaturated acids, and some containing either a hydroxyl or carbonyl group. Most of the unidentified peaks were small and the constituent acids were minor components. Judged subjectively as they eluted from the chromatograph, none of them possessed a pronounced odour which would contribute significantly to that of the extract. Further information from mass spectrum and infra-red studies would be required to achieve identification of these components.

In addition to the acids mentioned previously, other acids identified in the preputial fluid extract were hept-2-enoic and oct-2-enoic acids, n-nonanoic and n-undecanoic acids and three aromatic acids, benzoic, phenylacetic and β-phenylpropionic acids.

Quantitative results

During the course of the qualitative analysis, it became clear that the acids present in greatest quantity were the three aromatic acids and acetic, propionic and isovaleric acids. n-Butyric, isobutyric and n-valeric acids have very unpleasant odours at low concentration and, although present as minor constituents, they were considered to be potentially important contributors to the odour of the solutions.

The quantities of these acids present in the extracts of the three samples of preputial fluid were measured by comparing their peak heights with the corresponding peaks derived from standard solutions of the acids under identical conditions of injection and analysis. The chromatographic conditions

TABLE I

Relative retention values of organic acids extracted from boar preputial fluid, obtained on 10% diethyleneglycol adipate/2% phosphoric acid stationary phase

(a) at 110°C				(b) at 130°C				(c) at 160°C			
Acid	Vr_1/Vr_2	Unknown peaks		Acid	Vr_1/Vr_2	Unknown peaks		Acid	Vr_1/Vr_2	Unknown peaks	
		No.	Vr_1/Vr_2			No.	Vr_1/Vr_2			No.	Vr_1/Vr_2
Acetic	0·160	1	0·159	Composite peak		13	0·380	Unidentified		23	0·260
Propionic	0·230	2	0·230	Unidentified		14	0·398	Unidentified		24	0·359
Isobutyric	0·260	3	0·259	Hex-3-enoic	0·409			Undecanoic	0·469	25	0·465
n-Butyric	0·360	4	0·359	n-Heptanoic	0·413			Benzoic	0·572	26	0·574
Isovaleric	0·431	5	0·430	Unidentified		15	0·432	Unidentified		27	0·817
n-Valeric	0·605	6	0·608	Hex-2-enoic	0·477			Phenylacetic	1·00	28	1·00
α-Methylvaleric	0·673	7	0·673	Unidentified		16	0·545	β-Phenylpropionic	1·28	29	1·27
β-Methylvaleric	0·770	8	0·768	Unidentified		17	0·618				
Pent-4-enoic	0·855	9	0·848	Unidentified		18	0·695				
Caproic	1·00	10	1·00	Hept-2-enoic	0·748	19	0·742				
Unidentified		11	1·08	Unidentified		20	0·827				
Pent-2-enoic	1·22	12	1·22	n-Nonanoic	1·00	21	1·00				
				Oct-2-enoic	1·17	22	1·16				

TABLE II

Recovery of nine organic acids from boar preputial fluid by extraction and gas–liquid chromatography

	Boar 1						Boar 2		
	Weight 166 lb			Weight 193 lb			Weight 195 lb		
	(1) μg/ml	(2) as % of six aliphatic acids	(3) as % of all nine acids	(1) μg/ml	(2) as % of six aliphatic acids	(3) as % of all nine acids	(1) μg/ml	(2) as % of six aliphatic acids	(3) as % of all nine acids
Aliphatic Acids									
Acetic	184·54	74·59	12·79	140·30	76·35	12·93	179·11	92·36	79·53
Propionic	12·50	5·05	0·86	9·79	5·33	0·90	9·18	4·73	4·07
n-Butyric	1·05	0·42	0·07	0·56	0·30	0·05	0·39	0·20	0·17
Isobutyric	1·65	0·67	0·11	1·10	0·60	0·10	0·78	0·40	0·34
n-Valeric	1·62	0·65	0·11	0·95	0·52	0·08	0·90	0·46	0·39
Isovaleric	46·04	18·61	3·19	31·07	16·91	2·86	3·57	1·84	1·58
Sum of Aliphatic Acids	247·40			183·77			193·93		
Aromatic Acids									
Benzoic	820·21	68·63	56·85	591·07	65·60	54·47	14·93	47·74	6·62
Phenylacetic	311·53	26·07	21·59	268·51	29·80	24·74	15·06	48·15	6·68
β-Phenylpropionic	63·42	5·31	4·39	41·61	4·62	3·83	1·28	4·09	0·56
Sum of Aromatic Acids	1195·16			901·19			31·27		
Sum of Aliphatic and Aromatic Acids	1442·56			1084·96			225·20		

were the same as those used for the qualitative analysis. The standard solutions were prepared as weight or volume per cent in diethyl ether depending upon the physical state of the compound at room temperature. Values for these nine acids in the three experimental solutions are given in Table II, expressed as microgrammes per ml of preputial fluid, as percentages of the six aliphatic or three aromatic acids, and as percentages of all nine acids.

Discussion

The most striking feature of the quantitative results is the large difference in the concentration of the nine acids recorded for the three samples. The concentration of acidic material measured in the solution from Boar 2 (column 1) represents 21% of the total for Boar 1 at a comparable weight and only 16% of that for the same boar at 166 lb liveweight. Similar behaviour was found in the phenol analyses in which the total phenol content for Boar 2 was considerably less than that recorded for the two samples from Boar 1 and was approximately 40% in each case.

Although the relative weights differ, agreement is satisfactory between the percentage figures for each acid in the two solutions from Boar 1. On the other hand, the percentage figures in column 3 for Boar 2 bear little resemblance to those obtained for Boar 1. The percentage yield of acetic acid is increased by a factor of six, while the figures for the aromatic acids are decreased between three and eight times. However, the percentage figures in column 2 for Boar 2 agree more closely with those for the solutions from Boar 1. This shows that the relative proportions of the aliphatic acids, and of the aromatic acids, are reasonably similar, within their respective groups, in all three extracts of preputial fluid, but that the proportions of the two groups of acids differ in the solutions from the two boars.

These six low-molecular-weight aliphatic acids, and phenylacetic and β-phenylpropionic acids, all of which have characteristic odours, are the acids mainly responsible for the odour of the acidic fraction of preputial fluid. Small contributions will also come from some of the other acids in the mixture, especially the C_5 unsaturated acids, which have putrid odours at low concentrations.

There can be little doubt that these acids, and the phenols reported previously,[1] are the major contributors to the natural odour of a live boar and its environment. The exact quality of the odour, whether more phenolic or acidic in character, will depend largely on variable factors, for example, loss of ammonia by evaporation from expelled fluid, the pH of the local detritus, general conditions of animal hygiene, etc. Bacterial and enzymic decomposition of the secretions in the prepuce will vary between animals and influence the composition of the fluid. The reduced quantities of the aromatic acids and different phenol pattern observed for the fluid sample from Boar 2 can be explained on this basis.

It is quite feasible that these highly odorous compounds will also spread to the fatty tissue immediately surrounding the prepuce and thus impart an undesirable odour and flavour to the fat in that area. Normally, however, this fat is cut out and discarded during preparation of the carcass, thereby disposing of contamination arising from this source.

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CONTROLLED AERATION OF RICE IN BULK

By O. MYKLESTAD

Factors affecting the quality of rice during storage in bulk have been investigated, including environmental requirements for minimising microbial growth on the rice by convective and evaporative cooling. In laboratory tests, mould on Caloro rice was first detected after 1½ months' storage at 25° and 75% R.H., but was evident after only one week on rice stored at 25° and 90% R.H. Limiting conditions for drying (and hence evaporative cooling) of rice during ventilated storage were ascertained from a study of the sorption isotherms, determined in the laboratory which indicated that during the last stage an aerating atmosphere of 25° would require to have a humidity of 75% R.H. or less in order to reduce the moisture content of rice to the desirable final value of 14–15%.

Rice with an initial moisture content ranging between 16 and 22% was used during a full-scale aeration trial in which temperatures at various locations in the rice load (520 tons) were continuously recorded. Since these temperatures varied systematically with ambient temperature, heating of rice through microbial activity apparently did not take place during aeration—a fact confirmed when the rice was unloaded from the storage bin at the end of the aeration trial.

A specially designed humidity-controlling device was tested during the last 2½ weeks of the major aeration trial and functioned satisfactorily.

Introduction

Between being harvested and milled, Australian rice is stored in sheds where loads of rice differing appreciably in initial moisture content are kept apart in separate bins, each ventilated with unheated air. Such differentiation of the crops, especially at the peak of the harvest, may cause extensive holdup of transport vehicles at rice depots. There would therefore be a clear advantage if different loads of rice, irrespective of their initial moisture content, could be pooled in the storage bins without loss in quality during subsequent storage occurring. The feasibility of applying controlled aeration as a means of reducing the moisture content in a bin load of rice after batches differing in initial moisture contents were pooled has been studied by the C.S.I.R.O. Division of Food Preservation in Australia, and the results of this investigation are reported in the present paper.

Conditions for microbial growth on rice were first ascertained. It is well known that rates of microbial growth on foods in storage depend partly on nutritional factors and partly on storage conditions. It has, for instance, been demonstrated[1] that most micro-organisms proliferate readily on substrates containing suitable nutrients if the water activity (a_w) is above 0·80, that is, if the relative humidity (R.H.) of the air in contact with the surface of the food exceeds 80%. Also, the maximum growth rates attainable within certain intervals of R.H. depend on the surface temperature of the substrate.[2,3] More particularly, it has been shown that within the temperature range of 17–24° the growth rate of moulds on rice increases with moisture content of the rice when this exceeds 15%.[4] (Moisture contents are quoted on a wet-weight basis.)

Microbial growth was studied in relation to sorption isotherms, which represent the limiting moisture contents to which rice can be dried when in contact with an atmosphere at one constant temperature and various constant relative humidities. These sorption isotherms are therefore fundamental requirements for the design and satisfactory operation of automatic control devices to be utilised in any ventilation system for drying rice in bulk.

On the basis of the laboratory findings, a full-scale aeration trial was conducted on a load of 520 tons of rice, temperatures at various locations in this load within the storage bin being recorded continuously during the test. In the full-scale trial, final stages of aeration were controlled automatically with equipment specially designed for the purpose and described in a later section of this paper.

Experimental

Raw material

The raw material used for all the experiments was Australian-grown Caloro rice.

Microbial growth on rice

Experiments were carried out in this laboratory to ascertain which of a wide range of conditions most facilitated the growth of mould on rice having an original moisture content of 20·5%. Several 10 gram samples were kept in closed containers at various temperatures and relative humidities until mould was evident. During the experiments these containers were kept in constant-temperature storage rooms, while salt solutions present at the bottom of the containers produced the desirable R.H. of the air in contact with the rice samples.[5]

Sorption isotherms

The *adsorption* branch of the isotherm, which defines the relation between moisture content and relative humidity for initially dry material equilibrated at various relative humidities, was determined experimentally at 25° for Caloro rice (with an initial moisture content of 9%) by measuring the moisture contents of the rice gravimetrically[6] and ascertaining the corresponding relative humidities with the aid of a selenium oxide probe.[7]

Aeration of mixed loads of rice

Newly harvested Caloro rice ranging in moisture content from 16 to 22% was delivered by trucks to a storage shed at Murrami, New South Wales. Having first gone through a cleaning process in the lower region of the shed, the rice was elevated to the top of the experimental bin, into which it was evenly distributed by a mechanical grain thrower.

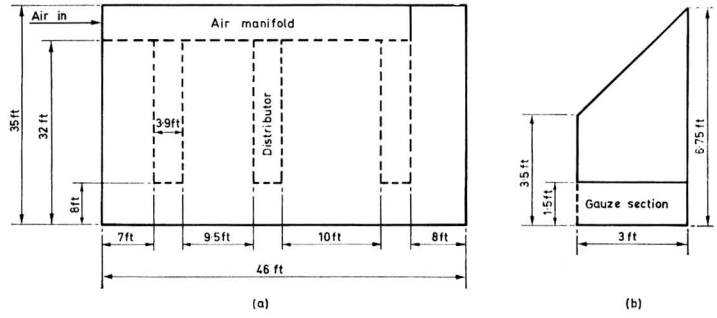

FIG. 1.—*Storage bin for experimental aeration of rice*
(a) top view of bin
(b) side view of manifold

Fig. 1 shows schematically the storage bin, which a total load of about 520 tons of rice filled to a height of 18 ft. Untreated air from the atmosphere was blown at a rate of 1 ft^3 air/min/ft^3 stored rice into the air manifold and was evenly distributed throughout the rice load by escaping from a 1·5 ft high vertical gauze section along the front of the manifold, a 1·5 ft high vertical gauze section along the front of the manifold, a 1·5 ft high gauze section along the side of the manifold, and three distributors formed as semi-cylindrical gauze tubes with a diameter of 46 in.

During the aeration trial the temperature in various locations of the rice bin was continuously recorded, before and after the trial the moisture contents of random rice loads were measured, and during the last period of the trial a humidity controller was brought into action.

Temperatures in the rice bin

Temperatures were continuously recorded at 45 positions within the load of rice, in accordance with the pattern shown in Fig. 2. The temperature-sensing elements were 24 gauge copper–constantan thermocouples.

Moisture contents of the rice

Samples of approximately 300 cm^3 of rice were analysed in a capacitance moisture meter having a maximum error of ±0·2% moisture.

Humidity control

The basic features of the humidity control device developed in this laboratory for use in the present investigation are illustrated in Fig. 3. Two sulphonated polystyrene humidity probes were connected to a Wheatstone bridge, one (the reference probe, R) being kept over a saturated salt solution at constant humidity in a sealed container while the other (the measurement probe, M) was placed in the air flowing into the rice bin. The resistance of M changed inversely with atmospheric humidity and produced a variable bridge current, which for this application was amplified and conducted to a relay. With the switch in the automatic position (A on the panel), the relay transmitted and maintained an energising current to the autotransformer of a fan, thereby keeping this fan in operation when the humidity of the ambient air was less than the constant reference humidity.

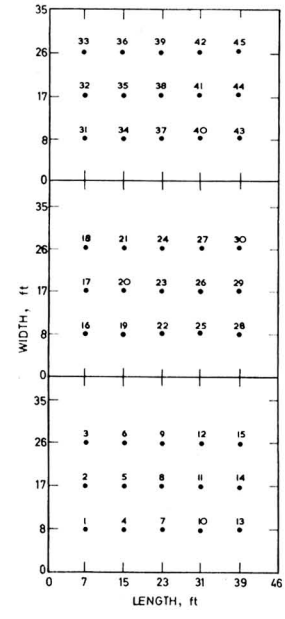

FIG. 2.—*Thermocouple positions in rice bed*
Top thermocouple positions 15·3 ft. above floor
Middle thermocouple positions 9·5 ft. above floor
Bottom thermocouple positions 4 ft. above floor

Results and Discussion

Microbial growth on rice

Fig. 4 shows, by numbers entered at appropriate intersections of a graph of temperature as a function of R.H., the time in days for the first visible sign of mould to appear on the rice being tested. At 27° and 75% R.H., close to the lowest

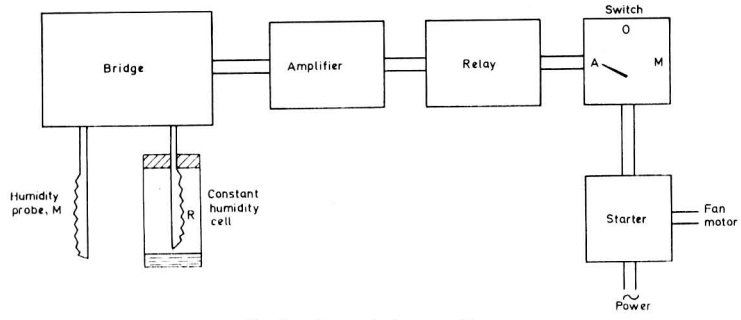

FIG. 3.—*Automatic fan controller*

R.H. value selected for this study, first indications of mould appeared after 46 days. Similarly, when storage was at 20° mould first became apparent after 25 days at 85% R.H., but in about 12 days at 90% R.H.; at 25° and 90% R.H. signs of mould were evident after only 7 days.

The experiments just mentioned involved storage of rice samples in closed containers, and represent conditions that might be somewhat less favourable than those obtained for bulk storage in ventilated sheds. However, the results indicated suitable combinations of conditions to be aimed at for satisfactory aeration and they also provided information needed to predict the consequences of uneven or intermittent aeration in commercial-scale operations if these factors were not adequately controlled.

Sorption isotherms

Fig. 5 shows the sorption isotherm for rice at 25° determined in this laboratory and elsewhere.[8-12]

In view of the many different kinds and varieties of rice to which Fig. 5 refers, the consistent differences evident in the equilibrium moisture contents at each level of relative humidity, though they rarely exceed 3%, are not unexpected. For instance, the sorption isotherm reported by Houston[11] for *brown* Caloro rice, consistently higher than that based on the experiments of the present work on *rough* Caloro rice at the same temperature, can readily be accounted for in terms of differences in sorptive capacity known to exist among different individual components of the rice grain. Since Karon & Adams[12] have shown that isotherms of rice hulls are considerably lower than the corresponding isotherms of rough rice of the same variety, removal of the hulls from rough rice would be expected to produce, under comparable conditions, a higher sorption isotherm for the resulting brown rice.

Fig. 5 indicates that rough Caloro rice should dry to a moisture content of 14–15% if the ventilating air used has a humidity not exceeding 75% R.H.

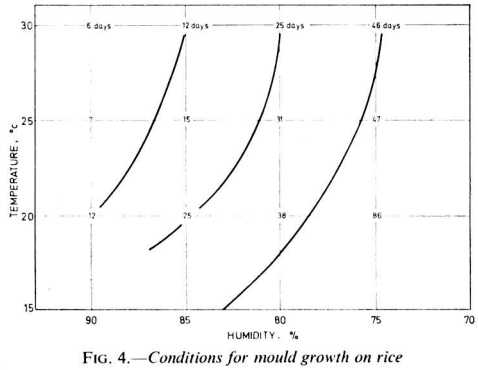

FIG. 4.—*Conditions for mould growth on rice*

Aeration of mixed loads of rice

The bulk aeration trial lasted for nearly three months (see sequence of operation in Table I).

Temperatures in the rice bin

In Fig. 6, the temperature of the atmosphere entering the bin (i.e. at Position 46) and that at the centre of each arbitrarily defined layer of rice within the bin (Position 8, 23, and 38—see Fig. 2) are plotted against time for the first four days of continuous recording. The centre of each layer tested showed periodical changes in temperature, but for each layer the temperature cycle was about 4 h out of phase with that of the corresponding measurement in the layer immediately below. The temperature in the centre of the bottom layer (see Fig. 2) was about 4 h out of phase with the temperature of the external atmosphere.

During this initial period of aeration the temperature gradient between Positions 8 and 23 was mostly 3°C/m but did decrease to a minimum value of 1°C/m. The temperature gradient between Positions 23 and 38 was poorly defined and frequently changed from positive to negative values, the cross-over times coinciding approximately with those when the atmospheric temperature was at a maximum or at a minimum.

Fig. 7 records all observations, at various times, of the maximum temperatures attained in each of the three rice layers investigated. The numerals inset in Fig. 7 in some of the symbols used to designate maximum temperatures in the bottom layer indicate actual positions in this layer (as defined

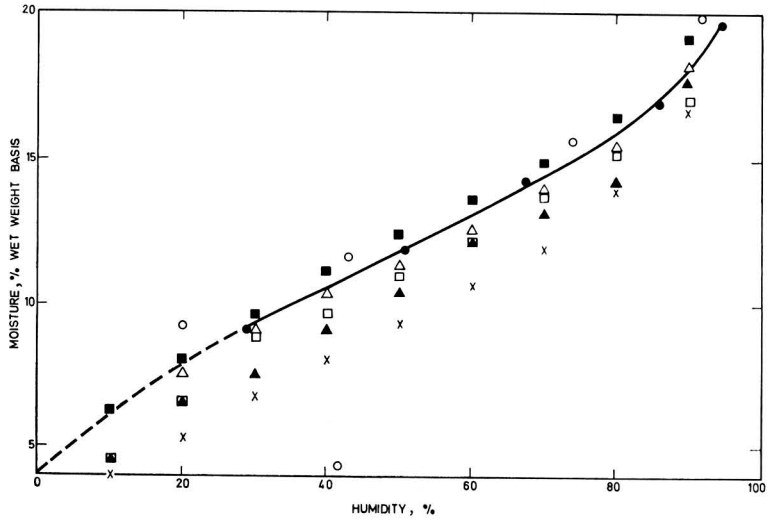

FIG. 5.—*Sorption isotherm of rice at 25°C*

Symbol	Reference	Variety
○	8	
□	9	Joya, desorption
×	9	Joya, adsorption
△	10	
●	C.S.I.R.O.	Caloro, rough
■	11	Caloro, brown
▲	12	Rexora

TABLE I

Operating schedule for the bulk aeration trial in 1965

Date	Operation
May 1	Started loading Caloro rice into storage bin at 10.30 a.m., ventilation started at 4.00 p.m.
May 3	Loading completed at 9.00 a.m.
May 4	Continuous temperature recording started at 10.00 a.m.
June 7	Automatic control device installed in fan circuit and put into operation at 3.45 p.m.
June 24	Switched to manual fan control at 9.00 a.m., only slight ventilation now needed
July 20	Ventilation stopped, started unloading the storage bin
July 26	Storage bin empty

by Fig. 2) where the particular maxima were recorded. For example, up to and including the eleventh day of the trial the maximum temperature in the bottom layer was recorded at Position 9 (see Fig. 2); but during the next two days it was observed in Position 12, and in the following three days in Position 9, 8, 9, and so on. The highest temperature measured at any location was 20·5°, and was observed after about eight days, but during about two-thirds of the trial the temperature of the rice remained below 15·5°. During the first month of the aeration trial the maximum atmospheric temperatures exceeded 21° but during most of the remaining period of the trial these temperatures varied between 15·5° and 21°.

It was a characteristic of these measurements that, in general, the highest maximum temperatures occurred in the bottom layer, usually in Positions 9 and 12, possibly because these were closest to the centre of the air manifold. However, close inspection of Fig. 7 shows that there were two periods, each of four days' duration, in which the maximum temperatures observed were not in the bottom layer. These periods coincided with unusually low atmospheric temperatures and were immediately followed by sharp increases in the temperature of the bottom layer when the atmospheric temperatures increased to their usual values.

The fact that temperatures measured in the numerous locations of the rice bed responded well to the periodic changes of atmospheric temperatures indicated even air distribution throughout the rice bin. This was a result of satisfactory duct positioning and good mixing of the loads of rice, which differed widely in original moisture content. If good mixing is not effected there is a tendency for the wet and dry portions of grain to pack unevenly, attain different bulk densities, and offer different resistance to air flow.[13] In such unevenly ventilated grain beds there is a risk of infestation and the activity of microbes and insects forming isolated colonies and producing hot-spots which are by-passed by the ventilating air. The problem of infestation in ventilated bins of grain becomes particularly serious during prolonged periods of warm and humid weather, because drying and evaporative cooling of the grain are then proceeding at insignificantly low rates.[14]

Moisture contents of the rice

Table II shows the distribution of moisture contents in the rice before the aeration trial, the figures being grouped according to initial moisture content but not according to the sequence of their placement in the bin.

Table III lists the moisture contents of random loads of rice transferred from the test bin to the rice mill at the end of the aeration trial. At this stage the moisture in various loads varied between 13·1 and 15·9%. If the average final moisture content of 14·5% is assumed, the average moisture loss from the rice during the aeration was about 293 kg/day.

Humidity control

The humidity controller was utilised for the last 2½ weeks of the aeration trial, and was set to keep the fan in operation when the atmospheric humidity was below 75% R.H., which

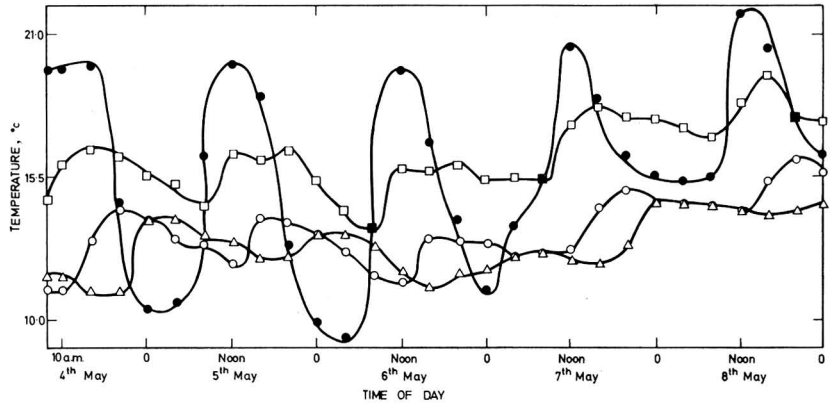

FIG. 6.—*Temperatures along central axis in rice bed*

□ Position 8 (see Fig. 2)
○ Position 23 (see Fig. 2)
△ Position 38 (see Fig. 2)
● Position 46 (see Fig. 2)

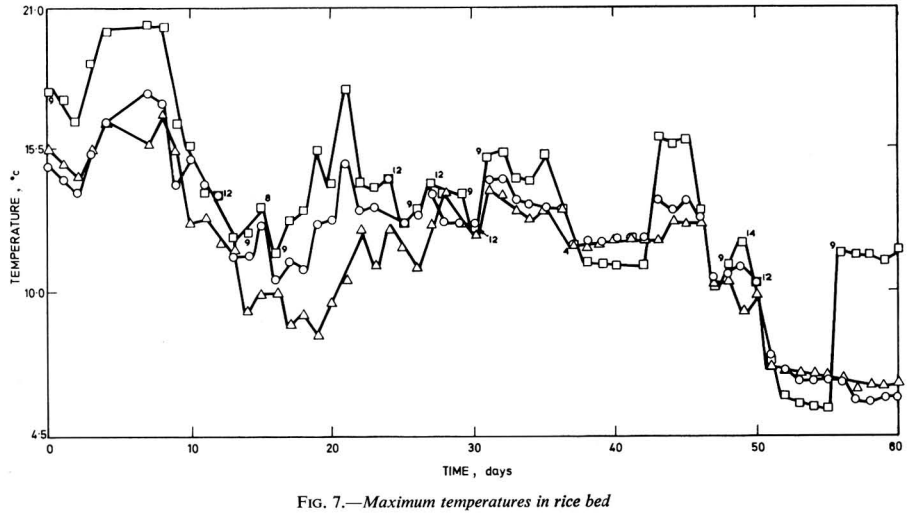

FIG. 7.—*Maximum temperatures in rice bed*

△ Top layer
○ Middle layer
□ Bottom layer

TABLE II

Distribution of initial moisture in batches of rice pooled in single bulk-storage bin

Moisture content, % wet wt basis	Number of batches	Weight tons	Percentage of total weight
16·1–17·0	22	149·49	28·77
17·1–18·0	13	108·90	20·96
18·1–19·0	15	109·45	21·07
19·1–20·0	10	93·65	18·03
20·1–21·0	5	41·76	8·00
21·1–22·0	2	16·25	3·10
Totals:	67	519·50	100·00

TABLE III

Moisture content of rice at the end of storage

Date	Moisture contents in random loads, % wet wt basis
July 20	15·9, 14·8
July 21	14·6, 14·3, 14·3, 14·1, 14·1
July 22	15·4, 14·1, 13·9, 13·6, 14·5, 14·1
July 23	13·8, 13·8, 13·4, 13·7, 13·6, 14·0
July 26	13·5, 13·1, 15·5

corresponds to an equilibrium moisture content of the rice of about 15%. During most of the 2½ weeks the device kept the fan in operation between 8·4 and 13·4 h per day. During five days of this period the average daily period of operation of the fan was only 2·1 h, owing to the rather moist and foggy conditions that prevailed in the Murrami area at the time.

Product

An analysis of rice fractions after the milling operation, carried out by the Rice Growers' Co-operative Mills Ltd., gave the following quantitative information on the grain components:

Whole grain:	55%
Hulls:	20%
Bran:	10%
Broken grain:	15%

These milling data for rice loaded into the storage bin at widely different moisture contents are roughly similar to the figures obtained for rice stored and ventilated in the conventional way.

Conclusions and Recommendations

Batches of rice which differ widely in initial moisture content may be admixed without loss of quality if the rice is properly aerated during storage. However, in order to minimise cracking, rice with a moisture content of less than 14% should not be stored with wetter rice.[15]

Care must be exercised to ensure even distribution by intimate mixing of loads that differ considerably in initial moisture content. This will prevent uneven packing of the grains and ensure proper aeration of all portions of the load.

The efficient ventilation coupled with favourable atmospheric conditions kept the temperature in the rice load below 15·5° for two thirds of the storage trial. At these low temperatures the rate of mould growth is generally retarded and the activity of the major insect pests is significantly reduced.[16]

Acknowledgments

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MICROBIOLOGICAL EFFECTS OF SYNTHETIC FOOD COLOURING MATTERS. SOME ASPECTS OF THE ADSORPTION OF AROMATIC SOLUTES AT BIOLOGICAL SURFACES*

By C. H. GILES and H. E. NURSTEN

Evidence is given to support the hypothesis that readily associating cationic dyes are liable to be inimical to biological cells because of the effects of their adsorption as ionic micelles at anionic sites, probably DNA. From an examination of the molecular structures of the food dyes now permitted in the United Kingdom it appears that microbiological activity in foodstuffs is likely to be affected only exceptionally.

Introduction

The microbiological effects of dyes have received attention ever since the work of Ehrlich, but those of food colours have been only little studied. In this paper some of the biological properties of dyes are surveyed, their mechanism is speculated on, and their relevance in the case of food colours is considered.

Dye molecules in general consist of complex aromatic hydrocarbon structures with a wide variety of substituents.[1-6] The majority of dyes belong to either the azo- or the anthraquinone classes, and a minority to the triphenylmethane and other classes. Their most common substituents include amino-, substituted amino-, halogeno-, hydroxy-, and sulphogroups. Sulpho- groups are introduced only to give solubility in water, the others mainly to influence the hue, though in many cases they also assist solubility in water.

Many *unsulphonated* azo- compounds are carcinogens, probably owing to their reduction in the animal body to a mixture of amines, although there does not seem to be any simple relation between constitution and potency.[7] Many amines, notably β-naphthylamine, are highly carcinogenic. Amino-compounds also form salts with acids, which are water-soluble and have the adsorptive properties to be described later. Sulphonation seems to inactivate the microbiological properties of smaller aromatic molecules, because by conferring high solubility in water it ensures ready elimination.[7] Many anthraquinones and other quinonoid compounds have microbiological activity, which is thought to be connected with their ease of reversible reduction, a property they share with the triphenylmethane dyes.[7] Potentially chelating structures in organic molecules confer antimicrobial activity, probably by combination with physiologically important metals.[8] Halogeno- substituents are often microbiologically significant.

Food is coloured with a limited range of dyes, nearly all ionisable. The mechanism of reaction between ionisable dyes and cells is therefore considered. There are on record many investigations upon dyestaining of micro-organisms,[9] but very few give quantitative physico-chemical data on which theories of staining mechanisms can be based.

* Presented to a Meeting of the Microbiology Group, 22 February, 1966

Association and microbiological activity

As a working hypothesis it is proposed that the microbiological effects of ionisable organic solutes, especially dyes, are in many cases due to association of the solute when adsorbed by the cell, i.e. to the adsorption of ionic micelles.

The adsorption takes place by an ion-exchange mechanism, and in many such adsorption processes at solid surfaces the adsorbed species is associated, i.e. an ionic micelle.[10,11] Evidence for the ion-exchange mechanism is: bacteriostatic activity of the acridine dyes depends upon their ionisability;[7] antiseptic activity is shown by cationic salts of long alkyl chain primary, secondary, and tertiary amines, but the quaternary salts are usually more effective, and these are more highly ionised;[7] there is an increase in adsorption of cetyltrimethylammonium bromide (CTAB) by particular micro-organisms with rise in pH of the treating solution (Fig. 1), and this parallels the adsorption of cationic dyes by wool fibre,[14] which is an ion-exchange process[14-16] (Fig. 2); there is a similarity between the normal isotherms for the adsorption of cationic dyes on alumina, which can readily be identified as an ion-exchange process,[17] and on yeast[18] (Fig. 3a, b) (there is experimental evidence that the cationic dye-adsorbing sites in fixed yeast cells are in the DNA molecule[18]); the effect of a cation, e.g. of a quaternary ammonium compound, can be neutralised by the competition of another cation.[7]

The coverage factor[10,11] for cationic dyes is similar whether measured for adsorption on yeast or on a variety of other substrates (Figs 4, 5). This factor, which is believed to be a measure of the degree of association of the dye ions in the adsorbed state, is calculated as follows. An estimate of the specific surface of the solid is obtained by adsorption of a substance with a small molecule, e.g. nitrogen or *p*-nitrophenol; this is assumed to be the 'true' value (V_t). A value is then similarly obtained* by adsorption of the dye under test. This value (V_d) proves to be higher than V_t, and the factor V_d/V_t is called the coverage factor. The factor depends upon the molecular weight of the colour ion of the dye, and in fact it increases with the cube of this weight.

The maximum amount of CTAB adsorbed by two specific organisms (*Escherichia coli* and *Staphylococcus aureus*) (Fig. 1)

* Assuming that the dye molecule lies flat on the substrate surface. For reasons and full discussion, see the original publications.[10,11] In the case of yeast cells the 'specific surface' measured probably represents the total number of anionic sites[18]

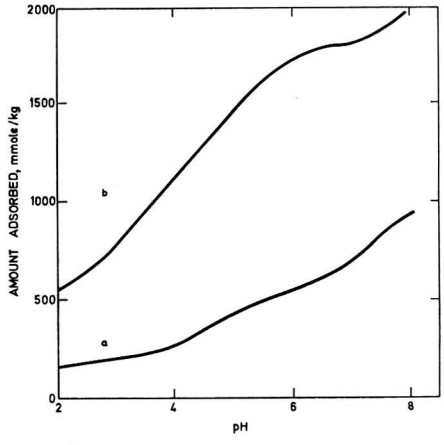

FIG. 1. *Adsorption isotherms (titration curves) of CTAB (cetyltrimethylammonium bromide) a typical quaternary ammonium antibacterial agent, on two micro-organisms*

Adsorption rises with rise in *p*H of the bath; this indicates an ion-exchange process. (Data from Salton[12], cf. Giles[13]).

[a] *Escherichia coli* [b] *Staphylococcus aureus*

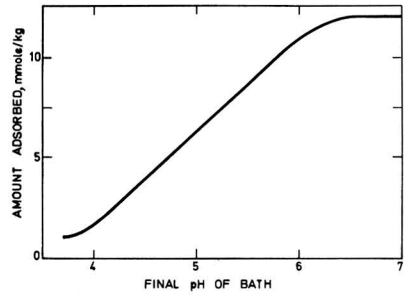

FIG. 2. *Adsorption isotherms (titration curves) of Crystal Violet (C.I. 42555) on wool (at 60°C). The effect of pH is similar to that shown in Fig. 1. (Data from Bird & Stancey)*[14]

Note. Adsorption is much less than for the systems shown in Figs 1 and 6, mainly because the solutions used in the present tests were of low dye concentration.

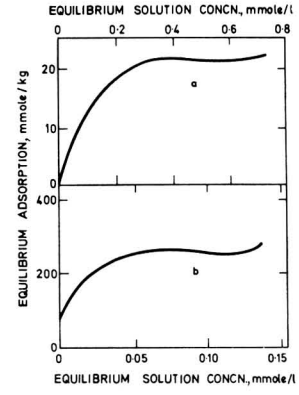

FIG. 3. *Adsorption isotherms for Crystal Violet at 20°C on* (a) *chromatographic alumina,*[17] *and* (b) *fixed yeast cells,*[18] *showing variation with dyebath concentration*

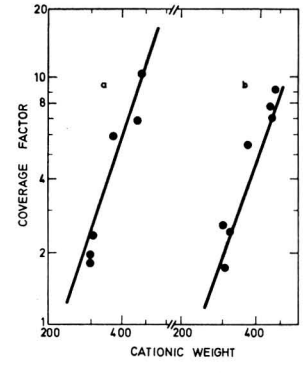

FIG. 4. *Plots*[11] *of* $\log_{10}$ *(coverage factor) against* $\log_{10}$ *(cationic weight) for adsorption of cationic dyes on* (a) *chromatographic alumina, and* (b) *fixed yeast cells. These data are believed to reveal an increase in size of adsorbed micelles with increase in weight of dye cation*

has been calculated[13] to be about four and two times as great, respectively, as that required to neutralise all the anionic groups in the whole organism; such an excess could only be explained by assuming an adsorption of ionic micelles.*

The adsorption process of the fungicide dodecylguanidine ('dodine') acetate, by conidia of *Alternaria tenuis* and *Neurospora crassa*, was found by Somers & Pring to show the characteristics of a cation exchange mechanism, the dodine cation being adsorbed at anionic sites in the fungal spores.[20] The adsorbed dodine, however, is remarkably resistant to repeated washing with water or even to treatment with an anionic surface-active agent or a competing metal cation; but it is removed by cetyltrimethylammonium bromide solutions sufficiently concentrated to disrupt the cell membrane and solubilise cell constituents. This behaviour appears to indicate the presence of micelles of dodine at the adsorption sites, formed probably after initial adsorption of individual dodine cations, though the authors themselves suggested that covalent bonding between dodine and cell constituents may be involved. Not all cationic antiseptics, however, are so resistant to removal from micro-organisms by water;[12] possibly this is due to differing cell wall permeability.

The activity of compounds in homologous series, e.g. alkyl thiocyanates as insecticides, or phenols as bactericides, has a maximum at an intermediate chain length, which is different

* Alkyl quaternary ammonium salts are adsorbed by quartz as two-dimensional aggregates[19]

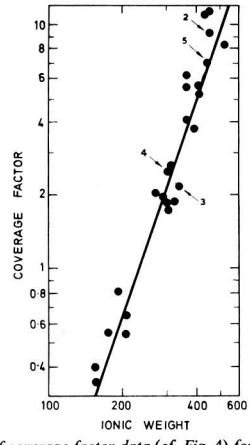

FIG. 5. *Plot of coverage factor data (cf. Fig. 4) for both anionic and cationic dyes on six different solid substrates*[10,11] *(viz. fixed yeast cells, graphite, and four inorganic materials). Arrowed numerals show the number of experimental results represented by the respective points*

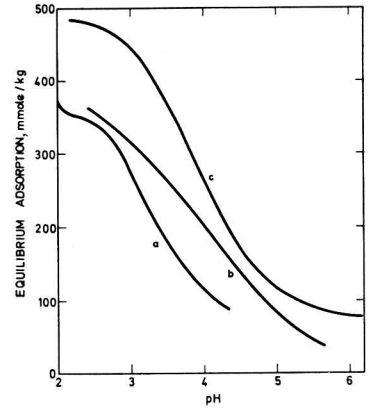

FIG. 6. *Adsorption isotherms of typical low-molecular-weight anionic dyes (as the free dye acids) on protein substrates,* (a) *C.I.* 16185 *on collagen at* 25°C; (b) *C.I.* 15510 (*a monosulphonated monoazo dye*) *on wool at* 60°*C*; (c) *C.I.* 16045 *on collagen at* 25°*C. These all show that adsorption decreases with rise in pH (cf. Fig.* 2, *above). (Data from Lemin & Vickerstaff and Coward & Nursten)*[24]

in different cases, but is usually between C_8 and C_{16}.[7] Sexton explains this is due to the variation in relative molar solubilities of the agents in the treatment solutions, but it could also be due to association effects, since a similar maximum, attributed to dye association, occurs in the light-fastness of homologous series of dyes adsorbed on dry solid substrates.[21]

In series of compounds of similar structure to direct cotton dyes, trypanocidal activity rises with ability to dye cotton.[22] Sexton[7] suggests that the agent may be adsorbed by a carbohydrate in the cell, but association effects may also occur, since it is known that cotton dyes readily associate in solution, and it has been suggested that they do so when adsorbed on cellulose.[16,23]

The possible active sites for adsorption of ionic dyes by living cells include carbohydrates, nucleic acids, and proteins, whilst lipids would adsorb non-ionic dyes. Figs 2 and 6 show that anionic dyes require acid conditions and cationic dyes require alkaline conditions for maximum adsorption on ionic substrates. Under the nearly neutral conditions of most biological fluids, adsorption of organic ions on ionic substrates will tend to be at a minimum. However, with increase in size of the hydrophobic portion of the molecule without increase in number of ionisable groups, adsorption under neutral conditions increases.[16]* Such a change in molecular structure; while increasing the affinity for biological materials, e.g. nucleic acids or proteins, also increases the tendency to association, so that if an ionisable organic solute has affinity for biological materials it probably has marked associative properties and may be adsorbed as an ionic micelle, thus causing disruption of membranes in a micro-organism.** This could account for the lysis and death of bacterial cells treated with quaternary ammonium surfactants.

Solutes without a specific toxic group in the molecule might therefore be expected to have the most marked microbiological activity if they associate readily. As stated before, sulphonated compounds are said to be usually non-toxic because their high water-solubility ensures ready elimination from the organism. Those dyes which are sulphonated and also can readily associate might be active, but, except for the cotton dyes already mentioned, there does not seem to be any published evidence that they are. The cationic dyes are adsorbed at acidic sites in DNA. Perhaps adsorption at these sites is more harmful to the organism than it is at basic sites in proteins.*

There is an exceptional situation in the case of food dyes. The pH value of stomach fluids is very low (about 1–2) and this would cause strong adsorption of anionic dyes to proteins. Most of the permitted food dyes are anionic and so will be subject to this action.

Microbiological effects of coal-tar food colours

Amongst the water-soluble ionisable compounds, microbiological activity would therefore be expected to be associated with: highly basic compounds with marked associative properties; anionic compounds with very marked associative properties; anthraquinones and other quinonoid compounds; chelating compounds (e.g. those with *O,O'*-dihydroxyazo-groups); and azo compounds which when reduced produce an unsulphonated amine with toxic properties.

Examination of the structure of the British permitted coal-tar food colours (Table I and Fig. 7) gives little reason for

* This increase in *affinity* of solute for substrate would shift the curve to the left in Fig. 2 and to the right in Fig. 6

** Sexton[7] suggests that cationic surfactants sever loose unions, e.g. between co-enzyme and protein in some enzymes. This process could be facilitated by adsorption of ionic micelles

* Cationic dyes act on oxidative enzymes and affect respiration in cells, and it has been suggested that their ease of reversible reduction may thus play a part in their microbiological action;[7] but such an explanation cannot account for the action of alkyl quaternary surfactants, which are not reversibly reducible

expecting microbiological activity: none is cationic, and, except for the two oil-soluble colours, all are anionic. C.I. 45430 is anionic through carboxy- and hydroxy-groups, the remainder through sulpho groups. On the other hand, hydroxy- groups not intramolecularly hydrogen-bonded are present in C.I. 11920 and 44090. C.I. 15970, 16150, 16230, 17200, 18050, and 18055, and Brown FK have structures with some tendency to give aggregating properties and surface-activity. Although no permitted dyes are anthraquinonoid, C.I. 44090 and 45430 and Violet BNP have quinonoid formulae and C.I. 73015 a closely related one.

Of the six colours not considered safe in recent years,[25,26] two (C.I. 13011, 42045) contain substituted amino-groups in unsulphonated benzene rings* and so are understandably suspect, as is C.I. 10316 which contains nitro-groups; C.I. 14700 has 2,4-dimethyl-** and C.I. 16155 has 2,4,5-trimethyl-substituents relative to the azo-group in the first component and these substituents would increase the basicity of the amine which would be formed if the dye were reduced; C.I. 14330 has 2,6-dihydroxy-substitution relative to the azo-group, conferring increased chelating properties on it.

Little experimental work seems to have been done to date on the microbiological effects of permitted coal-tar food colours. The following paragraphs summarise the reported results.

Lück and co-workers[27] have demonstrated that C.I. 45430 has a definite, though weak, mutagenic action on *E. coli*, as well as causing slight inhibition of growth in acid solution.

* But so do C.I. 44090 and Violet BNP, permitted

** But C.I. 16150, permitted, also has these substituents, and moreover has a molecule more likely to associate than C.I. 14700. On reduction it would give rise to an unsulphonated amine (which C.I. 14700 would not), and so would C.I. 11920, 12740, 15970, 16230, 17200, 18050, 18055, and 20285, and Brown FK and Chocolate Brown FB

TABLE I

Classification of permitted coal tar food colours[25,26]
(for formulae see Fig. 7)

C.I. No. or Name	Hue	Chemical Class	Group*	B.S.
11920	Yellow	Monoazo, unsulphonated	E	
12740	Yellow	Monoazo, unsulphonated	E	
14720	Red	Monoazo, disulphonated	B	3343 (1961)
14780**	Red	Monoazo, disulphonated	F	
15970	Orange	Monoazo, monosulphonated	E	
15985	Yellow	Monoazo, disulphonated	B	3340 (1961)
16045	Red	Monoazo, disulphonated	B	
16150	Red	Monoazo, disulphonated	B	3671 (1963)
16185	Red	Monoazo, trisulphonated	A	3341 (1961)
16230	Orange	Monoazo, disulphonated	E	3612 (1963)
16255	Red	Monoazo, trisulphonated	B	3342 (1961)
17200	Red	Monoazo, disulphonated	E	3610 (1963)
18050	Red	Monoazo, disulphonated	E	3611 (1963)
18055	Red	Monoazo, disulphonated	E	3780 (1964)
18965	Yellow	Monoazo, disulphonated	E	3614 (1963)
19140	Yellow	Monoazo, disulphonated	B	3211 (1960)
20285	Brown	Disazo, disulphonated	F	
28440	Black	Disazo, tetrasulphonated	B	
44090	Green-blue	Diphenylnaphthylmethane, disulphonated	A	
45430	Red	Xanthene	B	
73015	Blue	Indigoid, disulphonated	B	
Brown FK		Azo, sulphonated (with free amino group)	E	
Chocolate Brown FB***		Azo, sulphonated	E	
Violet BNP****		Triphenylmethane, disulphonated	E	
Black 7984*****		Azo, tetrasulphonated (with free amino-group)	B	

* A = Acceptable on available evidence
B = Provisonally acceptable
C = Possibly toxic
D = Probably toxic
E = Evidence inadequate for assessing acceptability
F = No information on toxicity
(From ref. 26).

** Ref. 25 positions the left-hand sulpho group wrongly[34]

*** Diazotised naphthionic acid coupled to a mixture of morin and maclurin. No formula in Fig. 7

**** The scientific name given in ref. 25 cannot be correct. It is surely intended to contain the grouping 4″-di(*p*-sulphobenzyl)amino and not 4″-di(*p*-sulphobenzylamino), but even this needs modification, since it is known that benzylamino groups sulphonate primarily in *m*-position[35] (see also C.I. 42580)

***** Ref. 25 does not indicate the position of coupling to the end component. Seven dyes appear in the Colour Index with 2R acid as end component and in six of these coupling has taken place in the 2-position; it seems the more likely here also

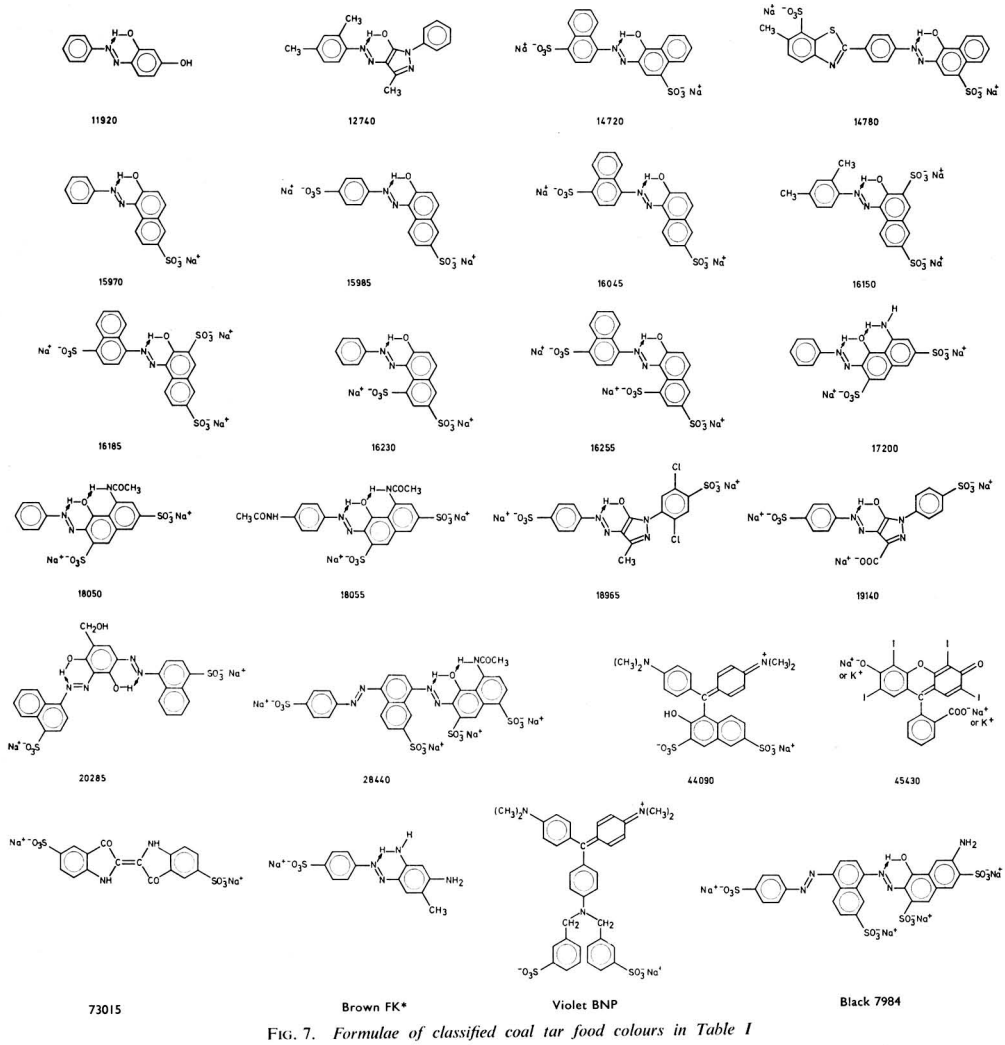

FIG. 7. *Formulae of classified coal tar food colours in Table I*

* This is not a homogeneous dyestuff and a high proportion of molecules of other formulae is present also

The same dye also inhibits lactic fermentation.[28,29] It falls into the quinonoid group and has phenolic and halogeno-substituents in addition, as well as not being as highly anionic as the other soluble food colours.

During lactic fermentation, dyes present are reduced and the products of reduction normally have a smaller inhibitory effect even than the dyes themselves. However, Eisenbrand & Lohrscheid[30] discovered that in some cases the products of reduction gave rise in presence of air to substances which are powerful inhibitors. Both C.I. 14720 and 16045 exhibited this effect. Later,[31,32] C.I. 15985, 16185, 16255, and 28440 were found to be active, but 19140 was not.

It seems that the substances responsible for the inhibition are oxidation products of 1,2- or 2,1-aminonaphthol derivatives. Red compounds are formed as intermediates to the real inhibitors by oxidation accompanied by loss of sulphogroups.[33] The result of the reactions is to produce larger molecules with a lower degree of sulphonation: exactly what is required to bring a non-toxic compound into the category of anionic compounds with marked associative properties.

Setting aside that C.I. 16150 apparently does not give rise to inhibitors, one would expect inhibition of lactic fermentation also from the oxidised degradation products of C.I. 14780, 15970, 16230, 17200, 18050, 18055, and Black 7984. If similar products could be obtained from aminophenols, C.I. 11920 and 20285 would have to be added to this list.

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EFFECTS OF LIMING AND VARIOUS FORMS OF ORAL COPPER SUPPLEMENTATION ON THE COPPER STATUS OF GRAZING SHEEP

By A. MACPHERSON* and R. G. HEMINGWAY

Sheep grazing limed pasture for periods extending to 14 months had consistently lower mean blood copper concentrations than similar sheep on comparable untreated pasture. Liver copper concentrations and total liver copper contents were also significantly lower for the sheep on the limed herbage. Liming did not affect the herbage copper concentration. Herbage copper concentrations of 8–9 ppm appeared to be insufficient to maintain the blood and liver copper status of sheep grazing limed pasture at adequate levels.

Prolonged oral supplementation providing 70 mg Cu per week as the sulphate, glycinate or EDTA salt increased blood and liver copper concentrations to similar degrees and with no apparent risk of toxicity.

Introduction

The improvement of hill pasture by liming has for a long time been implicated in the occurrence of 'swayback' in sheep. Stewart[1] has stated that the incidence of swayback was frequently increased following the application of lime. Russell & Duncan[2] and Wilson[3] have indicated that there is a high incidence of swayback in the limestone areas of the Scottish border and south-west counties which may be accentuated by the improvement of marginal land. Barlow, Purves, Butler & MacIntyre[4] supported the premise that swayback was more prevalent on recently limed or reseeded areas. They found no correlation between soil pH and the incidence of swayback and could not confirm an increased incidence on limestone soils in the region of the Scottish border.

Copper deficiency in lambs occurs on calcareous soils in South Australia but in these circumstances there is a real deficiency of copper and herbage copper concentrations are frequently as low as 2–3 ppm.

The application of lime to a pasture has generally been found to have little effect on herbage copper concentrations. MacPherson & Hemingway (data to be published) were unable to record any adverse effect on herbage copper status resulting from the application of up to 4 ton ground limestone per acre. Samples were obtained at frequent intervals over three years. Reith & Mitchell[5] have recorded that application of lime to herbage growing on copper-deficient soils may sometimes, but not always, depress the uptake of naturally occurring soil copper.

The observed effects on blood and liver copper concentrations of very high intakes of calcium compounds over prolonged periods have been variable. Dick[6] reported that high intakes of calcium carbonate (90 g/day) limited the storage of copper in the liver of sheep given 30 mg copper per day (as the sulphate) for 177 days. In a subsequent experiment however he could record no effect from feeding 120 g dicalcium phosphate per day. Hemingway, Brown & Inglis[7] gave 35 g calcium carbonate per day for thirteen weeks to growing sheep at pasture. There was no effect on liver copper concentration and only a small depressive effect on the level of blood copper.

It is important to recognise that liming may be only one of several features in the improvement of hill pasture. It may be associated with the use of basic slag and other fertilisers. There may be changes in herbage species and the intensity and duration of grazing. If liming increases the proportion of clover it might be expected that the herbage copper concentration would rise, because clover contains more copper than grass.[8]

Despite the prevalence of opinion that liming increases the incidence of swayback in sheep, there has been no published evidence on the effect of applications of ground limestone to the pasture on the copper status of the grazing animal. This paper presents information on the blood and liver copper status of sheep which have grazed limed or unlimed herbage. Grazing was for periods extending to fourteen months.

The most common form of oral copper supplementation is copper sulphate. The opportunity was taken to drench comparable sheep grazing the unlimed herbage with copper in the form of either sulphate, glycinate or EDTA salt for extended periods at weekly intervals.

Experimental

Nine plots, each of 0·8 acre were used for this experiment. The herbage, which was predominantly cocksfoot/perennial ryegrass with some crested dogstail, was considered typical of improved upland pastures. An application of 1·5 ton ground limestone per acre was given to three of the plots in the spring of 1964; the other six were left unlimed. Nitrochalk (1 cwt/acre) dressings were applied at intervals as required to meet the grazing requirements of the sheep.

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Thirty orphaned Blackface lambs which had been reared indoors from birth were randomly divided into five groups each with six lambs. One group grazed the limed plots while the other four groups grazed the unlimed pasture. Of these four groups one acted as a control. The remaining three groups were dosed once each week with a drench containing 70 mg Cu in the form of copper sulphate, copper glycine, or copper EDTA.

The lambs were introduced to the experimental plots on 18 August, 1964. Each plot was grazed in rotation until the herbage was virtually exhaused, after which the lambs were moved to an equivalently treated plot. The vacated plot was then re-fertilised with nitrogen in order to produce sufficient regrowth to allow the lambs to be moved back after about four weeks. By 25 January, 1965, however, the amount of herbage available in the plots was insufficient for the maintenance requirements of the sheep. Hay (about 1 lb/head per day; Cu content 5·0 ppm) was fed until 26 April, 1965 by which time there was sufficient growth of grass to maintain the sheep.

On 31 May, 1965 a further twelve lambs were introduced to the experiment. Six grazed the limed herbage and six grazed unlimed herbage.

Those sheep which had received a regular drench each week of a copper-containing salt were slaughtered on 19 July, 1965. By this date they had received a total of 3·15 g Cu. Two of the copper-treated sheep died of pulpy kidney within a month of commencement of the experiment. Analyses in respect of these two sheep have not been included.

The two larger groups each of twelve sheep continued to graze the limed and unlimed herbage until 7 October, 1965 when they were slaughtered.

Herbage samples for copper analysis were obtained at frequent intervals during the course of the experiment. Blood samples were taken on eleven occasions from the limed and control groups and on nine occasions from the three copper-treated groups of sheep. The whole livers of all the sheep were obtained at slaughter for the determination of dry weight, copper concentration and total liver-copper content.

Results

Herbage copper concentrations

The mean and range of herbage copper concentrations are presented in Table I.

There was no significant difference between the copper concentrations of the limed and untreated herbages. There were no marked seasonal changes.

TABLE I

Copper concentration of herbage samples from limed and unlimed plots between August, 1964 and September, 1965

	Limed	Unlimed
No. of Samples	17	35
Range. Cu ppm	6·1–13·5	5·7–15·3
Mean Cu ppm	8·9	9·6
S.E. of mean ±	2·28	1·69

Blood copper concentrations

The changes in the mean blood copper concentrations of the separate groups are illustrated in Fig. 1. The mean blood copper concentrations and total liver copper contents of the three groups of sheep which were given regular oral supplementation with copper salts were almost identical. For simplicity of presentation the various blood copper concentrations of these three groups have been combined.

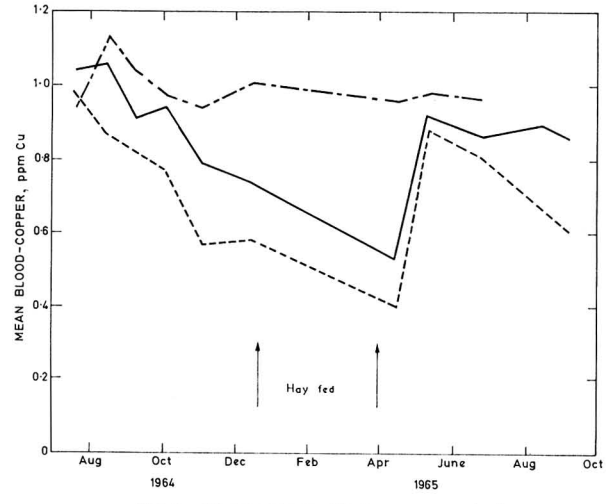

FIG. 1. *Changes in mean blood-copper concentration*

——— unlimed
– – – – limed
—— · —— Cu treated (unlimed)

At the start of the experiment the mean blood copper concentrations of the copper-treated, limed and control groups were 0·93, 0·98 and 1·04 ppm respectively. The mean blood copper concentration of the copper-treated sheep showed an initial increase to 1·14 ppm following the commencement of dosing. This level was not maintained, however, and the mean value thereafter varied little from 1·00 ppm throughout the remainder of the experiment.

The mean blood copper concentration of the group grazing the limed herbage exhibited a progressive decline during the nine months following introduction to the treated pasture. A minimum level of 0·40 ppm was reached on 12 May, 1965. The mean blood copper concentration of the control group followed a similar downward trend to that exhibited by the limed group. The decline, however, was not so rapid nor was the mean minimum level (0·53 ppm) attained so low. After 12 May, 1965 there was a rapid rise in the blood copper concentrations of both groups such that by 6 June the mean blood copper concentrations of the limed group was 0·88 ppm and that of the control group was 0·92 ppm. Thereafter the mean blood copper concentration of the limed group again decreased fairly rapidly so that two days before slaughter on 7 October, 1965 it was 0·61 ppm. During this period the control group maintained an almost constant blood copper concentration. The mean value never fell below 0·86 ppm which was the concentration on the last sampling date.

During the period of the experiment there were four sampling occasions (16 Sept., 1964, 12 May, 1965, 14 Sept., 1965 and 5 Oct., 1965) on which the mean blood copper concentration of the limed group was significantly ($P = 0·02$, 0·05, 0·01 and 0·01 respectively) lower than that of those sheep grazing unlimed herbage. On two further occasions (4 Nov., 1964 and 14 Jan., 1965) the difference in mean blood copper concentration between the limed and control groups approached significance ($P = 0·05$).

Liver copper concentrations and total liver copper contents

Table II presents the individual and mean liver dry weights, liver copper concentrations and total liver copper contents of the sheep which grazed untreated and limed herbage.

TABLE II

Individual and mean liver dry weights, copper concentrations and total copper contents of sheep grazing limed and unlimed herbage of comparable mean total copper concentration

Limed Herbage			Unlimed herbage		
Dry wt., g	Cu, ppm	Total Cu, mg	Dry wt., g	Cu, ppm	Total Cu, mg
268	8·3	2·2	160	16·6	2·7
274	10·8	3·0	108	18·4	2·0
157	11·3	1·8	186	18·4	3·4
212	11·8	2·5	133	23·1	2·9
225	14·6	3·3	122	23·5	2·9
218	14·9	3·2	209	28·2	5·9
152	15·2	2·3	192	30·8	5·9
185	17·0	3·1	172	45·2	7·8
163	18·8	3·1	215	98·4	21·1
171	21·9	3·7	171	141·4	24·2
190	26·6	5·1	168	156·6	26·3
151	60·0	9·1	127	157·2	20·0
Mean 197	19·3	3·5	164	63·1	10·4

Least Sign. Diff. ($P = 0·05$)

Mean Dry wt. 33·0 g
Mean Cu conc. 35·7 ppm
Mean Total Cu 5·8 mg

The mean liver copper concentration and total liver copper content of the sheep grazing limed herbage (19·3 ppm and 3·5 mg Cu, respectively) were both significantly lower ($P = 0·02$) than the amounts recorded for the control sheep (63·1 ppm and 10·4 mg Cu, respectively). These differences were found even although those sheep on limed herbage had significantly larger livers (197 g dry matter) than those which grazed unlimed herbage (164 g dry matter). This further emphasises that a real difference existed between the copper storage in these two treatments.

Seven of the twelve lambs grazing limed herbage had liver copper concentrations below the lowest value (16·6 ppm) recorded for the control sheep. A total of nine of the sheep which grazed limed herbage had liver copper concentrations below 20 ppm in contrast to only three of the control sheep. Values below 20 ppm have been associated with a markedly increased risk of swayback.

Table III details the mean liver copper concentration and the mean total liver copper content of those sheep which received 70 mg Cu per week in the form of either copper sulphate, copper glycine or copper EDTA. Liver copper concentrations were substantially increased compared with sheep which received no copper supplement. There was no difference in effectiveness among the three copper salts, the mean liver copper concentration of all the sheep being about 350 ppm. The range of individual values recorded was from 208 to 550 ppm. These levels are below concentrations of 1000–2000 ppm, which are associated with copper poisoning. It is concluded that such prolonged treatment with copper (averaging 10 mg Cu/day) conferred little risk of copper toxicity.

TABLE III

Mean liver dry weights, liver copper concentrations and total liver copper contents of sheep dosed with copper sulphate, copper glycine or copper EDTA

	Liver dry weight g	Liver Cu conc. ppm	Total Cu content mg	Dosed Cu in liver %
Cu sulphate	183	360·6	64·1	1·79
Cu glycine	192	346·8	66·4	1·80
Cu EDTA	172	345·3	59·3	1·61

The proportion of copper stored in the liver of these treated sheep has been calculated by comparison of the mean total liver copper content of these sheep with that of the sheep grazing unlimed pasture, expressed as a percentage of the total amount of supplementary copper which was administered. The amount stored was 1·6–1·8% of the total dose (Table II) and this did not depend on the form of supplementary copper. This proportion is similar to that which has been recorded by other workers.[6,7,9,10]

Discussion

The sharp fall in the mean blood copper concentrations of the limed and control groups during the period from January to May, 1965 may have been due, to some extent, to an insufficient intake of feed resulting from lack of herbage growth during this time. There were, however, similar rapid

reductions in the mean blood copper concentration of the limed group during October/November, 1964 and again in July/August, 1965, although at both times there was a plentiful supply of herbage to meet the nutrient requirements of the sheep. The blood copper concentration of the limed group was consistently lower than that of the control group. This indicates that liming impaired the ability of the sheep to absorb or retain the ingested copper when contrasted with similar sheep grazing unlimed herbage with a comparable mean copper concentration of about 9 ppm (Table I).

The finding that the liver copper concentration and total liver copper content of the limed group were both significantly lower than those of the control group substantiates the finding that the ability of the sheep to absorb copper from herbage had been reduced by the application of ground limestone to the herbage.

A daily supplement of 10 mg Cu was found to be fully sufficient to maintain blood and liver copper concentrations at adequate levels. This rate of supplementation was thought to be unlikely to cause the occurrence of chronic copper toxicity.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JANUARY, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Stone pavements in soils of Caernarvonshire, North Wales. D. F. Ball (*J. Soil Sci.*, 1967, **18**, 103–108).—Chemical and physical properties of a number of 'soil pavement' profiles are presented and their mode of formation and geological and pedological significance are discussed. A. H. CORNFIELD.

Origin and development of clay-with flints and associated soil horizons on the South Downs. J. M. Hodgson, J. A. Catt and A. H. Weir (*J. Soil Sci.*, 1967, **18**, 85–102).—Mineralogical and other soil profile studies are presented and the possible origin and development of the soils are discussed. A. H. CORNFIELD.

Pedoturbation by ants in a prairie soil. F. P. Baxter and F. D. Hole (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 425–428).—The mineral soil in the upper half of a representative mound of the ant *Formica cinerea* in a silt loam prairie soil consisted of about 85% of B horizon material and was unusually high in available P and K. The upward movement of soil material due to ant activity could account for the high clay content of the A horizon of this type of soil in contrast to nearby soil under forest. A. H. CORNFIELD.

Micropore size distribution of clay mineral systems. L. A. G. Aylmore and J. P. Quirk (*J. Soil Sci.*, 1967, **18**, 1–17).—The micropore size distribution of compacted clay mineral systems was studied by means of low-temp. N_2-sorption isotherms to saturation. Clay mineral systems exist with a high degree of parallel alignment of the plate-shaped crystals. This results in a high proportion of microporosity and in relatively discrete pore sizes. In montmorillonite clays much intercrystalline overlap area in the dry matrix is inaccessible to N_2 sorption. The structural configuration of these systems may be governed to some extent by the effect in suspension of electrostatic interactions between the charged particles on crystal size, but largely by mechanical interaction on sedimentation and the size of the exchangeable cations present. A. H. CORNFIELD.

Microaggregates in soils. A. P. Edwards and J. M. Bremner (*J. Soil Sci.*, 1967, **18**, 64–73).—Studies of dispersion of soil aggregates by sonic vibration, cation-exchange resin and chemical techniques suggest that microaggregates are formed by a solid-phase reaction involving linkage of electrically neutral clay mineral and org. matter particles by polyvalent metals on exchange sites. A. H. CORNFIELD.

Dispersion of soil particles by sonic vibration. A. P. Edwards and J. M. Bremner (*J. Soil Sci.*, 1967, **18**, 47–63).—Soils were as effectively dispersed by subjecting soil-water suspensions to sonic or ultrasonic vibrations as by chemical methods normally used. The pipette method for $< 2\ \mu$ material gave similar values whether dispersion was caused by sonic vibration or by chemical methods. The vibration method was effective for calcareous soils and also for those high in montmorillonite and org. matter. Dispersion occurs without dissolution of more than traces of org. and inorg. material and does not affect the pH or conductivity of the soil suspension. A. H. CORNFIELD.

Direct measurements of the distribution of oxygen in soil aggregates and in columns of fine soil crumbs. D. J. Greenwood and D. Goodman (*J. Soil Sci.*, 1967, **18**, 182–196).—A modified micro electrode polarographic method (*J. polagr. Soc.*, 1960, 22) was used to measure the distribution of O_2 in (a) agar which contained yeast and glucose to serve as a model of a respiring water-saturated soil, (b) saturated spherical soil aggregates, and (c) partly saturated columns of fine soil crumbs. Measurements in (a) and (b), but not in (c), agreed closely with predictions by diffusion theory. The disagreement appeared to result from water being unevenly distributed in the columns. A. H. CORNFIELD.

Water movement in soils as influenced by temperature gradients. G. W. Gee (*Diss. Abstr.* B, 1966, **27**, 1671).—Small columns of aggregates (0·5–1·0 mm.) of a silt loam treated with *VAMA*, were packed to a bulk density of 1·05 g/cc, the initial water content being 0·15–0·18 cc/cc (i.e., 3 and 1 bars tension). Periodically, the water contents were monitored by the attenuation of a highly collimated beam of thermalised neutrons until reaching an apparent steady state of zero net flow. Water flow induced by thermal gradients may be described by linear equations with constant coeff. The transfer coeff. (Q**) derived from irreversible thermodynamic considerations was a non-linear function of water content and apparently dependent on the imposed temp. gradient. Flow rates predicted by the Taylor-Cary flow equation show fair agreement with data obtained over 24 h but deviated considerably over longer periods. Both the Philip–de Vries equation and Fricks law under-estimated the first-day flow by factors 3 and 8 respectively. A. G. POLLARD.

Water flow in an unsaturated soil with a step-type initial water content distribution. R. D. Jensen and A. Klute (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 289–296).—A theory, based on experimental evidence, was developed which explains the flow of water against the moisture gradient observed when water was removed from the upper surface of soil columns made up of two layers of a given soil, with the upper layer at a higher moisture content than the lower half. It was shown that the initial direction of the pressure head gradient reversed without a corresponding reversal in the moisture gradient, and disturbance of the soil during the handling procedures and hysteresis were instrumental in reversing the pressure head gradient. A. H. CORNFIELD.

Seepage of steady rainfall through soil into ditches of unequal water level heights. W. L. Powers, D. Kirkham, and G. Snowden (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 301–312).—The technique of using a finite series of orthonormal functions generated from products of trigonometric and hyperbolic functions to solve a saturated water flow problem is illustrated. Several flow nets showing streamlines, equal potential lines, and the water table heights for different geometries of steady rainfall seeping through soil into ditches of unequal water level heights are presented. A. H. CORNFIELD.

Evaporation in forests. A. J. Rutter (*Endeavour*, 1967, **26**, No. 97, 39–43).—It is shown that areas covered with forests lose more water by transpiration than do areas covered with other vegetation. Thinning of forests can reduce evaporation appreciably. However, a conclusion whether forests are undesirable on catchments is premature because the evidence is still insufficient. (25 references.) I. DICKINSON.

Effect of black granular mulch on soil temperature, water content, and crusting. H. K. Qashu and D. D. Evans (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 429–435).—Application of a surface mulch of granular coke increased daytime temp. in the top 30 cm of soil, increased water content in the top 3 cm of soil, and maintained a friable crust compared with a rigid crust in the control. A. H. CORNFIELD.

Influence of texture on the moisture characteristics of soils. IV. Method of estimating the available water capacities of profiles in the field. P. J. Salter and J. B. Williams (*J. Soil Sci.*, 1967, **18**, 174–181).—The available-water capacity (AWC) of soil profiles in the field was measured by summing the products of the thickness of each horizon and the mean value of AWC for the relevant textural class (derived from average particle-size composition and from a relationship between particle size composition and AWC). When AWC estimated in this way was compared with measured values for 39 soils the mean error (the error of the estimated AWC value relative to the measured value) was $\pm 10\%$ when a 21 textural-class system and $\pm 17\%$ when a 12 class system was used. A. H. CORNFIELD.

Psychrometric measurement of soil water potential without precise temperature control. S. L. Rawlins and F. N. Dalton (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 297–301).—A theoretical analysis showed that by imposing certain boundary conditions it would be possible to eliminate the major effects of temp. fluctuation on psychrometric measurements of soil water potential. Tests on a psychrometer constructed to meet these boundary conditions were carried out on a soil column in the greenhouse. Although diurnal fluctuation of soil temp. was as great as 5° ,water potential was measured with an apparent accuracy of $\pm 0{\cdot}5$ bars throughout two irrigation cycles. A. H. CORNFIELD.

Correlation of surface area with other properties of nineteen British clay soils. D. M. Farrar and J. D. Coleman (*J. Soil. Sci.*, 1967, **18**, 118–124).—Correlation coeff. (r) among total surfaces area, liquid limit and cation-exchange capacity of nineteen British clay soils were 0·90 or higher. External surface was somewhat more poorly correlated with these three properties and also with plastic limit and clay content, which properties were not very well correlated with each other or with other properties.
A. H. CORNFIELD.

Measurement of soil moisture in the field by neutron moderation. I. F. Long and B. K. French (*J. Soil Sci.*, 1967, **18**, 149–166).—The design calibration working precautions and field use of an apparatus using neutron moderation for measuring soil water are described. Results using the apparatus for measuring extraction patterns of water in profiles of bare and cropped soils are presented.
A. H. CORNFIELD.

Comparison of water content–pressure head data obtained by equilibrium, steady state, and unsteady-state methods. G. C. Topp, A. Klute and D. B. Peters (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 312–314).—The water content–pressure head relationship for a small, well-confined, rectangular sample of fine sand was obtained during drying for different water flow conditions. At a given pressure head, the water content of the sand was higher in unsteady flow cases than in the equilibrium and steady-state cases.
A. H. CORNFIELD.

Ionic exchange behaviour in a loam soil as indicated by movement of calcium-45. M. A. Arshad and J. A. Carson (*Proc. Soil. Sci. Soc. Am.*, 1967, **31**, 321–324).—The movement of $^{45}Ca^{2+}$ placed near the soil surfaces as affected by leaching with solutions of different salts varied with the type of cation and of anion of the salt used. Movement was higher with SO_4^{2-} than with Cl^- solutions. The data may be useful in understanding the development of solonetzic soils. A. H. CORNFIELD.

Influence of adsorbed sodium and gypsum content on permeability of glacial till soils. J. C. van Schaik (*J. Soil Sci.*, 1967, **18**, 42–46).—The permeability of glacial till (montmorillonitic) soils was negligible when their exchangeable Na % (ESP) exceeded 15–20. In soils containing $CaSO_4$ permeability was reduced to low levels only when ESP exceeded 30–35. A. H. CORNFIELD.

Apparatus for automatically controlling solution flow rate through soil columns. I. J. Graham-Bryce, R. I. Davies, and A. A. H. Al-Rawi (*J. Soil Sci.*, 1967, **18**, 39–41).—A simple apparatus for automatically maintaining a constant flow-rate through several columns simultaneously is described. A. H. CORNFIELD.

Alcohol–water interactions on montmorillonite surfaces. R. H. Dowdy (*Diss. Abstr.* B, 1966, **27**, 1669–1670).—Vapour phase absorption of ethanol (**I**) and ethylene glycol (**II**), on homoionic montmorillonite surfaces was examined by *X*-ray diffraction spectroscopy and gravimetric methods. The surfaces were dehydrated by equilibration with **I** vapour at a relative pressure of unity or with **II** at 115° for 24 h. Adsorption of the two compounds was reversible to exchange with water at 40% R.H., with the possible exception of Al-glycol complexes which appear to retain small amounts of glycol in equilibrium with atm. moisture. The rate of loss of **I** from Cu-montmorillonite during re-hydration is a diffusion-controlled process; replacement of **II** by water conformed to the conditions of second-order kinetics. Evidence is advanced showing that the binding of **I** and **II** by montmorillonite is dependent on cation dipole bonds rather than on a OH·····O-clay type of interaction. A. G. POLLARD.

Clay mineralogy and mica-vermiculite layer charge density distribution in the Switzerland soils of Indiana (U.S.). D. F. Post and J. L. White (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 419–424).—Clay mineral weathering was studied in a loess mantled residual soil developed from micaceous Ordivician limestone and calcareous shales. A decrease in layer charge density of the vermiculite weathering product was traced from the parent material upward in the soil profile. A. H. CORNFIELD.

Cation-exchange reactions of soil and specimen vermiculites. J. D. Rhoades (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 361–365).—Vermiculites separated from soils differed considerably from specimen vermiculites (from 3 locations) in their ion-exchange and K-sorption properties, whereas no such differences occurred between soil and specimen monmorillonites. This indicates that the extrapolation of properties of specimen vermiculite to soil systems may not be justifiable. A. H. CORNFIELD.

Soil temperature patterns in surface-insulated containers in water baths related to maize behaviour. J. M. Walker (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 400–403).—Soil surface insulators did not establish isothermal conditions in soil in metal containers placed in constant temp. water baths. Maize seedling behaviour was significantly influenced by these nonuniform soil temp. conditions.
A. H. CORNFIELD.

Interstratification in vermiculite and biotite produced by potassium sorption. I. Evaluation by *X*-ray diffraction pattern inspection. II. Evaluation by one-dimensional Fourier analysis. J. D. Rhoades and N. T. Coleman (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 366–372, 372–377).—A study was made of the nature of the interlayer minerals formed when homoionic (Na^+ or Mg^{2+}) vermiculite and biotite were interacted with KCl to alter the interlayer cation composition to varying degrees of Na and K or Na and Mg saturation. A. H. CORNFIELD.

Potassium and ammonium fixation by vermiculitic soils. A. L. Page, W. D. Burge, T. J. Ganje and M. J. Garber (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 337–341).—Under wet conditions vermiculitic soils fixed approx. the same amounts of NH_4^+ and K^+, and cation exchange capacity reductions were essentially equal to the amounts fixed. When the amount of NH_4^+ or K^+ added to the soils subjected to oven drying exceeded the fixing capacity established for the soils under wet conditions, NH_4^+ and K^+ were fixed without further reduction in the cation-exchange capacity.
A. H. CORNFIELD.

Desorption of phosphate from kaolinite. U. Kafkafi, A. M. Posner and J. P. Quirk (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 348–353).—When an attempt was made to wash adsorbed PO_4^{3-} from kaolinite at pH 5·7 in 0·01 M-KCl, some of the PO_4^{3-} was desorbed while the remainder became fixed. The latter was not isotopically exchangeable with ^{32}P-labelled PO_4^{3-}. The process of fixation may involve a surface neutralisation of one of the protons of the adsorbed $H_2PO_4^-$, thus giving a double link to the surface. A large increase in the equilibrium concn. of PO_4^{3-} in solution renders the fixed PO_4^{3-} isotopically exchangeable. A. H. CORNFIELD.

[A] **Salt displacement and titration of aluminium chloride-treated trioctahedral vermiculites.** [B] **Salt displacement of acid-treated trioctahedral vermiculites.** M. T. Kaddah and N. T. Coleman (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 328–332, 333–336).—[A] The exchange acidity and its components (H_3O^+ and Al^{3+}), displaced from $AlCl_4$-treated vermiculites by neutral salt solutions, varied with the kind and concn. of the displacing salt, the source of vermiculite, the ageing period and Al-saturation, and the no. of Al-saturation and salt-displacement cycles. Potentiometric titration of Al-vermiculites in N-NaCl showed the presence of exchangeable H_3O^+, Al^{3+}, and probably hydroxy-Al ions. *X*-ray diffraction revealed interlayer deposits in vermiculite samples that had been Al-saturated and then leached with neutral salts.

[B] Solutions of NaCl, KCl, $CaCl_2$, and $MgCl_2$ displaced variable amounts of H_3O^+, Al^{3+} and Mg^{2+} from acid-treated trioctahedral vermiculites. The proportion of the displaced ions depended also on the source of vermiculite and the storage time of the treated vermiculites. *X*-ray diffraction patterns of K-saturated acidified samples showed interlayer deposits and reduced collapsibility.
A. H. CORNFIELD.

Infrared study of the adsorption of benzoic acid and nitrobenzene in montmorillonite. S. Yariv, J. D. Russell and V. C. Farmer (*Israel J. Chem.*, 1966, **4**, 201–213).—Montmorillonite (**I**) films in watchglasses were immersed in nitrobenzene (**II**) for 2 days over P_2O_5 in a desiccator then blotted between filter paper and excess **II** allowed to evaporate. Benzoic acid (**III**) was taken up by similar films from boiling CCl_4 or other solutions or by evaporating aq. dispersions of **I** containing dissolved **III**; or by absorption from the vapour phase at 100°. Layer spacing in the films was determined with an *X*-ray diffractometer. Ir spectra showed that water was completely displaced by **II** from K^+ and NH_4^+—**I** and that that remaining in **I** containing other cations (Mg^{2+}, Ca^{2+}, Cu^{2+}) was more weakly H-bonded than that present in the natural state. **II** was displaced fairly rapidly by water vapour at 45%. R.H. Films of **I** adsorbed 6–20% **III** from boiling saturated solution in

CCl_4. **III** was removed by cold CCl_4 containing atm. moisture. On **I** saturated with Al^{3+}, Cu^{2+}, Ca^{2+} and Mg^{2+} the **III** adsorbed after 2 weeks in 0·06% solution was almost entirely in the form of the benzoate anion; with Na^+ and K^+ adsorption was slower and there were significant amounts of interlayer acid as well as small amounts of benzoate anion. It is concluded that **II** and **III** are directly co-ordinated to NH_4^+ and K^+ and are co-ordinated through water mol. to the more highly polarising exchangeable cations in the interlayer space of **I**. (21 references.) J. I. M. JONES.

Ion exchange in soils from the ion pairs potassium-calcium, potassium-rubidium, and potassium-sodium. J. Deist and O. Talibudeen (*J. Soil Sci.*, 1967, **18**, 125–137).—Isotopic exchange of ^{42}K, ^{24}Na, ^{86}Rb, and ^{45}Ca was used to measure the equilibria between arable soils and mixed Cl^- solutions of the ion pairs, K–Ca, K–Rb, and K–Na; the results were interpreted by a thermodynamic treatment of the exchange isotherm (*J. Chem. Phys.*, 1953, **21**, 714). The cation-exchange capacities of the soils were not constant for the three ion pairs and decreased appreciably with K-saturation in the K–Ca systems because Ca ions were trapped inside the interlayer spaces. The soils' preference for K in the K–Na systems and for Rb in the K–Rb systems is explained in terms of the standard free-energy changes of the exchange reactions. The activity coeff. (f) of the adsorbed ions changed with K-saturation differently for the 3 ion pairs; f_{Na} and f_{Ca} decreased continuously with increasing K-saturation whereas f_{Rb} remained almost constant. With increasing K-saturation, f_K increased in the K–Na systems, remained unchanged in the K–Rb systems, and first increased and then decreased in the K–Ca systems. These changes are interpreted in terms of the effect of the various ions on the interlayer space of 2 : 1 type clay minerals and the possible distribution of adsorbed ions between the Gouy and Stern layers. A. H. CORNFIELD.

Thermodynamics of potassium–calcium ion exchange in soils. J. Deist and O. Talibudeen (*J. Soil Sci.*, 1967, **18**, 138–148).—Cation-exchange characteristics of the K : Ca saturated forms of five soils were measured at 25° and 50°. The rates of isotopic exchange of ^{42}K and ^{45}Ca were too fast to be measured except that of ^{42}K in the K : Ca Harwell soil at 25°. The slower isotopic exchange of K in this soil was attributed to the presence of a zeolite, clinoptilolite. The intra-particle diffusion coeff. of K in this soil increased with K-saturation to a max. at about 40% K, probably because of the blocking action of the more highly hydrated Ca ions at small K-saturation in clinoptilolite. The cation-exchange capacity, measured by istopic exchange along the K : Ca adsorption isotherm, decreased with increasing temp. probably because some interlayer spaces collapsed. The standard free energy, enthalpy, and entropy changes were negative for the reaction $Ca\text{-soil} + 2K^+ = 2K\text{-soil} + Ca^{2+}$. These results seem to show that K is more strongly bound than Ca by the soil and that the Ca-preference shown by the isotherm at small external electrolyte concn. is caused by entropy changes in solution. Calculated activity coeff. of the exchangeable ions changed with K-saturation similarly at both temp. but values at 50° were smaller than at 25°. A. H. CORNFIELD.

Positive adsorption from mixtures of three electrolyte solutions. A. K. Helmy (*J. Soil Sci.*, 1967, **18**, 35–38).—The double layer theory was used for estimating the relative amounts of positively adsorbed cations when a mixture of three salts with mono-, di-, and trivalent cations is present in clay electrolyte systems. When the salts are present at equal concn. the adsorbed cations are dominated by the trivalent cation. A. H. CORNFIELD.

Release of potassium from soils and clays by plant and chemical extractions and the effect on clay mineral structure. E. S. Conyers (*Diss. Abstr.* B, 1966, **27**, 1669).—Soils of varied ability to release K were treated with K and/or diluted with sand and cropped with millet or with lucerne (four cuttings). K was extracted by various chemical agents before and after cropping. Both plants and chemical extractants removed more K from soil–sand mixtures than was calculated from the proportional composition of the mixtures. Some correlations were established between K removed by chemical extractants before cropping and the K uptake by the plants; the latter was poorly correlated with Woodruffs $\triangle$F values. Cropping reduced the exchangeable K in the soils and also the K extracted by boiling HNO_3. The latter removed dry-fixed K effectively, but was less active in respect of wet-fixed K. When plants were grown in suspensions of Ca-saturated soil clays resulting changes in the structure of the clays included alteration of the interlayer surfaces, partial degradation of illite, diminution in kaolinite and, in one case, of vermiculite content of the clay. These changes were associated with a gradual decline in the uptake of K by the crops. The chemical 'weathering' of clays was examined by treatment with exchange resins alone or with Na, $NaBPh_4$ or boiling HNO_3 followed by X-ray diffraction pictures. The resin–$NaBPh_4$ treatment of illite removed considerable amounts of K holding the silicate layers together, thus degrading the illite. Treatments with HNO_3 and resin probably removed amorphous material only; resin alone had little effect. HNO_3 caused severe damage to a vermiculite clay, resin alone or with $NaBPh_4$ having little effect. The chemical 'weathering' treatments caused no structural alteration in kaolinite or montmorillonite. A. G. POLLARD.

Tracer study of nitrogen balance and residual nitrogen availability with twelve soils. J. O. Legg (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 403–406).—In pot tests using ^{15}N-labelled NO_3^--N (50–200 ppm, soil basis), the recovery of tracer N in an oat crop plus two succeeding Sudan grass crops averaged 94% for 12 soils, but was as low as 82% for one soil receiving low N. The recovery of applied N was not influenced much by N rates in most soils. The first Sudan grass crop removed 10–41% of the residual N from the oat crop, whilst the second crop removed about 4·5% of the residual N from the first two crops. A. H. CORNFIELD.

Soil particle-size fractions and nitrogen mineralisation. J. B. D. Robinson (*J. Soil Sci.*, 1967, **18**, 109–117).—Although the fractionation of East African soil samples into a no. of size groups <2 mm influenced mineralisable N values determined by incubation, no advantage would be gained by using refined soil fractions in incubation tests. Org. C, total N, and silt contents of the different particle size fractions were significantly correlated (positively), whilst the coarse + fine sand fraction was significantly correlated (negatively) with mineralisable N. A. H. CORNFIELD.

Nitrogen losses from urea as affected by altering soil urease activity. P. G. Moe (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 380–382).—Application of urease enzyme to soil treated with urea increased the rate of urea hydrolysis and NH_3 volatilisation losses early in incubation and also the rate of nitrification later in incubation. Application of an enzyme inhibitor, *p*-chloromercuribenzoate, decreased the rate of urea hydrolysis but prevented nitrification for the rest of the incubation period due to high soil levels of NH_4^+. Application of a maize mulch increased initial losses of NH_3 by volatilisation, but reduced leaching losses of N. NH_3 volatilisation losses were lower when lime and urea were applied on the surface of the mulch than when applied on bare soil. A. H. CORNFIELD.

Dynamics of nitrification in soils using a miscible displacement technique. K. T. Erh, D. E. Elrick, R. L. Thomas and C. T. Corke (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 383–389).—The miscible displacement technique for studying nitrification involves continuous leaching of the soil, maintained at a suction of 0·33 bar, with $(NH_4)_2SO_4$ solutions of selected strengths and with frequent analyses of the effluent for NO_3^-. The method is claimed to be superior to incubation and perfusion methods, since it permits nitrification in an open system with continuous removal of the products of nitrification, thus approximating conditions in the field. The method showed the presence of a lag phase before nitrification rate became high enough to convert all the NH_4^+ in the influent to NO_3^-. It also showed that NO_2^- accumulated temporarily when high concn. of NH_4-N (500 ppm) was present in the influent, and the ability of 'N-serve' greatly to reduce nitrification rate. A. H. CORNFIELD.

Nitrogen gradients and nitrification associated with decomposing maize plants and barley straw in soil. J. H. Smith (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 377–379).—During 16 days incubation at 27° of soil containing a layer of maize plant material, (2·1% N), NH_4-N accumulated near the plant material to such an extent that nitrification was inhibited. With plant material containing 1·78% N, NH_4-N accumulated, but not at sufficient concn. to prevent nitrification, whilst with plant material containing 1·27% N, soil NO_3^- near the plant material was immobilised. NH_4-N accumulation in the soil decreased rapidly with increasing distance from the plant material. Similar results, though of lesser magnitude, were found with layers of barley straw containing different N%. A. H. CORNFIELD.

Chemical availability of native and applied phosphorus in soils and their textural fractions. R. L. Halstead (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 414–419).—The Al-P fraction of several Canadian soils was correlated with % yield of oats in greenhouse tests and with $NaHCO_3$-extractable P. Ca-P was the main form of P in all but the Podzol soils, in which Fe-P predominated. Org. P, Al-P, Fe-P and $NaHCO_3$ sol. P increased with decreasing particle size, whereas Ca-P, total inorg. P and 0·002 N-H_2SO_4- extractable P

increased with decreasing particle size in the Podzols, but not in the other soils. A. H. CORNFIELD.

Soil factors affecting plant uptake of phosphate. D. Gunary and C. D. Sutton (*J. Soil Sci.*, 1967, **18**, 167–173).—Short- and long-term uptakes of P by ryegrass grown in pots were both well correlated with combinations of log [PO_4^{3-}] in solution, (log P), and with a capacity factor, (L-value). It is suggested that log P measures an intensity/kinetic complex that takes account of intensity, rate, and diffusion factors. The best correlation with a single parameter was with phosphate adsorbed by an anion-exchange resin, which gives a good measure of the capacity factor and also some measure of the intensity/kinetic complex. On soils of normal PO_4^{3-} status, the capacity factor tended to be the more important, suggesting local exhaustion of labile PO_4^{3-}. With soils treated with PO_4^{3-}, the intensity/kinetic complex measured by log (P) was dominant both for short- and long-term uptakes. A. H. CORNFIELD.

Metabolisable dyes for minimising phosphate sorption of Cecil soil. K. S. LaFleur and G. R. Craddock (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 324–327).—The addition of metabolisable anionic dyes (quercitin, gallein, and Mordant Violet 39) reduced the amount of phosphate adsorbed by Cecil soil, since they had a higher affinity than phosphate for the adsorbing sites. A. H. CORNFIELD.

'A' values of potassium related to other indices of soil potassium availability. W. F. Nuttall, B. P. Warkentin and A. L. Carter (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 344–348).—A method was developed to measure 'A' values of K with oat plants grown in the greenhouse and with $^{42}K^+$ applied to the soil 14–18 days after seeding. The total exchangeable + nonexchangeable K for 11 soils accounted for 56% of the variability of K uptake at the time of the tracer application, whereas 92% of the variability in 'A' values was accounted for by these measurements. A. H. CORNFIELD.

Effect of soil pH on the availability of magnesium to maize from magnesium sulphate and high-magnesium liming materials. N. R. Usherwood and J. R. Miller (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 390–393).—The application of Mg at 7·5–15 ppm (soil basis) in the form of $MgSO_4$, finely ground or burnt or hydrated dolomitic limestone increased dry matter yields of maize plants in pot tests to about the same extent irrespective of Mg source or initial soil pH (4·4–6·7). Coarse dolomitic limestone was inferior to the other sources. Rates of Mg application higher than 15 ppm did not increase yields further. Increasing soil pH from 5·3 to 6·7 decreased uptake of Mg by the plant where coarse and fine dolomitc limestone was applied, but not usually where $MgSO_4$ was applied. Mg uptake from $MgSO_4$ and burnt and hydrated dolomitic limestone was similar and higher than from finely-ground dolomitic limestone. A. H. CORNFIELD.

Determination of iron with thiocyanate. P. H. Hsu (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 353–355).—The effects of acidity and concn. of CNS^- on the intensity and stability of the colour obtained with Fe^{3+} were studied, and a procedure giving reproducible results is described. A. H. CORNFIELD.

Copper deficiency of wheat in the Rift Valley, Kenya. A. Pinkerton (*J. Soil Sci.*, 1967, **18**, 18–26).—Copper deficiency in wheat occurs on soils derived from pumice and ash and containing <3 ppm Cu as determined by bioassay with *Aspergillus niger*. Tomatoes were a good indicator crop in pot tests for indicating deficiency of Cu in these soils to wheat. Soil treatment with $CuSO_4$ and $CuOCl_2$ eliminated Cu deficiency in wheat and markedly increased grain Cu%. A. H. CORNFIELD.

Distribution of total and acid extractable (0·1 N-hydrochloric acid) zinc in Hawaiian soil profiles. Y. Kanehiro and G. D. Sherman (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 394–399).—Extractable Zn in Hawaiian soil profiles ranged from 0·1 to 17·9 ppm and in general increased with depth of soil. Total Zn (51 to 288 ppm) was not clearly related to depth of soil. Total and extractable Zn were significantly correlated, particularly in young and unweathered soils, but there was poor correlation between soil pH and extractable Zn. The occurence of Zn deficiency in plants was better correlated with extractable than with total soil-Zn values. Zn deficiency in plants was most commonly found in oxisols and ultisols that had undergone intensive weathering or had their subsoils exposed. A. H. CORNFIELD.

Contributions of fixed charge and mobile complexing agents to the diffusion of zinc. J. F. Hodgson, W. L. Lindsay and W. D. Kemper (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 410–413).—The presence in agar-agar gel of fixed negative charges (polygalacturonic acid) increased the diffusion transport of Zn from $ZnCO_3$ only slightly, whilst the presence of a sol. complexing agent (citrate) increased Zn transport several-fold. Sol. complexing agents, such as those known to be secreted by plant roots, may be important in increasing the transport of ions to roots. A. H. CORNFIELD.

Quantitative estimation of assimilable and reserve sulphate fractions of soil and their renewal rates. S. Fortini, G. Gardas and S. Panella (*Annali Staz. chim.-agr. sper., Roma*, 1965, [1966], Ser. iii, 238, 281–300).—Methods for the determination of sol SO_4^{2-} (extracted from soil with 10^{-3} M-$CaCl_2$ sol.) and adsorbed SO_4^{2-} (extracted after addition of $CaCO_3$ to the system), and the dynamic equilibrium of S in the soil (using $^{35}SO_4^{2-}$ as tracer) are reported. Renewal rates for available sulphates found were 2·7 μg S/g/day in volcanic and 4·6 μg/g/day in humic soil; the corresponding active fraction of the total reserves were 3·8 and 14% respectively. (17 references.) E. C. APLING.

Sulphate movement, adsorption, and desorption in three Costa Rican soils. E. Bornemisza and R. Llanos (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 356–360).—Movement of SO_4^{2-} in lysimeters was small and was enhanced only slightly by the presence of PO_4^{3-}. The soils adsorbed SO_4 from aq. solutions in proportion to the amount of SO_4 present even up to 1000 ppm. Adsorption was positively, and desorption negatively, correlated with fineness of soil texture. A. H. CORNFIELD.

Ecology and physiology of soil fungi involved in the degradation of lignin and related aromatic compounds. D. Jones and V. C. Farmer (*J. Soil Sci.*, 1967, **18**, 74–84).—Various species of soil micro-fungi were able to degrade lignin when this was the sole source of C in a mineral salts medium. The species of soil fungi able to metabolise vanillic acid in pure cultures was extended to include *Stilbium*, *Humicola* and two unidentified species. A. H. CORNFIELD.

Growth and survival of Rhizobium in peat culture. R. J. Roughley and J. M. Vincent (*J. appl. Bact.*, 1967, **30**, 362–376).—Factors affecting the use of peat in the prep. of legume inoculants are examined. Growth and survival of three strains of Rhizobia in peat culture were influenced by the source of the peat, the pH and the method of adjusting it, by the method of sterilisation, the drying temp. and the moisture content of the product. The choice of peat must be made only on actual tests of suitability. Sterilisation of the peat favoured the rapid growth of the rhizobia and subsequent viability during storage: it was essential in the case of the cowpea strain. Sterilisation by γ-irradiation was generally superior to autoclaving. Drying peat at >100° adversely affected the growth and survival of the organisms apparently as a result of the excessive heat of melting of the dried peat, which is sufficient to cause the death of many organisms and also the formation of inhibitory substances. Peat can be dried sufficiently and safely at temp. 80–100°. In the finished culture the optimal moisture content was 40–50% (wet wt. basis). A. G. POLLARD.

Interference by cells of *Nitrosomonas* with the estimation of nitrite. J. H. Anderson and H. Lees (*Chemy Ind.*, 1967, 1491–1492).—Low results obtained in the colorimetric (diazotisation-coupling) determination of NO_2 produced by oxidation of NH_2OH with washed-cell suspensions of *Nitrosomonas* are ascribed to part-replacement of the reaction between diazotised sulphanilic acid (**I**) and 1-naphthylamine by a reaction between **I** and a factor in the cell-extract. This is confirmed, in a prepared extract, by the slowly decreasing concn of **I** when present alone, the slightly slower decreasing concn. of NH_2OH when present alone, and the much more rapid decrease of each when **I** and NH_2OH are both present. It is probable that a factor in the extract reacts with **I** and that NH_2OH maintains this factor in a reactive form. W. J. BAKER.

Occurrence of carbazole in a peat soil. O. C. Braids, F. L. Himes and G. W. Volk (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 435–437).—Benzene–MeOH extracted 1·5% of the dry matter of Ap horizon material of Rifle peat. Carbazole was identified in the fractions of the extracted material sol. in either light petroleum or EtOH. A. H. CORNFIELD.

Determination of organic carbon and carbonates in soils. J. V. Anderson and W. Harris (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 341–343).—Carbonate is determined by treating the soils with 2N-HCl containing 12% $SnCl_2$ and weighing the CO_2 evolved after passage through a purification train to remove everything except CO_2. CO_3^{2-}-C + org. C are determined by boiling the soil with a mixture of H_2CrO_4 and H_3PO_4 and determining the CO_2 evolved. A. H. CORNFIELD.

Relationship between oxidation-reduction potentials and oxygen-diffusion levels in waterlogged organic soils. W. Armstrong (*J. Soil Sci.*, 1967, **18**, 27–34).—The ratio redox potential/O_2 diffusion rate

(both determined at the same electrode) in org. soils was not constant. At very low O_2 values there was a relatively large increase in redox potential per unit of O_2, but as O_2 diffusion rate approached 2×10^{-8} g cm^{-2} min^{-1} the ratio became much lower and variations became more obvious. In peat of high Fe content at pH 6 the first traces of O_2 corresponded with rH 1·10–1·20 (both reduced and oxidised Fe present), whilst above 2×10^{-8} g O_2 cm^{-2} min^{-1}, rH was >1·20 and oxidised Fe probably predominates. Thus oxidised conditions in soil may occur at very low O_2 levels. The O_2 diffusing from plant roots into peats will oxidise reduced soil products and thus protect the plant. A. H. CORNFIELD.

New phosphatic fertiliser from basic slag, hydrochloric acid, and rock phosphate. M. T. Shafik and A. F. Sabry (*J. agric. Fd Chem.*, 1966, **14**, 643–644).—In laboratory experiments a product containing 25% of citrate-sol. P_2O_5 was prepared by boiling ground phosphate rock with dil. HCl for 1 min., neutralising with basic slag to the di-Ca stage, filtering, and washing. P. S. ARUP.

Hydrothermal processing of naturally occurring phosphates. S. I. Wolfkovich (*Fertil. Soc.*, 1967, Reprint).—The potential value of the hydrothermal process is emphasised and the experimental data, on which the process is based, are described. Defluorinated phosphates are characterised by high purity and neutral reaction. Russian experimental work shows the fertiliser value of these products to equal that of superphosphate in nearly all soil types, as also is their efficiency as mineral supplements in stock feeds. Details of the manufacturing process are given. A. G. POLLARD.

Improved techniques for determining available phosphorus in fertilisers. V. C. Midkiff (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1207–1212).—Multiple pipettes, flasks, filtering carts, reagent dispensers and washing devices, which are illustrated, permit the determination of available P in fertilisers by the direct available gravimetric quinoline molybdate method to be carried out much more rapidly and easily. A. A. ELDRIDGE.

Alkalimetric 'quinociac' method for [determining] phosphorus [in fertilisers]. R. D. Duncan and J. A. Brabson (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1201–1207).—In the procedure using a reagent consisting of quinoline, Na molybdate, citric acid, HNO_3 and acetone, agglomeration of the ppt. is prevented by use of additional citric acid, and the resulting ppt. is readily sol. in alkali (cf. Hoffman and Wiles, *ibid.*, 1963, **46**, 579). Results are not as precise and accurate as those obtained by the corresponding A.O.A.C. gravimetric method. A. A. ELDRIDGE.

Plant Physiology, Nutrition and Biochemistry

[A] **Rates of photosynthesis and respiration in relation to stomatal movements in leaves treated with α-hydroxysulphonate and glycollate.** H. Meidner and T. A. Mansfield. [B] **Stomatal opening in light of different wavelengths; effects of blue light independent of carbon dioxide concentration.** T. A. Mansfield and H. Meidner (*J. exp. Bot.*, 1966, **17**, 502–509, 510–521).—[A] Treatment of leaves of tobacco and *Xanthium pennsylvanicum* with α-hydroxysulphonate (**I**) reduced photosynthesis and increased the [CO_2] in the intercellular spaces. The latter may cause the closure of stomata, the opening of which followed flushing the plants with CO_2-free air. Accumulation of glycollate was caused by **I** but glycollate alone did not affect stomatal opening or the internal accumulation of CO_2.

[B] With similar [CO_2] in the steady state, stomatal opening in *X. pennsylvanicum* was greater in blue than in red light. In daylight stomatal opening probably involves two factors; (*a*), removal of CO_2 by photosynthesis and (*b*), a response to blue light which is not dependent on removal of CO_2. With increase in night length from 2 to 14 h, the effect of blue as compared with red light became increasingly effective. The rapid initial phase of closure in darkness is probably independent of CO_2 accumulations. A. G. POLLARD.

Increased rate of net photosynthetic carbon dioxide uptake caused by the inhibition of glycolate oxidase. I. Zelitch (*Plant Physiol.*, 1966, **41**, 1623–1631).—Addition of an inhibitor of glycolate oxidase α-hydroxy-2-pyridinemethanesulphonic acid, to tobacco leaf disks at 35° stimulated $^{14}CO_2$ uptake at least 3-fold, but had no effect at 25°. The inhibitor did not increase photosynthesis in maize-leaf disks at either temp. High rates of photorespiration may limit the net CO_2 uptake in many plant species. E. G. BRICKELL.

Effect of hydrolytic enzymes on the photosynthetic efficiency and morphology of chloroplasts. E. S. Bamberger and R. B. Park (*Plant Physiol.*, 1966, **41**, 1591–1600).—Both lipase from runner beans and a protease (pronase) initially cause increased intensity-dependence of the 2:6-dichlorophenolindophenol Hill reaction of spinach chloroplasts. This is followed by an increase in the extrapolated zero intensity quantum requirement. E. G. BRICKELL.

Effect of red light on the phototropic sensitivity of maize coleoptiles. H. P. Chon and W. R. Briggs (*Plant Physiol.*, 1966, **41**, 1715–1724).—The reciprocity law proved valid for all curvatures obtained. With red light, the sensitivity of the first positive curvature was decreased over ten-fold and there was clear appearance of second positive curvature for which the reciprocity law was not valid. Within 1 h of the beginning of red light at 25°, reactions leading to the decrease in phototropic sensitivity of the first positive component had gone to completion irrespective of whether or not the red light was continuous. The effect of red light could be fully reversed by low dosages of far-red light; longer doses of far red were less effective. E. G. BRICKELL.

Effect of sink region cooling on translocation of photosynthate. D. R. Geiger (*Plant Physiol.*, 1966, **41**, 1667–1672).—*Beta vulgaris*, L. var. Klein Wanzleben was studied using ^{14}C-labelled photosynthate. On cooling the sink region four phases were observed; a temporary decline, a period of translocation at the pre-treatment rate, a period of decline, and a new steady rate at 35 to 45% of the original rate. The new rate persisted throughout 26 h of cooling. Data suggest that the translocation process includes active uptake into storage and growing areas. E. G. BRICKELL.

Removal of salt from xylem sap by leaves and stems of guttating plants. B. Klepper and R. Kaufmann (*Plant Physiol.*, 1966, **41**, 1743–1747).—Measurement of the osmotic potential of the guttation liquid and of exudates at various levels in guttating plants indicated that salt is removed from the xylem in the upper part of plants, particularly the leaves. However, the concn. of salt solutions forced through individual leaves by an artificial root pressure had no influence on the osmotic potential of the guttation fluid. E. G. BRICKELL.

Biomagnetic responses in Kharkov 22 M.C. winter wheat. U. J. Pittman (*Can. J. Pl. Sci.*, 1967, **47**, 389–393).—Pregermination exposure of seeds to introduced magnetic fields (0·5 to 100 oersteds for 240 h) resulted in a stable, temp.-independent enhancement of seedling growth rate. E. G. BRICKELL.

Absorption of silica from aqueous solutions by plants D. A. Barber and M. G. T. Shone (*J. exp. Bot.*, 1966, **17**, 569–578).—The uptake by plants of SiO_2 which exists in small concn. in essentially non-polar form in water at pH 7 is examined. The entry of SiO_2 into plants involves the expenditure of metabolic energy and, except under low-humidity conditions, the rate of entry exceeds that of the transpirational loss of water, thus causing higher concn. in the xylem sap than in the external solution. A. G. POLLARD.

Rôle of boron in plants. III Anatomical observations. S. G. Lee and S. Aronoff (*Plant Physiol.*, 1966, **41**, 1570–1577).—In B-deficient sunflower plants chloroplasts degenerate and cell walls undergo profound structural changes before any visual deficiency symptoms become apparent. Mitochondria frequently show myeline figures, while nuclei may develop dense rhombohedral structures. E. G. BRICKELL.

Cobalt requirement of non-legume root nodule plants. E. J. Hewitt and G. Bond (*J. exp. Bot.*, 1966, **17**, 480–491).—Growth of the modulated plants *Casuarina cunninghamiana* and *Myrica gale* in a N-free medium requires a source of Co. This requirement is restricted to the nodules, NO_3^- and NH_4^+ being effective N sources for other plant organs. The nodules contain notable amounts of vitamin B_{12}. Reduction in N fixation by Co-deficient nodules may result from retarded growth of the endophytes. A. G. POLLARD.

Effect of level of copper on the activity of enzymes in apple leaves. A. Perumal and J. M. Beattie (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 41–47).—Ascorbic acid oxidase activity in leaves of apple seedlings increased with level of Cu in the nutrient up to 0·05 ppm, but declined slightly with 0·1 ppm Cu. There was negligible activity of this enzyme when the substrate was free of Cu. Oxidase activity increased with level of Cu up to 0·02 ppm, and then decreased with further increasing Cu. Peroxidase activity decreased with increasing Cu up to 0·02 ppm in the nutrient and then increased with further increasing Cu. Catalase activity was max. with no Cu in the substrate and decreased with increasing Cu. A. H. CORNFIELD.

Contracted state as an energy source for Ca binding and Ca + inorganic phosphate accumulation by maize mitochondria. D. G.

Kenefick and J. B. Hanson (*Plant Physiol.*, 1966, **41**, 1601–1609).—Mitochondria in the contracted state will actively bind some ^{45}Ca but no real accumulation occurs until inorg. phosphate is available. Contraction appears due to X∼I formation to which Ca will bind. Subsequent reaction with phosphate produces Ca X∼P which is the transport moiety. E. G. BRICKELL.

Expansion of the leaf surface. VI. Senescence and the usefulness of old leaves. J. M. Hopkinson (*J. exp. Bot.*, 1966, **17**, 762–770).—In *Cucumis sativus* the effects of removal and of shading ageing leaves indicate that they act as sources of nutrient material for leaves more favourably placed with regard to light. Shading of leaves hastened senescence. The rate of senescence of lower leaves is influenced more by deficiency of light than by re-distribution of contained nutrients. A. G. POLLARD.

Release of volatile selenium compounds by plants. Collection procedures and preliminary observations. B. G. Lewis, C. M. Johnson and C. C. Delwiche (*J. agric. Fd Chem.*, 1966, **14**, 638–640).—The Se-accumulating plant *Astragalus racemosus* and lucerne (non-Se-accumulating) were grown in nutrient solutions to which traces of sol. ^{75}Se were added. Amounts of ^{75}Se measurable by radioactivity were absorbed by active C granules from the air surrounding the *Astragalus* plants within 1 day whereas measurable amounts from the lucerne appeared only after 1 week. The amounts of Se emitted by the leaves varied with the Se content of the plants and, in the case of lucerne, reached max. values at the period during the day of max. illumination. (16 references.) P. S. ARUP.

Metabolism of tryptophan in petioles of *Coleus*. J. G. Valdovinos and J. E. Perley (*Plant Physiol.*, 1966, **41**, 1632–1636).—The evidence presented suggests that tryptophan is converted in *Coleus* tissue to a compound, presumably IAA, which is capable of increasing the longevity of the petioles. E. G. BRICKELL.

Electrophoretic and immunological comparisons of soluble root proteins of *Medicago sativa* L. genotypes in the cold-hardened and non-hardened condition. E. A. Coleman, R. J. Bula and R. L. Davis (*Plant Physiol.*, 1966, **41**, 1681–1685).—A zone of highly charged and/or low mol. wt. protein components were found more prevalent in the protein complements of cold-hardened material than in non-hardened material. Immunodiffusion plate tests were not so definitive for identifying genotypes or physiological conditions but did corroborate the electrophoretic interpretations. E. G. BRICKELL.

Propionate in haem biosynthesis in soya-bean nodules. E. K. Jackson and H. J. Evans (*Plant Physiol.*, 1966, **41**, 1673–1680).—The supply of haem precursors from propionate is competitive with the supply of haem precursors from the citric acid cycle. The data support the hypothesis that propionate utilisation makes possible a mechanism for the formation of succinyl-CoA in addition to that provided by the citric acid cycle. E. G. BRICKELL.

Reduction of acetylene to ethylene by soya-bean root nodules. B. Koch and H. J. Evans (*Plant Physiol.*, 1966, **41**, 1748–1750).—Soya-bean nodules catalyse the reduction of acetylene to ethylene at relatively rapid rates, the conditions necessary being similar to those for optimum N fixation which suggests that the same enzyme system is involved. E. G. BRICKELL.

Potassium loss and changes in the fine structure of maize root tips induced by H-ion. H. Marschner, R. Handley and R. Overstreet (*Plant Physiol.*, 1966, **41**, 1725–1735).—At pH 5·5–8·0 losses of K decreased with decreasing H concn. Ca reduced K loss greatly in the lower part of the pH range. The effect of phosphate upon K loss was dependent on pH, temp., and the state of tissue development. In water or dil. HCl serious injury to the fine structure of the meristem cells occurred at pH 4 and below. E. G. BRICKELL.

Reaction products of the ascorbic oxidase from *Myrothecium verrucaria*. E. B. Lillehoj and F. G. Smith (*Plant Physiol.*, 1966, **41**, 1553–1560).—Oxidase activity results in O_2 uptake exceeding 0·5 mol. per mol. of ascorbic acid and in CO_2 evolution. Moreover, an average of 10% of the oxidised product disappeared. It is suggested the enzyme has peroxidative capacity on a reductant other than ascorbic acid. An intermediate of the oxidation appears to function as the substrate yielding CO_2 and L threonic acid on degradation. E. G. BRICKELL.

Identification of *Pyrus* species after paper chromatography of leaf and bark extracts. P. B. Catlin and E. A. Olsson (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 127–144).—There were differences in the patterns of chromatographs of polyphenolic compounds of bark extracts of different species of *Pyrus* used as rootstocks. *Pyrus communis* was distinguishable from three oriental species, among which one could be distinguished from the other two. Leaf extracts were superior to bark extracts for differentiating between species and results were sufficiently reproducible within a season and between seasons to provide an accurate and objective means of differentiation between species. A. H. CORNFIELD.

Changes in amino-acid content of excised leaves during incubation. I. Effect of water content of leaves and atmospheric oxygen level. J. F. Thomspon, C. R. Stewart and C. J. Morris. **II. Rôle of sugar in the accumulation of proline in wilted leaves.** C. R. Stewart, C. J. Morris and J. F. Thompson (*Plant Physiol.*, 1966, **41**, 1578–1584, 1585–1590).—I. Considerable proteolysis took place during incubation with a resultant increase in each amino-acid in the non-protein fraction but serine, proline (**I**), γ-aminobutyric acid (**II**), and methylcysteine sulphoxide were the only acids in which there was a net synthesis. Anaerobiosis abolished the accumulation of protein in wilted leaves and produced other significant changes notably an increase in alanine and a large increase in **II**.
II. A supply of sugar or starch is necessary for **I** accumulation in wilted leaves, oxidation of these carbohydrates furnishing γ-ketoglutarate and reduced nicotinamide adenine dinucleotide for synthesis. E. G. BRICKELL.

Anthocyanin pigments of the sweetpotato, *Ipomea batatas*. M. P. Imbert, C. E. Seaforth and D. B. Williams (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 481–485).—The pigments identified were caffeoylated glucosides of cyanidin and peonidin substituted in the 3- and 5-C positions on the flavonoid nucleus. A. H. CORNFIELD.

Relation of taxonomic, climatic, and tissue maturity factors to the essential oil constituents in leaves and fruits of the *Aurantioideae*. R. W. Scora and S. Torrisi (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 262–271).—Gas–liquid chromatograms of the essential oils of the leaves and fruits of the *Aurantioideae* indicated that different genera and species could be identified by this technique. Many replications were necessary to secure reliable results and varietal differences could be detected only within climatic parameters. The composition of the essential oils varied with the physiological stage of the tissues. A. H. CORNFIELD.

Auxin activity of substituted benzoic acids and their effect on polar auxin transport. G. W. Keitt jun., and R. A. Baker (*Plant Physiol.*, 1966, **41**, 1561–1569).—Tobacco pith was used as the study media. Triiodobenzoic acid affected growth through IAA inhibition. The best growth promoters were the least inhibitory to transport and the most effective transport inhibitors were at best poor auxins. E. G. BRICKELL.

Hormonal regulation of seed dormancy in hazel (*Corylus auellanal*) and beech (*Fagus sylvatica*). B. Frankland and P. F. Wareing (*J. exp. Bot.*, 1966, **17**, 596–611).—The after-ripening of hazel and beech seed involves low-temp. and moist conditions for ∼12 weeks. Germination of dormant seeds is stimulated by gibberellic acid (**I**), kinetin or thiourea, provided the pericarp is not intact. The activity of gibberellin-12 in this respect is 10-times that of **I**. No changes in concn. of auxin or inhibitors were apparent during the after-ripening period. Some differences in distribution of gibberellin like substances in chilled and dormant seeds are recorded and discussed. A. G. POLLARD.

Two gibberellin-like substances in young shoots of tomato (*Lycopersicon esculentum*, Mill.). T. A. Hill and I. W. Selman (*J. exp. Bot.*, 1966, **17**, 534–545).—Two substances resembling gibberellins were isolated from the acidic fraction of tomato shoots and separated by paper chromatography. Some characteristics of the substances are described. The proportions of the two substances in the shoots varied with the age of the tissues. A. G. POLLARD.

Uptake of growth substances. VIII. Accumulation of chlorinated benzoic acids by *Avena* segments: possible mechanism for the transient phase of accumulation. IX. Further studies of the mechanism of uptake of 2,3,6-trichlorobenzoic acid by *Avena* segments. M. A. Venis and G. E. Blackman (*J. exp. Bot.*, 1966, **17**, 771–789, 790–808: cf., *idem.*, *ibid.*, 270). VIII. The uptake of the various chlorinated acids by segments from the mesocotls of *Avena sativa* was affected differentially by preliminary treatment of the segments with a 0·01 M-K maleate buffer at pH 5·5. The uptake of 2,3,6-trichlorobenzoic acid (**I**) by the segments becomes negative a few h after excision; this effect is prevented by addition of streptomycin, the action of which on the uptake of other acids of the series, shows apparent differences.

IX. The actions of several physiologically active substances on the uptake of **I** are examined and a mechanism for the accumulation of the acid within the mesocotls is suggested. A. G. POLLARD.

Effects of gibberellin and growth retardants on bud development and cold hardiness of peach. L. J. Edgerton (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 197–203).—Spraying peach trees with K gibberellate (50–80 ppm) increased vegetative growth, sharply reduced the numbers of flower buds, delayed differentiation, produced smaller buds during the dormant season, and increased cold hardiness of the buds. Flowers on treated trees appeared normal the following spring and had a higher % fruit-set than on unsprayed trees. The fruits were larger on treated than on comparable untreated, thinned trees. Application of 2000 ppm N-dimethyl amino succinamic acid (Alar) in July stopped terminal elongation of shoots but did not prevent the breaking of lateral buds near the shoot apex. Flower bud formation was increased slightly, whilst cold hardiness of buds and wood during the winter was not affected.

A. H. CORNFIELD.

Independence of morphactin and gibberellin effects on higher plants. J. D. Mann, H. Hield, Kung-Hing Yung and D. Johnson (*Plant physiol.*, 1966, **41**, 1751–1752).—Experiments with sweet orange seedlings showed that the action of morphactins (fluorene-9-carboxylic acid deriv.) is at least partially independent of both gibberellin synthesis and action. Combinations of morphactin with gibberellin, used as general foliar sprays, may be useful in the chemical shaping of plants. E. G. BRICKELL.

Technique for growing deciduous fruit trees in water culture. H. Mori (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 253–261).—The technique for growing apple trees up to 7 years of age in solution culture is described. A. H. CORNFIELD.

Analysis for aflatoxins. M. Wiley (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1223–1224).—In thin layer chromatographic separation using methyl acetate as solvent the R_F values of impurities and aflatoxins differ from those obtained in a methanol–$CHCl_3$ system, so that aflatoxins are readily separated from interfering fluorescent compounds (cf. Pons and Goldblatt, *J. Amer. Oil Chemists Soc.*, 1965, **42**, 471). A. A. ELDRIDGE.

Determination of dienestrol diacetate in mixed feeds. V. B. Hill, E. L. Schneider and E. E. Martin (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1233–1236).—The residue on evaporation of an ethyl ether extract of the sample is refluxed with methanol and NaOH; the solution is washed with $CHCl_3$, the pH is adjusted to 9·5, and a $CHCl_3$ extract is evaporated to dryness. The residue is dissolved in AcOH, an aliquiot is heated with anisaldehyde and HCl and the extinction is measured at 610 nm. Recoveries of 92·6 to 107% are reported. Acetone interferes, forming a red-orange complex.

A. A. ELDRIDGE.

Assay and preparatory separation of aflatoxins from groundnut products. R. M. Eppley (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1218–1223).—Clean, quant. extracts of aflatoxins are obtained by extraction of a water-wetted sample with $CHCl_3$; the extract is passed through a column of SiO_2 gel and Na_2SO_4, which is eluted with hexane, followed by diethyl ether, and then aflatoxin is eluted with $CHCl_3$ containing 3% of methanol. Determination, down to 1 μg per kg, is accomplished by thin layer chromatography.

A. A. ELDRIDGE.

Isolation and purification of aqueous solutions of aflatoxins from fermentation medium. N. D. Davis, A. W. Hayes, D. W. Eldridge and U. L. Diener (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1224–1225).—Aflatoxins B_1, G_1+B_2, and G_2 were separated by paper chromatography from the filtrate from a yeast extract-sucrose medium inoculated with *Aspergillus flavus* and identified under u.v. light. The bands were cut out and eluted with methanol, aflatoxin B_1 being determined spectrophotometrically at 363 mμ.

A. A. ELDRIDGE.

Crops and Cropping

Measurement of evapo-transpiration potential and water balance of certain crops. L. Tombesi, E. Romano and E. Lauciani (*Annali Staz. chim.-agr. sper., Roma*, 1965 [1966], Ser. iii, 234, 24 pp. + folded chart).—Values of evapotranspiration potential (ETP) calculated according to Thornthwaite (*Georg. Rev.*, 1958, 38), Blaney and Criddle (*U.S. Dept. Agr. Tech. Bull.*, 1962, 1275) and Turc (*Ann. Agron.*, 1961, 12) agreed well with experimentally determined values for wheat, grain maize, lucerne and autumn herbages of rape and *Vicia faba*, minor cultivated in the Agro Romano, but large discrepancies were observed with spring and summer herbages of *Vigna sinensis* and fodder maize. Cultivation coeff. (cf. Blaney and Criddle) agreed well with those reported for wheat, grain maize and lucerne, but were >1·7 for *Vigna herbensis* and fodder maize. The practical application of ETP values in the calculation of water balance is briefly discussed. (24 references.)

E. C. APLING.

Manufacture of coated seed with delayed germination. K. Schreiber and L. J. LaCroix (*Can. J. Pl. Sci.*, 1967, **47**, 455–457).—Seeds are enclosed in three layers (i) 'Spring jacket' containing various materials such as loam and talc in an aq. org. solvent system together with binders and plasticisers such as sugar, glycerol, and propylene glycol (ii) 'Lining' consisting of methyl cellulose or a mixture of ethyl and methyl cellulose in ethanol and (iii) 'Winter jacket' which is inpermeable to moisture but subject to fission by frost during the winter. Materials used for this purpose are primarily polystyrenes, ethyl cellulose of various viscosities, and beeswax in different combinations. E. G. BRICKELL.

Yield response of spring wheat and barley to nitrogen fertiliser in relation to soil and climatic factors. R. A. Young, J. L. Ozbun, A. Bauer and E. H. Vasey (*Proc. Soil Sci. Soc., Am.* 1967, **31**, 407–410).—The response of spring wheat and barley to application of N was related to moisture and NO_3^- content of the soil at seeding time and also to growing season rainfall and temp. Regression equations relating response to these factors were developed. Plant response was not related to available N as determined by mineral N accumulation during incubation for 2 weeks or by NH_3 released by boiling the soil with alkaline permanganate.

A. H. CORNFIELD.

Relation of silicon in barley to disease, cold, and pest-resistance. F. C. Lanning (*J. agric. Fd Chem.*, 1966, **14**, 636–638).—The average SiO_2 content in five varieties varied from 0·5% in the heads developing in spring, to 5·8% in the spring roots. The average SiO_2 content in the leaves and roots increased ~2-fold from autumn to spring. The results showed no relationship between the total SiO_2 and resistance to pests, cold, or diseases. The SiO_2 deposits were examined petrologically. (12 references.)

P. S. ARUP.

Evaluation of methods to determine sweet maize maturity for processing. J. D. Campbell and E. M. McKerlie (*Can. J. Pl. Sci.*, 1967, **47**, 381–387).—The refractive index of the kernel juice was an accurate, simple, and rapid method of determining maturity, and was considered superior to all other methods studied. Moisture content, alcohol-insol. solids content, and trimetric rating were also adequate indices of maturity but too time-consuming. Pericarp content was neither reliable nor rapid and shear-press ratings did not give accurate or consistent results. E. G. BRICKELL.

Relationship between photosynthetic area and grain yield per plant in wheat. H. D. Voldeng and G. M. Simpson (*Can. J. Pl. Sci.*, 1967, **47**, 359–365).—Shading treatments indicated that the ear and flag leaf contributed the major proportion of grain dry wt., the correlation coeff. ranging from +0·54 to +0·90. The combination of a large flag leaf plus a large ear area shows promise as an index for selecting higher yielding individuals from a mixture of genotypes.

E. G. BRICKELL.

Effect of (2-chloroethyl) trimethylammonium chloride (CCC) on certain agronomic traits of barley. E. N. Larter (*Can. J. Pl. Sci.*, 1967, **47**, 413–421).—A triple application of CCC by spray (10^{-1}M.) (at 3-, 5-, and flag leaf stages) reduced height to about 75% of the control and retarded maturity. Grain yields, kernel wt., tiller no. per plant, % protein, and total β-amylase activity were not affected.

E. G. BRICKELL.

Salt hardiness and dye reduction by potato tissue and mitochondrial fractions as influenced by previous storage of the tubers. C. C. Craft (*Plant Physiol.*, 1966, **41**, 1662–1666).—Uptake of O_2 and reduction of tetrazolium occurred at higher rates in discs from potato tubers (*Solanum tuberosum* L) stored at 0° than in those from tubers stored at 12·8°. Likewise tetrazolium reduction was higher in mitochondrial fractions from tubers at 0° than in that at 12·8°. Inhibition of O_2 uptake and tetrazolium reduction progressively increased with increasing concn. of KCl in tissue and mitochondrial fractions from tubers stored at 0 and 12°, but inhibition was most severe and occurred at lower concn. of KCl in the material from tubers stored at 12·8°. E. G. BRICKELL.

Effect of temperature on invertase, invertase inhibitor, and sugars in potato tubers. R. Pressey and R. Shaw (*Plant Physiol.*, 1966, **41**, 1657–1661).—Detailed studies demonstrate the high dependence of the accumulation reducing hexoses and invertase in the tubers on temp. and suggest a direct rôle for invertase in hexose accumulation in tubers at low temp. E. G. BRICKELL.

Fertilisation of sugar-beet. V. Morani (*Annali. Staz. chim.-agr. sper., Roma*, 1965 [1966], Ser. iii, 232, 22 pp.).—Italian research on

the fertilisation of sugar beet since 1950 is reviewed. The generally recommended pre-emergence treatment is 100–125 kg of P_2O_5 ha and 45–60 kg of N/ha (N/P ratio: 1 : 2 to 1 : 3), or in some specific areas, an NPK fertiliser (from 1 : 2 : 1·5 to 1 : 1 : 1) at a rate equivalent to 15–20 kg of N/ha. Post-emergence broadcasting of nitrates is advocated on soils of low permeability, and of NP fertiliser (2·5 : 1 or 2 : 1) on other soils. (48 references.)
E. C. APLING.

Effects of different methods of stubble cultivation on the growth of sugar beets. O. J. Furrer (*Schweiz. Landw. Forsch.*, 1966, **5(3–4)**, 456–468).—Trials were carried out over a period of 3 years and in different locations to determine the effect of (*a*) no cultivation, (*b*) rotovating, (*c*) skimming and (*d*) skimming and green manuring on sugar beet growth. (*a*) Generally reduced yield but increased sugar content so that profits were not affected, (*b*) and (*c*) were equal in yield and sugar content; (*d*) showed no advantages to offset extra cost although long term effects were not studied. In two cases this treatment increased the output of misshapen beet. Stubble cultivation effectively helped to prevent soil drying out.
J. B. WOOF.

Growth of natural swards at different altitudes. J. Caputa (*Schweiz. Landw. Forsch.*, 1966, **5(3–4)**, 393–426).—A report of an international group studying mountain grasslands in Austria, Italy, West Germany and Switzerland between 1963 and 1965. Productivity, seasonal evolution of grass, growth and quality of forage harvested at different times were recorded for 17 plots at altitudes of 430–1900 m. Spring growth increased with altitude, 15 q/ha being harvested in 45 days at 430 m but in only 13 days at 1900 m and this may cause problems with excess of early forage. Pasture yield decreased with altitude and the forage quality varied with the botanical composition of the grass and the cutting time. Young forage contained less protein in summer than in spring but the decrease with growth was less marked at this time. N fertilisers were effective but only economic if applied in suitable weather and the forage used efficiently. Under similar conditions a stocking rate of 3·7 units/ha could be achieved at any altitude but the summering period varied. (22 references.) J. B. WOOF.

Competition between legumes and grasses. C. T. de Wit, P. G. Tow and G. C. Ennik (*Versl. Landbrouwk. Onderz. Ned.*, 689, 30 pp.).—The grass *Panicum maximum* and the legume *Glycine javanica* were mutually exclusive when grown together in pots, with or without N-fertilisation, but without *Rhizobium*. In the presence of the appropriate strain of *Rhizobium*, however, the grass profited from the presence of the legume without detriment to the latter except under N-scarcity. A study of the N-yields of the plants indicated that N-fixation might be suppressed by N-fertilisation or *vice versa*. Perennial ryegrass and white clover were mutually exclusive when grown together in pots with a water-level 99 cm below the surface. At higher water-levels, however, the grass profited from the presence of the clover without detriment to the latter; the transference of N from the clover to the grass was indicated. P. S. ARUP.

Growth of *Hordeum jubatum* under various soil conditions and degress of plant competition. D. B. Wilson (*Can. J. Pl. Sci.*, 1967, **47**, 405–412).—When grown alone *H. jubatum* developed best under high soil moisture, high soil fertility, and low soil salinity. On wet, non-saline soils its growth was restricted by *Dactylis glomerata L.* and by *Agropyron elongatum* (Host)., *Festuca arundinacea* Schreb. and *Phalaris arundinacea L.* Growth was enhanced as soil temp. were increased. E. G. BRICKELL.

Effects of organic and mineral fertilisers on the botanical composition, yield and quality of the forage from *Arhenatherum* meadows. W. Künzli (*Schweiz. landw. Forsch.*, 1967, **6**, 34–130).—The botanical composition of the sward was best assessed by hand sampling of species. Relationships of temp. and rainfall data with yields of individual species are recorded. The capacity to increase yields under favourable growth conditions is not a characteristic of species but is largely dependent on the nature and extent of competition with other species in the sward. Regardless of the nature (or mineral) of the manurial treatment the same total amount of nutrients always gave the same yield. Applications of N gave the greatest increase in yield per unit N in the first cut of the season. Responses to N of different sub-associations of grass species in relation to temp. and rainfall varied with the time of year. The effect of mineral N fertilisers on yields was less dependent on weather conditions than was the total yield. Yields of crude protein, crude fibre, starch equivalent, P, K, Ca and Mg were influenced more by the quant. botanical composition of the sward and, in some cases, by the reserve of plant nutrients in the soil, than by the nature of the sub-associations of species present. The nutritive value of the herbage is considered in relation to the proportion of grasses, clovers and 'weeds' in the herbage.
A. G. POLLARD.

Control of potassium value on permanent pastures. P. de Vries (*Versl. landbouwk. Onderz. Ned.*, 1966, **675**, 44 pp.).—Trends in K-values (dependent on the 0·1 N-HCl-extractable K_2O, the org. matter content of the soil, and a soil factor) in the top 5 cm of four different soils under standard management were studied in long-term experiments at different levels of K_2O-fertilisation. A formula was developed in which the annual changes in K-value are calculated as $(B-B_O-A)/F$ where B = the annual application of K_2O, B_O = the amount of K_2O necessary to maintain equilibrium, $A = K_2O$ removed from soil (being dependant on management), and F = a factor dependant on soil type. (24 references.)
P. S. ARUP.

After-effects of varying rates of spring application of nitrogen and of dates of first defoliation on the yield and botanical composition of a grass–clover sward. R. G. Heddle (*J. Br. Grassld Soc.*, 1966, **21**, 251–257).—Mid-season depression of grassland yield following spring application of N was due almost entirely to clover depression, the grass fraction showing no after-effects of the treatment. The effect became more marked and lasted longer in successive years. Varying the date of first defoliation in spring did not affect the clover depression significantly. A. H. CORNFIELD.

Cultural and symbiotic properties of Rhizobia from Egyptian clover (*Trifolium alexandrinum*). T. M. el Essawi and A. S. A. Ghaffar (*J. appl. Bact.*, 1967, **30**, 356–361).—Morphological, cultural, biochemical and symbiotic properties were examined and compared with strains from other countries. All were microscopically similar showing normal growth on agar slopes, broth and milk. Some local, but no foreign strains, grew at 35° in 5% NaCl. Differences in NO_3^- reduction, fermentation of carbohydrates and utilisation of organic acids were apparent. Most Egyptian clover strains (*EC*) improved growth of the host plant as measured by dry wt. and N-content. *EC* strains formed only ineffective nodules on red clover, (*T. pratense*). In general *EC* was effectively nodulated by foreign clover rhizobia. (16 references.) C.V.

Calcium content of various apple cultivars as affected by rootstock. J. W. Sistrunk and R. W. Campbell (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 38–40).—Leaves from three cultivars (Winesap, Rome and Jonathan) grown on Hibernal rootstock were higher in Va than those from the same cultivars grown on French crab rootstock. Liming the soil or foliar sprays of $Ca(NO_3)_2$ increased Ca% in the leaves of all cultivars on Hibernal, but not on French crab, rootstock. A. H. CORNFIELD.

Effects of chemical thinning on repeat bloom of McIntosh apple trees. M. W. McKee and C. G. Forshey (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 25–32).—There were significant differences between years in the effectiveness of thinning and in the amount of repeat blossom when post-bloom sprays of naphthyl-1-acetic acid and 1-naphthyl *N*-methylcarbamate (Sevin) were applied to mature trees. All treatments greatly increased repeat bloom, but there was no relationship between the degree of thinning and the amount of repeat bloom. About half of the increase in flowering associated with chemical thinning was from increased flowering of non-fruiting spurs and half from increased flowering of fruiting spurs and the development of new spurs. A. H. CORNFIELD.

Laboratory studies of anthocyanin development in McIntosh apples. R. M. Smock (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 80–88).—Treatment of whole apples or skin disks with sucrose, galactose, $CaCO_3$, and $NaHCO_3$ in solution or suspension stimulated anthocyanin production. Responses were extremely variable. Fruit harvested from limbs that had prior night heating gave the most consistent responses to the treatments. A. H. CORNFIELD.

Volatiles from apple fruit as related to variety, maturity, and ripeness. D. S. Brown, J. R. Buchanan and J. R. Hicks (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 98–104).—Volatiles emanating from apple fruit were characterised by gas chromatography. The production of volatiles changed characteristically during ripening. Certain volatiles reached max. at a time nearly coincident with the respiratory climacteric. The production of volatiles following cold storage differed notably from that observed immediately after harvest, being especially influenced by the degree of maturity at harvest. A. H. CORNFIELD.

Gas chromatographic analysis of apple aroma compounds. J. E. Fargas (*Diss. Abstr.* B, 1966, **27**, 1670–1671).—Compounds con-

cerned in apple aroma, obtained by high-vac. distillation or by sampling the head-space gas accumulating in closed containers, are examined chromatographically. Among various liquid phases tested for the gas–liquid chromatographic technique, best resolution was obtained with 15% Carbowax 1000 on Chromosorb W, 60/80. Head-space analyses showed the principal aroma compound in McIntosh apples to be ethyl n-butyrate and that in Rome Beauty to be n-butyl acetate. Head space analyses provide useful data for determining varietal differences and for establishing indices of maturity and ripening. A. G. POLLARD.

Relationship of sugars and sorbitol to watercore in apples. M. W. Williams (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 67–75).—Sorbitol was a major component of sap exudates of severely watercored apple fruit. Sorbitol content of the main vascular bundles always increased as watercore developed and, in Delicious, coincided with a decrease in leaf sorbitol. Watercore was not always associated with high levels of sugar in the tissues. A. H. CORNFIELD.

Carbon dioxide fixation in preparations from Tunisian sweet lemon and Eureka lemon fruits. E. Bogin and A. Wallace (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 298–307).—*In vitro* studies involving ^{14}C-labelled CO_2 fixation indicated that both phosphoenolpyruvic acid (Pep.) carboxylase and NADP malic enzyme exist in both Tunisian sweet lemon and the Eureka sour lemon fruits. *Pep* carboxylase was inactivated when extracted from an acetone powder prep. while malic enzyme was not affected. The two enzymes are involved in org. acid synthesis. There were differences in the levels of CO_2 fixation and in amino-acid content between the sweet and sour lemon. A. H. CORNFIELD.

Organic acids in the juice vesicles of orange and grapefruit. S. V. Ting and H. M. Vines (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 291–297).—At least eight different org. acids, five of which could be measured quant. were found in the juice vesicles of Hamlin orange and Marsh Seedless grapefruit. Changes in the concn. of the org. acids through the season are reported. A. H. CORNFIELD.

Factors affecting the mineral element content of leaves and fruit of Wolcott blueberries. W. E. Ballinger and L. J. Kushman (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 325–330).—The effect of date of harvest, harvest interval, N application and crop load on the N, P, K, Ca and Mg levels of leaves and fruit of the blueberry are reported. A. H. CORNFIELD.

Effect of cultural practices on processed cherry quality. W. O. Harrington, J. F. Robinson, C. H. Hills and F. N. Hewetson (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 184–189).—The effect of orchard cover (Sudan-grass, rye-grass, or rye-grass–vetch) and N fertilisation (2·3–21 lb $NaNO_3$ per tree over 6 years) on yields and processed quality of cherries was studied. The largest fruit was produced with Sudan-grass and the smallest with rye-grass–vetch cover. The N rate had little effect on fresh cherry size or sol. solids content. Orchard treatments had little effect on drained wt. and processed yields of canned cherries. The canned cherries from the low-N plots had the least, and those from Sudan-grass plots the most, red colour. Canned cherry quality was affected more by season than by orchard treatment. A. H. CORNFIELD.

Fatty acids in the bark of Halehaven peach in relation to winter hardiness. D. O. Ketchie (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 204–207).—Flower buds collected on Oct. 7th withstood temp. approx. 10° lower than those collected on Sept. 25th. On Oct. 7th palmitic, stearic, oleic, linoleic and linolenic acids in the bark were significantly higher and palmitoleic and two odd-chain saturated fatty acids were significantly lower than on Sept. 25th. The ratio of unsaturated to saturated fatty acids was 0·56 on Sept. 25th and 0·91 on Oct. 7th. A. H. CORNFIELD.

Effect of temperature and various CO_2 and O_2 concentrations on growth of *Typhula* sp., a parasitic fungus of strawberry plants. C. L. Lockhart (*Can. J. Pl. Sci.*, 1967, **47**, 450–452).—Pot experiments with Cavalier and Sparkle plants showed that to reduce mould growth from *Typhula* sp. it is important to have at least 5 to 10% CO_2 and 5% O_2 in controlled atm. storage. Min. optimum and max. temp. for growth of *T.* sp. were −1, 10 and 10° respectively. E. G. BRICKELL.

Experiments in the deep fertilisation of vines using the injector pole. A. Baroccio and M. Palieri (*Annali. Staz. chim.-agr. sper., Roma*, 1965 [1966], ser. iii, 235, 16 pp.).—Results obtained in 4 years of vineyard trials comparing the effects of N P and N P K fertiliser, applied to a depth of 20–50 cm using an injector pole, and by surface distribution, are reported. Yields, sugar concn. and grape valuation were generally favoured by deep application of fertiliser, and the K content of foliar tissues responded particularly well to underground treatment. E. C. APLING.

Ammonium-induced stem and leaf lesions of tomato plants. D. N. Maynard, A. V. Barker and W. H. Lachman (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 516–520).—Distinctive, previously unreported, necrotic leaf and stem lesions occurring on the Heinz 1350 tomato variety were non-pathogenic in origin and were traced to high concn. of NH_4^+ in the stem and leaf tissue of plants supplied with high levels of NH_4^+ fertilisers. A. H. CORNFIELD.

Fertilisation of lettuce under glass with dried blood and with Nitro-chalk. J. P. N. L. Roorda van Eysinga (*Vers. landbouwk. Onderz., Ned.*, 1966, 681, 18 pp.).—Lettuce responded more favourably to Nitro-chalk at low soil-N levels, and to dried blood at high levels of soil-N. Directions are tabulated for the use of Nitro-chalk with respect to the sol. soil-N. The effect of blood-N in soil was ~72% of that of Nitro-chalk. P. S. ARUP.

Variety research on the resistance of groundnuts to drought. III. Experiments out of doors and under glass. J. Gautreau (*Oleagineux*, 1967, **22**, 25–29).—The varieties of groundnuts giving the best results in the last 4 years in tests for drought resistance were compared. At Bambey, under artificially-induced drought conditions, outdoor and under-glass tests were made. At Louga and Tivauane, which are situated in an area where the rains are limited and irregular, variety tests were made with very encouraging results. Three varieties, two early and one late, were and sometimes superior to the standard variety for this part of North Senegal. M. DUDLEY.

Treatment of tapping holes of sugar maple with paraformaldehyde. M. Lortie (*Naturaliste can.* 1966, **93**, 963–971).—To prevent the undesirable development of micro-organism in the tap holes, pellets of paraformaldehyde were applied prior to the sap flow. The treatment slightly delayed the subsequent callus formation but no ill-effects were observed over a 4-year test period. A. G. POLLARD.

Floral initiation of upland cotton, *Gossypium hirsutum*, L. in response to temperatures. J. R. Mauney (*J. exp. Bot.*, 1966, **17**, 452–459).—Night temp. <28° increased the height at which the first floral branch was formed, the response being influenced by germination temp. and by the length and intensity of daylight to which the plants were exposed. With 8 h periods of high-intensity daylight the initiation was delayed more by high night temp. than after exposure to high light intensity for 14 or 24 h. The inhibitory effect of high night temp. was increased by high day temp. (28–32°). High day temp. delayed floral initiation if the night temp. was high (28–32°), but caused the formation of the first floral branch at a lower level than when the night temp. was 20–22°. The period from planting to floral initiation was shortened by day temp. of 32° and night temp. 20°. A. G. POLLARD.

Rôle of nitrogen in wood deterioration. IV. Relationship of natural variation in nitrogen content of wood to its susceptibility to decay. W. Merrill and E. B. Cowling ((*Phytopathology*, 1966, **56**, 1324–1325).—The susceptibility of poplar stems to invasion by wood-rotting fungi (*Lenzites trabea*, *Polyporus versicolor*) is examined in relation to the N content of the wood. Data obtained accord with the hypothesis of a direct relationship between N content of wood and susceptibility suggested in published information on other timbers. A. G. POLLARD.

Pest Control

Effects of three species of aphids on barley, wheat or oats at various stages of plant growth. J. U. Apablaza and A. G. Robinson (*Can. J. Pl. Sci.*, 1967, **47**, 367–373).—*Schizaphis graminum* (Rondani), *Macrosiphum avenae* (Fabricius) and *Rhopalosiphum maidis* (Fitch) were tested out on Parkland barley, Selkirk wheat and Rodney oats. The maize leaf aphid did not establish large populations on wheat or oats, but most seedlings of barley infested prior to heading were killed. The green bug and the English grain aphid severely injured or killed seedlings of barley, wheat and oats, and reduced the kernel wt. of harvested grain. E. G. BRICKELL.

Fluctuation in amount of starch in host plants invaded by rust and mildew fungi. C. J. Mirocha and A. I. Zaki (*Phytopathology*, 1966, **56**, 1220–1224).—In leaves of *Phaseolus vulgaris* the starch constant fell after inoculation with *Uromyces phaseoli* by 50% in 4 h and by 90% in 24 h. Just before and during sporulation of the fungus the starch content and the dry wt. of the leaves increased rapidly, falling again sharply after sporulation without diminution in leaf dry wt. *Avena sativa* inoculated with *Puccinia graminis* and *Hordeum vulgare* inoculated with *Erysiphe graminis* also showed alterations in starch content but with different rhythms. A. G. POLLARD.

Occurrence and nature of antimicrobial substances in plants with special reference to the susceptibility of certain insects to *Bacillus thuringiensis*, Berliner. B. Maksymiuk (*Diss. Abstr.*, B, 1966, **27**, 1672).—Antimicrobial substances inhibiting the growth of the pathogen, *B. thuringiensis* var. *thuringiensis*, Berliner, were detected on leaves of various families of Gymnospermae and Angiospermae. In the Virginia and loblolly pines the toxic material occurred throughout the year on old and young growth; expressed juices were toxic, *in vitro*. to *B. thuringiensis* and two other species. Analysis of the anti-bacterial material suggests that that in pitch pine is a hydroxy-carboxylic acid. The action of the extracted toxin on a range of insects is examined. A. G. POLLARD.

Synthesis of dialkyl (1-methyl or phenyl, 2-thiophenoxy vinyl) phosphates. A. Arcoria and S. Fisichella (*Annali Chim.*, 1966, **56**, 1504–1511).—Some phosphoric esters $(RO)_2P(O)[OC(Y) = CHSC_6H_5]$ of potential biological interest were obtained in high yield (90%) from Me_3 or Et_3 phosphite and 1-chloro-1-thiophenoxy-acetone or -acetophenone. (14 references.) L. A. O'NEILL.

Synthesis of carbon-14-labelled 2-methyl-2-(methylthio)propional-dehyde O-(methylcarbamoyl)oxime. W. J. Bartley, D. L. Heywood, T. E. N. Steele and W. J. Skraba (*J. agric. Fd Chem.*, 1966, **14**, 604–607).—The synthesis is described of this insecticide, nematocide, and acaricide (Temik) with ^{14}C in three different positions, and of the corresponding (labelled) sulphoxide and parent oxime. P. S. ARUP.

Interaction of dialkyl esters of phosphoric acid and alkyl esters of alkylphosphinic acid with perfluorochloroacetones. S. Z. Ivin, V. K. Promonenkov and E. A. Fokin (*Zh. obshch. Khim.*, 1967, **37**, 1642–1643).—Reactions were studied of dealkylphosphonates (**I**) and alkyl phosphinates (**II**) with symmetrical and unsymmetrical perfluorochloracetone (**III**) in search for new materials possessing insecticide characteristics. The (**I**) or **II** (0·1 g-mol) were added dropwise to **III** and temp. of reaction was maintained at 60–70°. Mixing was continued 4 h at room temp. and reaction mixture then distilled. Nine vinyl esters of phosphoric acid were prepared in 60–70% yields, namely *O,O*-diethyl-*O*-dimethyl-*O*- and -di-isopropyl-(α-difluorochloromethyl-β-fluorochloro)vinyl phosphate, 0,0-diethyl- and di-isopropyl-*O*-(α-fluorodichloromethyl-β-fluorochloro)vinyl phosphate; *O,O*-diethyl and -dimethyl-*O*-(α-difluorochloromethyl-β-dichloro)vinyl phosphate and *O*-ethyl-*O*-(α-fluorochloromethyl-β-fluorochloro)vinyl methylphosphonate. A.L.B.

Quaternary salts of bipyridyl—a new agricultural tool. W. R. Boon (*Endeavour*, 1967, **26**, No. 97, 27–32).—A new class of herbicide, based on the bipyridylium ion, has important consequences for agriculture throughout the world. The new herbicides can eliminate certain traditional steps in cultivation, such as ploughing, they reduce the risk of soil erosion and are almost instantaneously inactivated in the soil. Toxic hazards are not apparent. (23 references.) I. DICKINSON.

Effect of DMPA [O-(2,4-dichlorophenyl) O-methyl isopropyl-phosphoro-amidothioate] on groundnut yields. E. W. Hauser (*Weeds*, 1967, **15**, 84–85).—Application of DMPA as a pre-emergence spray or mixed with DNBP (4,6-dinitro-*o*-s-butyl-phenol, alkanolamine salt) at the emergent stage increased groundnut yields over 5 years. Yields from the treatments were significantly better than from cultivated and handweeded plots or from plots sprayed with standard herbicide mixtures. A. H. CORNFIELD.

Properties and biological activity of some *N*-aryl derivatives of aminoacetohydroxamic acid. Z. Eckstein and M. Sak (*Bull. Acad. pol. Sci. Ser. Sci. Chim.*, 1966, **14**, 745–750).—The relationship between chemical structure and fungicidal activity of hydroxamic acids was studied. Ten *N*-phenylglycine ethyl ester deriv. and 24 *N*-arylaminoaceto-hydroxamic acid were synthesised. Only nine compounds of these showed biological activity. T. M. BARZYKOWSKI.

Effect of temperature on the ability of *Colletotrichum graminicola* to form appressoria and penetrate barley leaves. W. P. Skoropad (*Can. J. Pl. Sci.*, 1967, **47**, 431–434).—Appresoria are formed at temp. ranging from 15 to 35° but can penetrate barley leaves only at a range of 25 to 35°. E. G. BRICKELL.

Epidemiology and control of *Ascochyta pinodes* on field peas in Canada. V. R. Wallen, T. F. Cuddy and P. N. Grainger (*Can. J. Pl. Sci.*, 1967, **47**, 396–403).—Results of seed examinations from 1953 to 1964 show that blight is now epiphytotic and *A. pinoes* is the principal pathogen. Optimum range for infection was 15 to 18°, Thirma 75, Orthocide 75, Panogen 15 and Spergon as seed treatment were effective in controlling the organism in sand, sandy loam and sterilised sandy loam. E. G. BRICKELL.

Inactivation of veinbanding and latent C viruses in strawberries by heat treatment. A. T. Bolton (*Can. J. Pl. Sci.*, 1967, **47**, 375–380).—Treatment of strawberry roots with aq. $KMnO_4$ and a gradual increase in temp. made it possible to subject some varieties to 48°. This temp. inactivated veinbanding. Latent C virus was inactivated at 46·5°. E. G. BRICKELL.

Structure-activity relationships in alkyldinitrophenols and their derivatives. M. Pianka (*Chemy Ind.*, 1967, 1625–1634).—Effects of (i) substituents on acaricidal activity of dinitrophenols and their Et carbonates, with special reference to the effectiveness of Dinobuton and Dinoterbon in protecting certain crops, and (ii) of lengthening the normal-ester chain on biological activity and of 2-alkyl substitution and esterification on pre-emergence herbicidal activity. The mode of action of these esters is explained and the value of Dinoterb acetate and Medinoterb acetate for weed control in high-org. soils or low-rainfall areas is indicated. (iii) The relative activites of *e.g.*, 4-alkyl-2,6-dinitrophenols and 2-alkyl-4,6-dinitrophenols, against powdery mildews on cucumbers, apple rootstocks, or barley seedlings are considered. Based on % eradication of the disease, no. of C atoms, and nature of substituents in the mol., relationships between biological activity and structure are discussed fully. W. J. BAKER.

Toxicological interactions of chlorinated hydrocarbon and organophosphate insecticides. A. J. Triolo and J. M. Coon (*J. agric. Fd Chem.*, 1966, **14**, 549–555).—Single doses of aldrin, dieldrin, or chlordan at first increased, but after 4 days decreased the toxicity of parathion to mice; the protective effect continued for 12 days. Aldrin had a similate diphasic effect on hexobarbital sleeping time, and also against paroxone and several similar compounds. Protection was accompanied by increases in the secretion of certain esterases; both effects were nullified by the administration of ethionine together with the aldrin. (28 references.) P. S. ARUP.

Effect of *N*-dimethylaminosuccinamic acid (Alar) on watercore and harvest drop of apples. L. P. Batjer and M. W. Williams (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 76–79).—Spray application of 1000–2000 ppm Alar either 14–20 or 110 days after full bloom delayed the development of watercore and reduced harvest drop. The early treatment was the most effective. The treatments produced fruit which was firmer and somewhat lower in sol. solids. A. H. CORNFIELD.

Persistence and mobility of *N*-dimethylaminosuccinamic acid (Alar) and its effect on anthocyanin metabolism in sweet cherries, *Prunus avium*. K. Ryugo (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 160–166).—When cherry trees were sprayed with Alar (500–4000 ppm) the residue levels in green fruit decreased initially, but then increased in the ripening fruit, indicating that movement into the fruit exceeded its rate of catabolism. At 2000 ppm Alar curtailed shoot elongation and induced early production of anthocyanins in the fruit. There were varietal differences in the extent to which the treatments altered the ratio of keracyanin to chrysanthemin, but leucoanthocyanin metabolism was not affected. The treatments did not affect fruit size or sol. solids content. A. H. CORNFIELD.

Interaction of γ-irradiation and controlled atmospheres on *Botrytis* rot of strawberry fruit. E. Chalutz, E. C. Maxie and N. F. Sommer (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 365–371).—Controlled atm. (O_2-CO_2-N_2) inhibited lesion development of *Botrytis* rot in stored strawberry fruit, but was generally less effective than irradiation with 200 krad of γ-rays. The combined effects of controlled atm. plus irradiation did not give enough benefit over irradiation alone to justify the cost. A. H. CORNFIELD.

Laboratory examination of organic fungicides against 300 pathogenic-fungi in soil. A. L. Morehart and H. W. Larsh (*Appl. Microbiol.*, 1967, **15**, 1248–1251).—Seven commercial and five experimental org. fungicides were tested against *Hertiplasma capsulatum*, *Crypticoccus neoformans*, *Allescheria boydii* and *Sporotrichum sobenckii* Lanstan (48% 1-chloro-2-nitropropane) (**I**), DAC 649 (**II**), DAC 469 (**III**), DAC 2787 (**IV**), He 3944, maneb (**V**) {80% [ethylene bis(dithiocarbamate)]Mn and Nabam (**VI**) 90% [Ethylene bis(dithiocarbamate)]Na}, in order of decreasing activity, inhibited all test fungi. The most marked results were obtained with **I**, vapam (**VII**) and **II, V, VI, and IV** were fungicidal to a lesser degree. It is considered that **I, II, VII** merit further study. (20 references.) C.V.

Metabolism of carbon-14 Diphenamid in strawberry plants. T. Golab, R. J. Herberg, S. J. Parka and J. B. Tepe (*J. agric. Fd Chem.*, 1966, **14**, 592–596).—Plants were grown in soil treated with 3·8 lb of ^{14}C-labelled Diphenamid per acre; the radioactivity found

in the fruit was ~1% of that found in the leaves and calyxes. The major metabolite *N*-methyl-2,2-diphenylacetamide was found in comparatively much smaller amounts. (12 references.)
P. S. ARUP.

Parathion absorption, translocation, and conversion to paraoxon in bean plants. A. El-Rafai and T. L. Hopkins (*J. agric. Fd Chem.*, 1966, **14**, 588–592).—Parathion (**I**) on leaf or glass surfaces disappeared with half-lives of 1 day, but the **I** on the leaves was finally more persistent. After 2 days ~ 30% of the **I** had entered into the plant whilst ~ 46% had mostly evaporated; conversion to paraoxon amounted to >1% of the original application. Of the **I** absorbed by the roots from nutrient solutions, <2% was translocated to the leaves. (18 references.) P. S. ARUP.

Metabolism of 2-methoxy-3,6-dichlorobenzoic acid (Dicamba) by wheat and blue-grass plants. N. A. Broadhurst, M. L. Montgomery and V. H. Freed (*J. agric. Fd Chem.*, 1966, **14**, 585–588).—The metabolites extracted with EtOH from both plants and freed from their conjugates with plant constituents by acid or enzymic hydrolysis, were identified as 5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid (90%), 3,6-dichlorosalicylic acid (5%) and the parent substance (5%). P. S. ARUP.

Mode of action of organophosphate antithelmintics. Cholinesterase inhibition in *Ascaris lumbricoides*. C. O. Knowles and J. E. Casida (*J. agric. Fd Chem.*, 1966, **14**, 566–572).—The relative toxicities as inhibitors of cholinesterase activity (*in vivo* and *in vitro*) in the anterior section of the worms are tabulated for 56 organophosphates. Detoxification appears to take a course different from that occurring in insects and mammals, and proceeds at a much slower rate. (31 references.) P. S. ARUP.

Mode of action of carbamate synergists. R. L. Metcalf, T. R. Fokuto, Christopher Wilkinson, M. H. Famy, S. Abd El-Aziz and E. R. Metcalf (*J. agric. Fd Chem.*, 1966, **14**, 555–562).—Data on the synergising of insecticidal carbamates by methylenedioxyphenyl compounds and their detoxification by phenolases (tyrosinase) support the view that the synergistic action is due to the inhibition of the enzymes that would otherwise detoxify the carbamates (largely by ring-hydroxylation). (50 references.) P. S. ARUP.

Interactions between DDT analogues and microsomal epoxidase systems. J. W. Gillet, T. M. Chan and L. C. Terriere (*J. agric. Fd Chem.*, 1966, **14**, 540–545).—Liver microsomes of rats (male only and quails (female only) dosed with DDT showed greatly increased (probably abnormal) epoxidase activity (as detected by gas chromatography) when incubated at 38° in an NADPH system in the presence of aldrin or heptachlor. Much lower responses were obtained with DDD, DDE or Kelthane. (29 references.)
P. S. ARUP.

Synthesis and herbicidal properties of new substituted 2-(m-tolyl)-acetamides and related compounds. H. C. Godt, jun., P. C. Hamm and R. E. Wann (*J. agric. Fd Chem.*, 1966, **14**, 602–604).—Particulars are given of 14 compounds. P. S. ARUP.

Effect of four herbicides on growth of *Rhizoctonia solani*. R. Rodriguez-Kabana, E. A. Curl and H. H. Funderburk jun. (*Phytopathology*, 1966, **56**, 1332–1333).—Liquid cultures of *Rh. solani* were incubated with various concn. of atrazine, diuron, EPTC or paraquat, and the mycelium produced was weighed. In general growth inhibition of the fungus increased with the concn. of the herbicide used and varied with the nature of the herbicide and with time after inoculation. A. G. POLLARD.

Effect of herbicides on asparagus and weeds, and residues retained in the soil. S. S. Sandhu and J. K. Greig (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 372–377).—Of herbicides tested simazine (2–4 lb per acre) was the most effective for controlling annual weeds in asparagus, although linuron (2 lb), diphenamid (6–8 lb) monuron (1 lb), and DCPA (16 lb per acre) were also effective. The herbicides had no toxic effects on asparagus plants, as measured by dry wt. Nine months after the second application monuron was found in the 0–3 in. soils layer and simazine in the 3–6 in. layer.
A. H. CORNFIELD.

Effects of herbicides on the performance of sweet potatoes. J. K. Griig and A. S. Al-Tikriti (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 466–471).—The application of amiben (2–4 lb), DCPA (8–16 lb), diphenamid (3–8 lb), and trifluralin (3–6 lb per acre) to sweet potatoes controlled weeds early in the season and increased yields 10-fold. In general herbicide treatments did not alter the chemical composition of sweet potato foliage and roots.
A. H. CORNFIELD.

Effects of herbicides and weed competition on growth of orchard trees. W. M. Mellintnin, G. Crabtree and F. D. Rauch (*Proc. Am. Soc. hort. Sci.*, 1966, **88**, 121–126).—Combinations of amitrole with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) or simazine gave the best weed control and most satisfactory tree growth. Max. retardation of tree growth due to weed competition occurred in the early years. The extent to which weeds reduced tree growth varied with location. A. H. CORNFIELD.

Effect of shading and temperature on loss of herbicidal phytotoxicity. R. E. Eplee (*Diss. Abstr.* B, 1966, **27**, 1670).—To assess the interaction of rate of application of herbicide and crop population, a pre-emergence application of simazine to maize and a post-planting treatment of diphenamid on tobacco were given, plant populations being varied in each case. Where crops had no competitive advantage (e.g., by cultivation treatments or use of selective herbicides) they were unable completely to control weeds even at high population density. Where competitive advantage existed the crop reduced weed growth significantly. Artificial shading of crops reduced the phytotoxicity of diuron, simazine trifluralin and diphenamide and increased the time required to kill the weed plants. Tentative explanations of these effects are offered. Shading (50%) or increase in temp. >110°F caused destruction of diuron deposits on glass plates or on soil. Under similar conditions simazine was lost from glass and soil surfaces with all degrees of shading and with temp. >110°F. Diphenamide responded similarly except that it was lost from a soil surface at >150°F. With all three herbicides losses were greater with decreased shading and with increased temp. Incorporation of simazine or diphenamid with soil prevented loss at all temp.; with diuron loss was prevented at temp. >110°F.
A. G. POLLARD.

Photodecomposition of 2,4-dichlorophenoxyacetic acid. D. G. Crosby and H. O. Tutass (*J. agric. Fd Chem.*, 1966, **14**, 596–599).—When aq. solutions of the herbicide were irradiated with u.v. light or sunlight the rapidly formed decomposition products were 2,4-dichlorophenol, 4-chlorocatechol, 2-hydroxy-4-chlorophenoxyacetic acid, 1,2,4-benzenetriol, and (as insol. end-product) polyquinoid humic acids. (10 references.) P. S. ARUP.

Separation and identification of substituted urea herbicides by paper chromatography. L. C. Mitchell (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1163–1166).—Nine substituted urea herbicides and/or their trichloroacetates were separated by the use of two solvent systems: (*a*) heavy mineral oil in ethyl ether (immobile) and aq. tetrahydrofuran (mobile), (*b*) 2,2,4-trimethylpentane containing glucial acetic acid. Monuron and 3-(2-chlorophenyl)-1-methylurea are exceptions. The compounds are detected in u.v. light or (except fenuron) by first spraying with $AgNO_3$ and 2-phenoxyethanol with 1 drop of H_2O_2, in acetone. A. A. ELDRIDGE.

Lactone derivatives of organic thiophosphorus acids. Imperial Chem. Industries Ltd. (Inventor: A. J. Floyd) (B.P. 1,063,071, 17.8.62).—The title compounds have pesticidal activity and the formula $RR^1PX{\cdot}SA$ (R and R^1 are alkyl or alkoxy of 1–6 C or R^1 is alkyl- or dialkylamino of 1–6 C; X is O or S; and A is γ- or δ-lactone ring. E.g., $(OEt)_2PS_2H$ (3·16) is added dropwise to a solution of Na (0·37) in EtOH 30, then 3-bromo-2-oxotetrahydrofuran (2·75 g) is introduced slowly. The mixture is boiled during 1·75h., cooled, then filtered, and the filtrate is evaporated. The residue is extracted with ether and the washed extract is distilled, to give 3-*diethoxyphosphinothionylthio-2-oxotetrahydrofuran*, b.p. 105°/0·005 nm. F. R. BASFORD.

Insectical compounds of the phenyl or naphthyl carbamoyl oxime type. African Explosives & Chem. Industries Ltd. (B.P. 1,063,363, 10.3.64. S. Africa, 14.3.63).—Compounds claimed are active against *Cimex lectularius*, *Anthronus vorax*, *Musca domestica*, etc., and have the formula $CHR{:}N{\cdot}O{\cdot}CO{\cdot}NR^1Me$ (R^1 is H or Me; R is Ph which may contain OH, halogen, alkyl, halogenoalkyl, alkoxyalkyl, alkenyl, cycloalkyl, optionally, substituted aryl, aralkyl, $CONH_2$, CN, CO_2H, CHO, acyl, aroyl, aryloxy, alkoxy, alkylthio, arylthio, SH, alkyl- or dialkylcarbamyl, CNS, or NO_2. As an example of method of prep., by keeping a mixture of PLCH:NOH (0·01), ether, MeNCO (0·11 mol.), and NEt_3 (3 drops) overnight, then removing solvent, there is obtained O-(*methylcarbamyl*)-*bezaldoxime*, m.p.h. 97·4–97·7°. F. R. BASFORD.

Cyclotriphosphazatriene derivatives for combating plant growth. N. V. Philips' Gloeilampenfabrieken (B.P. 1,063,704, 12.9.63. Neth., 14.9.62).—Typified by pentachloro-ethylamino-cyclotriphosphazatriene, the title compounds are prepared by reacting hexachlorocyclotriphosphazatrine with a primary aliphatic amine (1–8 in the hydrocarbon radical) at low to room temp. Thus, an aq.

solution of $EtNH_2$ is added to hexachlorocyclotriphosphazatriene in Et_2O, over 30 min., with stirring which is continued for 25 min. after the addition. The Et_2O layer is separated, dried over Na_2SO_4 and concentrated to leave a residue for dissolving in Pr^iOH. After treatment with activated C and filtering, the filtrate is concentrated, cooled to —25° and the cryst. product, m.p. 33–34°, filtered off and dried. A smaller amount of the pentachloro-ethylamino-cyclotriphosphazatriene is obtained by fractionally distilling the mother liquor so that a total 59% yield is obtained. Mixed with 95 parts of kieselguhr, the dust produced inhibits undesirable plant growth. S. D. HUGGINS.

Manufacture of organic mercury compounds. Metalsalts Corp. (Inventors: S. J. Lederer, H. E. Jecker and J. Houston) (B.P. 1,063,995, 12.9.63).—Org. Hg compounds, having a high degree of activity against various fungi and bacteria and a powerful herbicidal action, particularly a selective action against crab-grass, of empirical formula $R_2Hg_2NH_2X$, in which R is a substituted or unsubstituted phenyl or naphthyl group, the substituting groups, when present, being halogen (Cl) nitro, hydroxy, carboxy, or aliphatic hydrocarbon (alkyl C_{1-6}) and X is the residue of an inorg. or org. compound having an active acidic H (including hydroxyl), are prepared by reaction of dry NH_3 gas with an aryl Hg hydroxide or salt, in an anhyd. org. solvent and further treatment of the resulting diaryl dimercuric ammonium hydroxide with a compound having an acidic H atom or with a salt of such compound, e.g., anhyd. NH_3 is bubbled through a suspension of phenyl mercuric hydroxide in methanol. When complete solution is obtained, propionic acid is slowly added with agitation. The ppt. of diphenyl dimercuric ammonium propionate is separated and dried. J. M. JACOBS.

Cyclotriphosphazatriene derivatives as plant growth inhibitors. N. V. Philips' Gloeilampenfabrieken (B.P. 1,063,703, 12.9.63. Neth., 14.9.62).—The claimed pentachloro-alkyl- or phenoxy-cyclotriphosphazatriene is mixed with a solid or liquid carrier. Thus, 5 parts by wt. of an active ingredient and diatomite are ground to form a composition for crop protection. The active ingredient is obtained by adding Bu^nONa in dioxan over, 40 min., to Cl_6-cyclotriphosphazatriene in C_6H_6 and the reaction mixture washed with water and the C_6H_6 distilled off. The residue is fractionally distilled *in vacuo* (0·3 mm. Hg), the fraction boiling at 120–127° being collected and allowed to stand for 2 days. Crystallised Cl_6-cyclotriphosphazatriene is then filtered off, leaving crude Cl_5-*n*-butoxy-cyclotriphosphazatriene containing 43·75% Cl. S. D. HUGGINS.

Agricultural chemical compositions. Fisons Pest Control Ltd. (Inventors: G. S. Hartley and R. Howes) (B.P. 1,063,714, 13.12.62).—The claimed composition, which forms a solid gel on standing, consists of a solid agricultural chemical preferably insol. or slightly sol. in water, the Na salt of a long chain (12–24 C) saturated aliphatic carboxylic acid and water (50–200 times the wt. of Na salt). Thus, stearic acid, NaOH and water are heated, with stirring, to 65–70° when finely divided Cu oxychloride is added and the mixture cooled, with stirring, to 30°. Me_3N and water are then added and the mixture allowed to stand to form a thin gel, which is sufficiently stable to resist normal transport handling but can be readily fluidised on stirring. The gel is fluidised by stirring, diluted with H_2O (2 : 1) and sprayed using a low vol. sprayer from a height of 7 m on to banana leaves under atm. conditions of 45% humidity and 36°. The droplets reach the leaves in a moist condition and the chemical is not removed from the leaves by the application of 10 cm. of artificial rain. S. D. HUGGINS.

Oxadiazolone compounds. Rhône-Poulenc S.A. (B.P. 1,063,799, 11.12.64. Fr., 13.12.63).—The claimed 5-t-butyl-3-phenyl-2-oxadiazolone and the corresponding oxadiazolones in which the Ph ring has 1 or more substituents (halogen, alkyl or alkoxy groups of 1–4 C and $-NO_2$) have herbicidal properties. The appropriate hydrazide is reacted with $COCl_2$, in C_6H_6 or Et_2O, at 20–120°. Thus, 1-trimethylacetyl-2(4-methoxyphenyl)-hydrazine (prepared from Me_3-acetyl chloride and 4-methoxyphenylhydrazine in C_6H_6, in the presence of Et_3N) and 20% $COCl_2$ in Ph·Me are heated at 75° until gas evolution ceases. After concn. of the Ph·Me solution under reduced pressure, the residual solid is recrystallised from EtOH to give 5-*t*-butyl-3-(4-methoxyphenyl)-1,3,4-oxadiazol-2-one, m.p. 92°. S. D. HUGGINS.

Animal Husbandry

Xanthophyll and carotene stability during lucerne dehydration. A. M. Livingstone, R. E. Knowles, M. Israelsen, J. W. Nelson, A. C. Mottola and G. O. Kohler (*J. agric. Fd Chem.*, 1966, **14**, 643–644).—In pilot-scale the carotene content of lucerne meal remained fairly constant whilst considerable losses occurred in the xanthophyll that were much increased by raising the temp. of the outgoing air from the drier from 105° to 160°, or by decreases in the moisture content of the meal to be dried. (17 references.) P. S. ARUP.

Proline as factor in distillers' dried solubles which stimulates cellulose digestion by rumen micro-organisms. G. D. Potter, C. O. Little and G. E. Mitchell, jun. (*J. agric. Fd Chem.*, 1966, **14**, 647–649).—The stimulatory factors (examined *in vitro*) were completely pptd by ethanol, and an acid hydrolysate of the ppt. was fractionated by paper chromatography. All active fractions contained proline but not in sufficient quantity to account for the stimulating effect. During the first 12 h of incubation before cellulytic action had commenced the proline was converted into valeric acid which was metabolised by the micro-organisms. The importance of this acid as a key substance is suggested. P. S. ARUP.

Relationship between stem diameter and *in vitro* digestibility of forages. D. N. Mowat, R. S. Fulkerson and E. E. Gamble (*Can. J. Pl. Sci.*, 1967, **47**, 423–426).—When lucerne was cut at the first-flower stage, stem dia. had no effect on *in vitro* dry matter digestibility and no consistent relationship occurred between stem width and lignin or acid-detergent fibre content. Stem width had no influence on the digestibility of various bromegrass entries cut at the heads-elongated stage but the narrower stems did have a slightly higher lignin content. E. G. BRICKELL.

Copper sulphate-induced fermentation changes in continuous cultures of the rumen microbial ecosystem. L. L. Slyter and M. J. Wolin (*Appl. Microbiol.*, 1967, **15**, 1160–1164).—$CuSO_4$ (50 mg/500 ml culture vol.) was introduced twice daily and caused marked inhibition of fermentation of the concentrates although fermentation of lucerne hay was not inhibited by the same $CuSO_4$ concn. when the inoculum for the culture was obtained from a cow maintained on normal concentrated rations. When the inoculum was from a cow on a high concentrate ration, fermentation was partially inhibited. Concn. of $CuSO_4$ that did not inhibit these fermentations caused preferential production of propionic acid and decreased production of CH_4. C.V.

Influence of variations in roughage—concentrates ratio on dairy cows. N. D. Dijkstra (*Vers. landbouwk. Onderz. Ned.*, 1966, 683, 39 pp.).—Cows receiving daily 3–5 kg of hay with ~9–10 kg of concentrates throve as well as cows on larger hay and smaller concentrate rations, without significant differences in milk production. P. S. ARUP.

Comparison of different methods which estimate nutritive value of forages. A. S. Mohammed (*Diss. Abstr. B.*, 1966, **27**, 1679–1680).—Methods compared were: average daily gain in wt. in feedlot, *in vivo* digestibility, voluntary feed intake and calculated nutrient value index (*N.V.I.*). Two *in vitro* methods, using whole rumen liquor or washed cell suspensions, and fermentation periods of 8, 12 or 24 h, were used for predictive purposes together with determinations of digestibility of dry matter and of cellulose. Supplementary laboratory determinations were made of lignin content and H_2SO_4-solubility of forages either alone or in combination with concentrates. *In vivo* digestibility was predicted satisfactorily by the 24-h cellulose digestibility (using rumen liquor or washed cells) or the 24-h dry matter digestibility (washed cells) or by the H_2SO_4 solubility or, particularly, the lignin content (correlation coeff. −0·99). Voluntary intake was well predicted from the 8-h, *in vitro* dry matter digestibility by artificial rumen methods or by 12-h cellulose digestion (washed cells). The lignin content and H_2SO_4 solubility values afforded a fair prediction of voluntary intake. A valuable prediction of body-wt. gains, from forages alone or from mixed rations was given by the *N.V.I.* Data from feeding trials using different qualities of lucerne hay are presented. The four methods for determining digestibility were closely correlated and could be inter-converted with reasonable accuracy. Where species-differences between digestibility by sheep and cattle were apparent, the only consistent differences were the superior digestion of crude fibre by steers and the somewhat better digestion of protein by sheep. A. G. POLLARD.

Methods of estimating the digestibility and voluntary intake of range forage consumed by grazing cattle. C. L. Streeter (*Diss. Abstr.* B, 1966, **27**, 1680–1681).—The reliability of various methods is examined. Standard digestion trials with cows and steers using different indicators showed quant. recovery of Cr_2O_3 from impregnated paper and of Delrin particles in faeces from steers in metabolism crates but not in that from cows fitted with total faecal

collection bags. In grazing studies with oesophageal-fistulated cattle low faecal recoveries resulted when Cr_2O_3-impregnated paper was administered and collection bags were used. This is attributed to incomplete collection by this means. Reliable estimates of total faeces production were obtained by use of Cr_2O_3-impregnated paper twice daily and by taking grab samples from the rectum at the same time. Use of this method and also examination of dietary and faecal samples from 12 oesophageal-fistulated grazing cattle for 6 consecutive days together with determinations of digestibility by the lignin ratio method provides data from which variation of intake and dry matter digestibility between animals can be calculated. Evidence was obtained indicating that the digestible N and digestible energy contents of the intakes were not affected similarly by dietary differences resulting from varying environmental conditions. A. G. POLLARD.

Factors affecting nutrient intake by ruminants. A. W. Mahoney (*Diss. Abstr. B.*, 1966, **27,** 1678).—Physical and physiological factors affecting voluntary intake and termination of feeding are examined using rumen fistulated steers. The steers had continuous access to good quality timothy hay except during experimental periods when the forage was fed to satiation in two daily meals. Wt. of ingesta and dry matter in the rumen were measured at the beginning and end of feeding and compared with corresponding data during continuous feeding periods. During continuously offered forage the cellulose, lignin and gross energy content of the reticulo-rumen dry matter at the commencement and termination of feeding showed no significant differences; a definite difference in these respects was apparent with the daily two-meal system of feeding. The average rate of movement of the dry matter of the reticulo-rumen (lignin ratio method) was 153·7 g/h; rates of flow of liquid ingesta through the reticulo-omasal orifice (measured by disappearance of polyethylene glycol in the 3 h period following initiation or termination of eating) were 4·56 and 4·63 kg/h respectively. Acetate, propionate and butyrate, but not valerate in the ruminal fluid increased progressively during feeding. The timing of these changes and those in the acetate concn. of peripheral blood is examined and a theoretical explanation of the mechanism of control of the initiation and termination of feeding by ruminants is suggested. A. G. POLLARD.

Fattening beef cattle on pasture and high-silage rations. D. L. McIlroy jun. (*Diss. Abstr. B.*, 1966, **27,** 1679).—High-energy supplements were fed to steers on pasture and to others on high-silage rations; comparison was also made with the effects of adding long hay to a high-maize silage drylot ration. Ground maize was an effective supplement for steers on pasture in respect of gain in wt. and financial return: its efficiency was not increased by pelleting. Hominy pellets were less satisfactory for this purpose. When maize supplements were delayed until the latter half of summer the fattening period was increased by 50 days and the live slaughter-wt. by 135 lb (but with increase in rib-eye area) as compared with feeding the supplement throughout the year. Beef cattle fattened in drylot produced carcasses with lower moisture content but more fat than did pasture-fattened beef or drylot-fattened steers, but there was no difference in meat quality (tenderness, juiciness). A. G. POLLARD.

Sex-associated characteristics of the beef carcass and muscle growth in the live animal. W. E. Meyer (*Diss. Abstr.* B, 1966, **27,** 1673).—The performances of 224 bulls from 12 sires were tested over a 3-year period. Carcass data from the bulls and from a group of heifers receiving different levels of total digestible nutrients (*TDN*) were obtained ultrasonically to measure differences in the *longissimus dorsi* area. Corresponding groups of bulls, steers and heifers were slaughtered at similar live-wt. for direct measurements of carcass characteristics. Muscle area as measured by the two methods showed general agreement; differences apparent in the early stages were maintained until slaughter. In heifers the muscle area increased with the dietary level of *TDN*; with a low level of *TDN*, heifers lost wt. and the muscle area diminished correspondingly. Bulls gained wt. faster than heifers and had heavier hides and lower dressing %. Leg length and circumference of round were smaller in the heifers but other linear carcass measurements could not be associated with sex. Bull, as compared with heifer carcasses contained the lower subcutaneous, inter- and intra-muscular fat, gave higher yields of retailable meat, less ether-extractable matter and higher water contents in the *longissimus* muscle. Values for steers were intermediate between those for bulls and for heifers. No sex-related differences were apparent in %N, tenderness, flavour or juiciness of the *longissimus* and *semi-tendenosus* muscles. Less 'finish' and a higher % of retailable meat is recorded in 900 lb than in 1100 lb cattle. Significant differences between sires were apparent in nearly all untrimmed and trimmed cuts and sire–year interactions for marbling score, carcass grade and trimmed primal cuts were shown. A. G. POLLARD.

Quality tests on pig carcasses; possible use in breeding practice. P. Steinegger (*Schweiz. landw. Forsch.*, 1967, **6,** 1–33).—The unreliability of the assessment of carcass quality from external observations on live pigs is discussed. Among a large no. of carcasses examined, the body lengths and lengths of sides of those from gilts were greater (by averages of 0·82 and 0·73 cm respectively) than those from castrate pigs of the same wt. In all cases the no. of ribs was within the range 13–17. In growing pigs the average daily gain in wt. and the rate of fat deposition were greater, and the noticeable increase in fat thickness began earlier in castrates than in gilts. The time and rate of fat deposition varied with the breeding selection. A. G. POLLARD.

Comparison of the biological efficacy of D- and DL-α-tocopheryl acetate in chickens. W. L. Marusich, G. Ackerman and J. C. Bauernfeind (*Poultry Sci.*, 1967, **46,** 541–548).—The biological potencies of D- and DL-α-tocopheryl acetates in the chick were compared using plasma tocopherol levels and incidence of encephalomalacia with three methods of administration as indicators of potency. The data confirmed the accepted biological potency ratio of 1·0 mg D-α-tocopheryl acetate being equal to 1·36 i.u. and 1·0 mg DL-α-tocopheryl acetate being equal to 1·0 i.u. of vitamin E activity. A. H. CORNFIELD.

By-products of the refining of soya-bean oil as pigment sources for poultry. I. Pigmentation studies with broilers. B. Lipstein, S. Bornstein and P. Budowski (*Poultry Sci.*, 1967, **46,** 626–638).—Acidulated soya-bean soapstock (ASS), containing 168–260 ppm xanthophylls was a satisfactory pigmenter for broilers. Soya-bean lecithin (32–45 ppm) xanthophylls was unsatisfactory. The pigmenting ability of ASS-derived xanthophylls was about half that of maize xanthophylls. The retention of ASS-derived xanthophylls was not affected as was that from lucerne meal by age of the birds. Changes in rate of retention, as well as effects of age and sex, were reflected in the concn. of blood plasma xanthophylls, but not in toe-web xanthophylls levels. A. H. CORNFIELD.

Calcium and phosphorus in the diet of Coturnix quail. B. F. Miller (*Poultry Sci.*, 1967, **46,** 686–692).—Coturnix quail made satisfactory wt. gains from hatch to 6 weeks of age when fed an all vegetable diet (maize–soya-bean meal–lucerne meal) containing 0·44% Ca and 0·59% P. Diets containing higher Ca and P contents did not result in better wt. gains, but caused somewhat poorer feathering in birds, especially when the Ca : P ratio was 2 : 1 or wider. A. H. CORNFIELD.

Calcium and phosphorus in menhaden fish meals in relation to ash content. C. H. Kurtzman and M. E. Ambrose (*Poultry Sci.*, 1967, **46,** 718–726).—The Ca and P contents of 29 samples of menhaden fish meal were highly correlated with ash content. Within prescribed limits of accuracy the ash content of the fish meals can be used to indicate their Ca and P contents. A. H. CORNFIELD.

Niacin requirement of the laying hen. R. L. Adams and C. W. Carrick (*Poultry Sci.*, 1967, **46,** 712–718).—A maize–soya-bean protein diet containing 6 ppm niacin was as effective in supporting egg production and wt., hatchability, feed consumption and health of the hens over 32 weeks as were diets containing up to 55 ppm niacin. The treatments had no effect on niacin% in the eggs. The niacin requirement of the laying hen is $<0{\cdot}00073$ g per bird per day. A. H. CORNFIELD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Fine grinding of wheat millfeeds and whole wheat for industrial use. A. C. Stringfellow, A. J. Pepinski, and V. F. Pfeifer (*Cereal Sci. Today*, 1967, **12,** 43–45, 48, 60).—Conditions and economic aspects are investigated for the pin-milling of the milling by-products red dog, middlings, and bran and of whole wheat. The proposed uses of the products with max. particle-size 64μ include paper-making and as material for xanthation reactions and protein extraction. (11 references.) P. S. ARUP.

Sorption of hydrogen chloride by wheat flour. A. C. Teng (*Diss. Abstr.*, B, 1967, **27,** 3918–3919).—The rate of sorption of HCl by soft white winter wheat flour from a gaseous mixture of HCl and N_2 was determined under various process conditions. The

apparent rate of sorption was affected by factors such as partial pressure, temp. flour moisture content, sample depth and gas flow rate inside the microbalance enclosure. Equilibrium sorption isotherms were recorded for the flour with a set of different moisture levels at 34°F. The apparent sorption rate increases as the flour moisture content and HCl partial pressure increase. It appears that local equilibrium exists between the free and immobilised sorbate at the gas–solid interface in this particular sorption process. F. C. SUTTON.

Reduction of microbial population of flour during milling. C. Vojnovich and V. F. Pfeifer (*Cereal Sci. Today*, 1967, **12**, 54–55, 58–60.)—Experiments are described in which bacterial counts of wheat (containing 13% of moisture) were reduced, without damage to the grain, from ~ 1,000,000 to < 500; better results were obtained by damping the heated grain to 15% of moisture with water containing Cl (250 ppm) followed by holding for 1 h and re-heating at 60°. Successful experiments are also described in which the grain was treated with air containing ethylene oxide or propylene oxide at 48°. (10 references). P. S. ARUP.

Sugars and confectionery

Determination of total acid phosphatase in honey. F. Gunther and O. Burckhart (*Dt. LebensmittRdsch.*, 1967, **63**, 41–44).—A phosphomonoesterase with a pH optimum between 4·9 and 5·3 was detected in honey and assayed. A 50% honey solution (1 ml) was added to 1ml of substrate consisting of 25 mg of Na *p*-nitrophenyl phosphate dissolved in 10 ml of 0·1M Sorensen citrate buffer, pH 5·3. After 3 h incubation at 37°, 10 ml of 0·02N-NaOH were added to stop the reaction. A blank was prepared in which the substrate was incubated at the same temp. and the honey and alkali added together after 3 h. The extinction of the reaction mixture relative to the blank was determined at 400 mμ. A calibration curve was constructed to obtain the amount of *p*-nitrophenol released from the extinction obtained. (21 references.) J. B. WOOF.

Determination of sugar alcohols in dietetic foods by gas–liquid partition chromatography. I. Analysis of dietetic biscuits. H. G. Jones, D. M. Smith and M. Sahasrabudhe (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1183–1187).—The total hexitol content is determined as the trimethyl silyl ethers with 10% SF–30 (silicone gum rubber) on Chromosorb W, and the individual hexitols are determined as the acetates on 7% QF–1 (trifluorsprophylmethyl siloxane) and 1·7% BDS (butanediol succinate polyester) on Chromosorb W. Recoveries were all in the range 94·0 to 105·5%. A. A. ELDRIDGE.

Fermentation and Alcoholic Beverages

Fluorimetric determination of malvin in must and wine. H. Bieber (*Dt. LebensmittRdsch.*, 1967, **63**, 44–46).—A sample (1ml) was acidified with 0·05ml of 0·1N HCl and 1·0 ml of a 1% solution of $NaNO_2$ added. After shaking and standing for 2 min., 10 ml alcoholic NH_3 were added. After filtration, malvin was detected qual. under a 366 mμ lamp. Quant. determination was carried out in a fluorimeter using Hg lamp excitation at 365 mμ and measuring the emission at 490 mμ selected by means of a filter. Levels found in a no. of wines are quoted. In certain hybrid wines the malvin content may be as much as 275 mg/100 ml. J. B. WOOF.

Determination and evaluation of the nitrate content of grape musts and wines. H. Rebelein (*Dt. LebensmittRdsch.*, 1967, **63**, 233–239).—The methods of Tillmans, Jankowic and Grau for determining NO_3^- are compared. The first method was found apparently to overestimate by up to 50% in some cases and the second took longer. A method based on that of Grau is described in which the NO_3^- is reduced by spongy Cd formed in situ in the wine and the resulting NO_3^- determined colorimetrically as a diazo compound. Other wine components do not interfere. 5 ml wine, 5 ml water and 2 ml conc. aq. NH_3 are mixed and 500 mg Zn powder and 1 ml 5% Cd acetate are added. After making up to 50 ml and filtering, 10 ml of a mixture of Griess reagents I and II (sulphanilic acid and naphthylamine in AcOH respectively) in equal proportions are added to 10 ml of filtrate. The extinction is measured at 530 mμ against a blank of 10 ml filtrate and 10 ml AcOH. The concn. is given by reference to a standard curve prepared with KNO_3. A standard deviation of ±2% was obtained. A no. of wines and musts were analysed and the ratio of NO_3^- to K and ash determined. J. B. WOOF.

Preparation of red wine with and without fermentation of skins. J. Prehoda (*Mitt. Klosterneuberg Ser. A. Rebe u. Wein*, 1966, **16**, 463–468).—In comparison with several processes, preference was given to that described by Peyer in which the unfermented mash is heated to 50° by passage through a plate-heater, and, after standing for 8 h, pressed; the cooled must is stored in barrels. The system avoids risks of undue oxidation, and is economically advantageous. The flavour of the resulting wine is less pronounced than usual, but smoother owing to its low content of tannin. P. S. ARUP.

Detection of alcohol additions to table wines. A. Patschky (*Dt. LebensmittRdsch.*, 1967, **63**, 197–200).—The detection method is based on the measurement of the concn. of the fermentation by-products glycerin and 2,3-butylene glycol. They can be measured rapidly by standard colorimetric methods (H. Rebelein: *Z. Lebensmittel-Untersuch. u.-Forsch.*, **105**, 296, 1957). In the light of results collected over 4 years, average glycerin and butylene factors have been determined and the relationships $A = 12{\cdot}5 \times Gl$ and $A = 100\sqrt{Bu}/0{\cdot}85 \pm 0{\cdot}09$ were found to be generally applicable. (10 references.) J. B. WOOF.

Determination of ascorbic acid in presence of sulphurous acid with use of glycolaldehyde. R. Burkhardt and A. Lay (*Mitt. Klosterneuberg Ser A. Rebe u. Wein*, 1966, **16**, 457–462).—In the iodimetric titration of Schneyder and Kain (cf. *Analyt. Abstr.*, 1962, **9**, 5445) glycolaldehyde can with advantage be substituted for propionaldehyde for the purpose of masking the free SO_2 in the second titration, in which the ascorbic acid alone is determined. P. S. ARUP.

Increase in size and sugar content of grapes in relation to photosynthesis and relevant factors. V. Hartmair (*Mitt. Klosterneuberg Ser. A. Rebe u. Wein*, 1966, **16**, 435–444).—A review with 39 references. P. S. ARUP.

Fruits, Vegetables, etc.

Storage of apples in polyethylene film [box] liners of different densities. K. Klemm (*Mitt. Klosterneuberg Ser. A. Rebe u. Wein*, 1966, **16**, 487–491).—Three films of thickness 38μ and $5 = 0{\cdot}918$, 0·922, and 0·940, respectively, were compared in storage trials at 0°. Whilst differences in changes in atm. composition were observed in the packages as between the two lighter foils (the CO_2 increasing up to ~5%) the CO_2 content in the packages lined with the heaviest foil quickly rose to ~9%. The acid content of the apples decreased most in fruits stored without liner foil, and least in those protected by the heaviest liner. Differences in the liners has no effect on the firmness of the tissue or on the *n* of the press juice. P. S. ARUP.

Determination of starch syrup in fruit syrups and calculation of their content of invert sugar and sucrose. J. Schneyder and G. Vlcek (*Mitt. Klosterneuberg Ser. A. Rebe u. Wein*, 1966, **16**, 469–477).—The method of calculation is described and conversion tables are presented for the determinations from the optical rotation of the sample and its reducing sugar content before and after sucrose inversion. The calculation is based on the (approx.) known composition of the starch syrup that has been used. (10 references.) P. S. ARUP.

Effect of blanching on quality and storage stability of dried vegetables. I. Browning of dried turnip cabbage. E. Engl (*Dt. LebensmittRdsch.*, 1967, **63**, 35–40).—The extent of browning during storage was determined spectrophotometrically on samples of turnip cabbage which had been blanched for 10 min. and kept under air or N_2 in the presence of various promotors and inhibitors. These were compared with unblanched controls. Asccorbic acid induced and EDTA and cysteine inhibited browning. U.v. spectra of extracts from fresh and stored samples indicate that phenolic compounds of the flavonoid type are involved. The extent of browning depended directly on the formation and degradation of dehydroascorbic acid. Min. browning occurred after 3 min. blanching which corresponded to the max. content of dichlorophenolindophenol reducing substances and 50% destruction of peroxidase activity. (21 references.) J. B. WOOF.

Bacteriological investigation of deep-frozen vegetables and prepared meals. W. Adam (*Dt. LebensmittRdsch.*, 1967, **63**, 229–233).—The occurrence and temp. resistance of *Salmonella, Staphylococci, Clostridium botulinus* and *C. perfringens, Bacillus cereus* and coliform organisms in prepared vegetables are considered with particular reference to deep freezing. For a no. of commercial prep., total organisms were determined by plating on tryptone-glucose-extract agar and the no. in each group by

plating on selective media. On the basis of the findings permissible limits are suggested. (15 references.) J. B. WOOF.

Investigations into flavour chemistry with special reference to synthesis of volatiles in developing tomato fruit *(Lycopersicon esculentium)* Mill under field and glass greenhouse growing conditions. K. B. Dalal (*Diss. Abstr.*, B, 1967, **27**, 3982–3983).—Two varieties of tomatoes grown in the field and in the greenhouse were studied for non-volatile changes and volatile components. The progress in development of volatile reducing substances, reducing sugars, water-sol. pectins, and org. acids during the process of maturation is described. Four alcohols, isobutanol, isopentanol, n-hexanol, and 2-methyl-3-hexanol were identified, together with certain esters and aldehydes. It is fairly evident that creation of typical tomato odour is greatly influenced by the nonvolatile compounds, such as, total titratable, and org. acids. F. C. SUTTON.

Mechanical behaviour of the sweet potato under slow loading and impact loading. F. S. Wright (*Diss. Abstr.*, B, 1967, **27**, 3909).—The sweet potato was studied from three aspects: mechanical behaviour of the internal tissue (storage parenchyma); mechanical behaviour of external tissue (periderm and cortex); and the effect of load application and skin removal on the wt. loss of the whole root. An Instron instrument was used to obtain force-deformation relationships of root tissue under slow loading. For impact loading an accelerometer and oscilloscope were used to obtain acceleration-time curves. Wt. loss of the sweet potato was not affected by a load application without skin rupture. Removal of the skin tissue caused a considerable increase in wt. loss. F. C. SUTTON.

Non-alcoholic beverages

Production of sterile apple and cabbage juice. W. Mischon (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 86).—The juices were filtered through sterile filters under CO pressure and, after bottling, were stored for 6 months. The apple juice was brown through oxydase activity and no longer fresh tasting similar to pasteurised products. The cabbage juice showed no abnormality. J. B. WOOF.

Tea, coffee, cocoa

Chlorogenic acid content of raw coffee, roast coffee and coffee extract powders. G. Lehmann, H-G. Hahn and O. Luzuriaga (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 273–275.—About 1 g of powder or finely ground bean is extracted with 70% aq. methanol for 24 h in darkness at room temp. or for 1 h on a water bath at 80°. Aliquots of the extract are loaded on to micro columns of polyamide. After washing with water and aq. methanol, the chlorogenic acid is eluted with alkaline methanol and diluted to a standard volume with aq. methanol. The absorption at 324 mμ is measured and compared with that of a standard chlorogenic acid solution treated in exactly the same manner. Values for several coffees and coffee products are quoted and compared with those quoted in the literature. (17 references.) J. B. WOOF.

Compatability concept of coffee varieties. K. Mulhens and L. Graf-Riekmann (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 177–179).—A discussion of the coffee components of pharmocological, flavour and aroma significance with special reference to treatments (e.g. steam treatment before roasting and decaffeinating) which modify such properties and make them more suitable for blending or particular types of consumer. (24 references.) J. B. WOOF.

Milk, Dairy Products, Eggs

Microbial and enzymic activity in raw milk held at low temperatures. T. A. McCaskey (*Diss. Abstr.*, B, 1967, **27**, 4046).—The investigation was conducted to determine the rate of growth of bacteria in milk held at low temp. and to study some of the changes produced by them. Criteria used were pH, titratable acidity, curd tension, fat acidity, and loss of protein in whey derived from milk that was coagulated by rennet. *Pseudomonas fluorescens* appeared to be an important organism in milk held at low temp. The changes, proteolytic activity of *P. fluorescens*, and the greater lipolytic activity in a broth culture of *Achromobacter lipolyticum* are recorded. F. C. SUTTON.

Incidence and significance of thermoduric bacteria in farm milk supplies. S. B. Thomas, R. G. Druce, G. T. Peters and D. G. Griffiths (*J. appl. Bact.*, 1967, **30**, 265–298).—A reappraisal and review. Methods of assessing the incidence of thermoduric bacteria in milk and dairy equipment are discussed. The resistance of these organisms to detergent sterilisers is considered in the light of data obtained in several Welsh surveys. The influence of thermoduric bacteria on the keeping quality of pasteurised milks and the relation of these organisms to the bacterial content of milk powders are also discussed. (165 references.) A. G. POLLARD.

Factors influencing microbial stability in butter-cream-type fillings. J. H. Silliker and S. A. McHugh (*Cereal Sci. Today*, 1967, **12**, 63–65, 73–74).—The concn. of sucrose occurring in the fillings when present in 'devil's-food' cakes permit the growth of (inoculated) Staphylococci, but not of strains of *Escherichia coli* or Salmonella. As fillings containing 2·1–3·0 pt. of sucrose to 1 pt. of water were, in themselves (or when protected by icing in the cakes) bactericidal to Staphylococci it is assumed that their growth is due to dilution of the dissolved sucrose by moisture migration from the pastry crumb. Infections with Staphylococci would not occur with normal sanitary precautions. P. S. ARUP.

Commercial possibilities of acid cream prepared using joghourt bacteria. F. Ahrens and H. Maier-Bode (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 181).—A discussion of the possibility of producing a commercial product by acidifying cream with *Lactobacillus bulgaricus*. J. B. WOOF.

Dehydration induced interactions of egg yolk lipoproteins and low molecular weight carbohydrates. J. R. Schultz (*Diss. Abstr.*, B, 1967, **27**, 3984).—Free lipid, remaining easily extractable with non-polar fat solvents after rehydration of the dried yolk, was implicated as being responsible for the lack of foaming powder in yolk dried without added sugars. Egg yolk dried with added sugars contained no easily extractable lipid after being rehydrated. When the moisture content of the isolated low-density lipoprotein was reduced to below 30%, that lipid began to be released. The effect of these factors on the micellar structure of the lipoproteins is discussed. F. C. SUTTON.

Mechanism of formation of jellies during denaturing of egg albumin. A. S. Zholbolsynova and V. N. Izmailova (*Dokl. Akad. Nauk. SSSR*, 1967, **172**, 130–132).—Three dimensional structure (I) of jelly formed in thermal denaturing of egg albumin (II) was studied. When temp. increased by 1°/10 min., sp. optical rotatory power $[\alpha]_{546}$ of 3·1% aq. solution of **II** at $[H^+]$ 2 was constant up to 30° and then increased to new constant value at 55°. If 2·2 and 3·1% aq. solutions of **II** with $[H^+] < 2$ are kept at 55°, $[\alpha]_{546}$ rises to 90° in 2 h and **II** is fully, irreversibly denatured. Jelly is formed by interaction of carbohydrate chains formed in process. Kinetics of growth of increase in strength of **I** in jelly was determined from ultimate static shear stress. Jelly strength is capable of reversible restoration after mechanical destruction. Strength-time curves for concn. of 2·2 and 3·1% **II** show 60 and 10 min. induction period, after which strength increases steadily to over 10 times in few h to 1–3 and 10^3 dynes/cm^2. Curve and table show that, after complete loss of strength by mechanical breakdown, jelly $[H^+] < 2$ can regain almost its full strength on three successive occasions, when kept 12–48 h. Weak structure which occurs in jellies is similar to thioxotropic coagulation structure networks in which particles of dispersed phase of macromol. aggregates are limited by van der Waals forces acting between hydrophobic carbohydrate groups of **II** mol., set free during thermal denaturing.

P. W. B. HARRISON.

Edible Oils and Fats

Classification and evaluation of margarine in relation to food value determination. R. Ristow (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 73–79).—Analyses including essential and other fatty acids and tocopherol are quoted for a no of margarine samples from the upper middle and lower price groups in the Summer of 1962 and Summer and Winter of 1965. Analyses vary widely within each category. J. B. WOOF.

Stable salt water suspension of [butyrated hydroxyanisole] BHA. L. J. Henthorn (*Cereal Sci. Today*, 1967, **12**, 49–50).—In order to counteract the pro-oxidant effect of NaCl, added in aq. solution to cereals before the final processing, the 23% aq. NaCl is treated before use with a mixture of BHA (595 ppm) and sufficient of a mixture of Tween 60, Tween 80 and Atmos 300 to give an adequately stable suspension. P. S. ARUP.

Polycyclic hydrocarbons in crude vegetable oils. G. Grimmer and A. Hildebrandt (*Chemy. Ind.*, 1967, 2000–2002).—Many samples of unrefined vegetable oils and fats were analysed for contents of 13 polycyclic hydrocarbons (**I**) by extraction of a 50% cyclohexane solution of the oil with water–dimethylformamide (**II**) (1:9), re-extraction of **I** from the **II** phase with aq. cyclohexane, and determination of **I** spectrophotometrically after concn. and purific-

tion (*J. Chromatogr*, 1964, **20,** 89). Contents of **I** in copra were determined similarly. Recoveries were from 67% (anthracene) to 92% [dibenz (a, h) anthracene] and deviation between extreme and mean values for concn. were < 10%. Results showed that **I** in cocoanut oil originated mainly from smoke-drying the copra, and that sunflower-seed and palm-kernal oils are contaminated more than other kinds (e.g. rapeseed, groundnut, soya-bean, linseed), but always less than cocoanut oil, which contained ~ 2450 μg of **I** per kg. W. J. BAKER.

Polycyclic aromatic hydrocarbons in cocoanut oil and their removal. G. Biernoth and H. E. Rost (*Chemy. Ind.*, 1967, 2002–2003).—Reports concn. of 13 of the hydrocarbons in oil from (i) fresh coconuts, (ii) copra treated $Ca(OH)_2$, (iii) hot air-dried copra, and in commercial oil from smoke-dried copra, as determined by a slight modification of the Grimmer-Hildebrandt method (*J. Chromatogr.*, 1965, **20,** 89). The sensitivity is ~0·5 μg per kg, recovery is ~55%, and the coeff. of variation 5–20%. Values show that any further handling and treatment (especially smoke-drying) of copra increases the concn. of hydrocarbons in the oil. They can be removed almost entirely by stirring the oil for ~30 min. at 90–95° with activated C followed by or after steam deodorisation at 180° in vac. Deoderisation removes mainly hydrocarbons having ⩾4 rings, whilst C removes those of higher mol. wt. W. J. BAKER.

Catty odours in food: their production in meat stores from mesityl oxide in paint solvents. R. L. S. Patterson and D. N. Rhodes (*Chemy Ind.*, 1967, 2003–2004).—When hung for 48 h at ~2° in a newly-painted cold store carcasses were contaminated by a lipophilic catty odour. One component of the thinners used in the paint contained ~4% of mesityl oxide, and when treated with H_2S formed a substance with catty odour identical with that in the meat. Attention is drawn to the danger inherent in food stores when paints or solvents containing mesityl oxide are used. Formation of compounds having catty odours at high dilution by reaction of mesityl oxide with H_2S or S-containing amino-acids is described by Aylward *et al.* (*ibid.*, 1967 1562, 1563). W. J. BAKER.

Meat and Poultry

Lipid oxidation and pigment changes in fresh and irradiated beef. B. E. Greene (*Diss. Abstr.*, B, 1967, **27,** 3983).—Lipid oxidation was studied in radiation sterilised cooked beef by the thiobarbituric acid (TBA) test and by manometric experiments. Malonaldehyde (MA), the lipid oxidation product measured by the TBA test, decreased during storage of meat in sealed cans. Exposing the meat to air did not result in the rapid increase in this product that was noted in unirradiated samples. To determine whether MA might react with free amines in the meat, solutions of MA and lysine were incubated for several days. This resulted in a coloured reaction product (possibly a Maillard reaction), and changes in absorbence spectra indicated a new compound. The development of off odours and colours, resulting from non-microbial deterioration of prepacked raw meats, were investigated in relation to these changes to polyunsaturated fatty acid oxidation. Propyl gallate and butylated hydroxyanisole inhibited rancid odours in both fresh and irradiated samples, and effectively inhibited lipid oxidation. F. C. SUTTON.

Effect of stretch-tension during rigor on fibre diameter, fibre extensibility, and tenderness of bovine *longissimus dorsi* muscle. E. M. Buck (*Diss. Abstr.*, B, 1976, **27,** 3982).—It is concluded from the experiments described, that state of muscle contraction during rigor can influence the post-rigor extensibility dia. of individual muscle fibres and ultimately tenderness as determined by shear force. Tenderness in beef apparently does not involve a simple one, two, or three component system but is dependent on many factors. Production of consistently tender beef will depend on elucidation and control of these factors. F. C. SUTTON.

Bacteriology of pre-packed pork with reference to the gas composition within the pack. G. A. Gardner, A. W. Carson and J. Patton (*J. appl. Bact.*, 1967, **30,** 321–333).—Pork was stored at 16° or 2° in open-topped tins sealed with film permeable or impermeable to gasses. Gas concn. (CO_2, N_2, O_2) were determined initially and at hourly intervals together with counts of various groups of micro-organisms present. Comparison was made with similar material under 'aerobic' conditions, tins being covered with perforated film. Relationships between the distribution of groups of organisms, storage temp. and gas concn. are discussed. A. G. POLLARD.

Lipolytic micrococci in pork fat. C. Cantoni, M. R. Malnar, P. Remon and G. Giolitti (*J. appl. Bact.*, 1967, **30,** 190–196).—Various strains and sub-species of micrococci were grown on media containing pork fat. Of 33 free fatty acids subsequently detected 6 contained C-chains of <8 C atoms, none of these being present in the original fat. Fifteen other carbonyl compounds were also found. The data presented are considered in relation to the microflora of dry sausages. A. G. POLLARD.

Scatter in raw protein contents in meat sausage batches. R. Thalacker (*Dt. LebensmittRdsch.*, 1967, **63,** 65–68).—Protein contents of sausages in batches prepared by manual, semi- and fully automatic processes were measured and the results evaluated statistically. 120–200 sausages were examined per batch. The scatter was small in each case, standard deviations being ±0·11, ±0·13 and ±0·13 respectively. J. B. WOOF.

Fish

Effects of ethylene oxide on the tissue and flora of fresh and freeze-dried cod fillets. R. A. Herbert and R. D. Haight (*J. appl. Bact.*, 1967, **30,** 224–229).—Use of ethylene oxide (**I**) to sterilise cod fillets resulted in denaturation of the protein and exudation of sol. material from the tissues. Numbers of surviving organisms were lowered to 0–100 per g of tissue, but sterilisation was never complete. Freeze-dried fillets, treated with **I** and reconstituted in sterile Ringer's solution, yielded a sterile product which, when inoculated with a natural bacterial flora, produced normal spoilage odours. A. G. POLLARD.

Effect of oxygen tension on the spoilage microflora of irradiated and non-irradiated haddock *(Melanogrammus aeglefinus)* fillets. J. J. Licciardello, L. J. Ronsivalli and J. W. Slavin (*J. appl. Bact.*, 1967, **30,** 239–245).—Irradiated (γ-irradiation, 150,000 rad.) and non-irradiated, air-packed and vac.-packed fillets were examined after storage at 35°F. The non-irradiated air-packed or vac.-packed product carried a spoilage flora consisting principally of pseudomonads. Achromobacter formed the main group of spoilage organisms of irradiated air-packed samples and lactobacilli (or possibly *Microbacterium thermosphactum*) predominated in irradiated vac.-packed material. A. G. POLLARD.

Spices, Flavours, etc.

Investigation of nutmegs and nutmeg powder. D. Strauss (*Dt. LebensmittRdsch.*, 1967, **63,** 239–342).—Of the nutmegs investigated 50% of micro-organisms and insects, their cocoons or excrement could be detected. *Aspergillus* and *Mucor* species were common of which *A. flavus* was especially noted because of its association with aflatoxin. *Orycaephilus mercatir* larvae and insects were identified. Photographs of the infestations are shown. In nutmeg powder, parts of insects and larvae and chitin fragments were identified. J. B. WOOF.

Gas chromatographic method for measuring pungency of *Capsicum* species. J. I. Morrison (*Chemy Ind.*, 1967, 1785–1786).—The method, which differentiates capsaicin (**I**) from other pungent substances, *e.g.* vanillyl-n-nonamide, is an improvement over the organoleptic method of Scoville (*J. Am. pharm. Ass.*, 1912, **1,** 453). An aliquot of a benzene-MeOH (1 : 1) solution of the sample, *e.g.* Mombasa oleoresin, is chromatographed on a short column of 2% G.E. SF-96 on Gas. Chrom.-Q (100–120 mesh) at 208° with N_2 as carrier gas and a dual H_2-flame detector at 250°. The concn. of **I** is calculated (by reference to predetermined peak-area per μg of pure **I**) from the peak at 4·25 min. The concn. of **I** is related directly to the organoleptic Scoville Heat Value. W. J. BAKER.

Colouring matters

Carotenoid pigments in plants. Major interfering substances in determining 2,4-D, a metabolite of 2,4-DB. R. D. Hagin, D. L. Linscott, R. N. Roberts, and J. E. Dawson (*J. agric. Fd Chem.*, 1966, **14,** 630–632).—The gas chromatographic peak, formed on analysis of 2,4-DB residues from brome grass and timothy grass has been assumed to be due to 2,4-D formed by the beta-oxidation of 2,4-DB. It is shown here that this peak can equally well be due to the presence of methylated deriv. of the carotenes lutein and violoxanthin that occur in the grasses. The peak in question cannot, therefore, be assumed to be formed entirely by 2,4-D. (13 references.) P. S. ARUP.

Carmine dyes and archil. M. Ney (*Dt. LebensmittRdsch.*, 1967, **63,** 167–170).—Review of the history of the chemistry and use of

these dyes. Analytical procedures and compositions of various commercial prep. are summarised. J. B. Woof.

Pesticides in Foods

Pesticide quest: residue surveys and tolerances. H. Egan (*Chemy Ind.*, 1967, 1721–1730).—An extensive review is presented of the extent to which pesticide residues occur in food and of the consequent degree of their control by limits or tolerances, statutory or otherwise, in Britain and other countries. The problem of establishing residue tolerances is discussed in respect of whether these are based on good agricultural practice (as in Britain) or on systematic knowledge of dietary patterns and acceptable daily human intakes (as adopted by international bodies). Pesticide residues in various foods and diets, permissible daily intakes and some tolerances are listed for various countries and organisations. Though continued caution is necessary in the use of pesticides in agriculture and veterinary practice, there are no immediate risks in Britain but accurate assessment of long-term risks (if any) is much more difficult. (58 references). W. J. Baker.

Pesticide residues in Food. FAO and WHO, Nov. 1966 [Rome 1967] No. 370.—The report comprises the outline of the study covering general considerations, acceptable daily intake, food consumption figures, tolerances and methods employed in estimating; evaluation of consumer hazards; conclusions and recommendations. There is an annex, high food consumption figures based on USA survey (1955). E.M.J.

Pesticide standard transfer canopy: an apparatus for protection and transfer of pesticide reference standards in an inert atmosphere. A. R. Glasgow, jun. and P. Lombardo (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1175–1176).—The cylindrical glass container for the sample vial is provided with a perforated ring for the distribution of dry N. A. A. Eldridge.

Determination of residual amounts of *o*-phenyl phenol on citrus fruits. E. Kröller (*Dt. LebensmittRdsch.*, 1967, **63**, 242–244).—After a brief description of the methods previously described the use of one based on reaction with 4-aminoantipyrine is given in detail. 250 g. of whole citrus fruit is homogenised until oil cells can no longer be discerned. Water, 10 ml 70% H_3PO_4 and antifoam are added and the flask containing the whole mixture connected to a distillation apparatus with a trap containing 10 ml diisopropyl ether. After 6 h distillation, the receiver was washed out and the combined distillates extracted with alkali. After neutralisation, an aliquot of the aq. phase containing 10–70 μg of phenol is added to 0·5 ml of 4-aminoantipyrine (1 g in 100 ml water) and 0·5 ml of $K_4Fe(CN)_6$ (2 g in 100 ml water). The mixture is made up to 25 ml and the extinction determined at 510 mμ. 0·5 to 100 ppm can be estimated with an error of ±150%. (32 references.) J. B. Woof.

Determination of Imidan with Imidoxon in sweet maize by gas chromatography with flame photometric detection. M. C. Bowman and M. Beroza (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1154–1157).—Imidan (*OO*-dimethyl *S*-phthalimidomethyl phosphorodithioate) and Imidoxon (the phosphorothioate) are separated in a SiO_2 gel column and then gas chromatographed at 200° using a glass tube packed with 10% DC-200 on Gas Chrom Q, with a flame photometric detector. The column must be conditioned for 4 to 8 h before injection by repeated injection of maize extract containing the insecticide. Recoveries of 94 to 100% at 0·1 and 5 ppm levels are recorded. A. A. Eldridge.

Gas chromatographic determination of compound 4072 and Shell SD-8447 by electron capture and flame-photometric detection. M. Beroza and M. C. Bowman (*J. agric. Fd Chem.*, 1966, **14**, 627–627).—Conditions are described under which determinations can be made of these insecticides viz., the vinyldiethyl phosphates of 2-chloro-1-(2,4-dichlorophenyl) and of 2-chloro-1-(2,4,5-trichlorophenyl, respectively. The min. detectable amounts are 0·02 ppm by electron capture, and 0·002 ppm by flame photometric detection. The latter method offers several operative advantages over the former. The product of the acid hydrolysis of compound 4072 was confirmed as 2,2′,4′-trichloroacetophenone, and that from SD-8447 as 2,2′,4′,5′tetrachloroacetophenone. P. S. Arup.

Determination of fenthion residues in plant and animal tissues by electron capture gas chromatography. R. J. Anderson, J. S. Thornton, C. A. Anderson, and D. B. Katague (*J. agric. Fd Chem.*, 1966, **14**, 619–622).—Methods are described for the extraction and cleanup of fenthion and its 5 metabolites (fenthion sulphoxide and sulphone, fenthion O-analogue and its sulphoxide and sulphone). The procedure comprises the oxidation of fenthion and its metabolites to the sulphone of the O-analogue, the alkaline hydrolysis of this sulphone, the bromination and acetylation of the liberated phenol, and finally the determination of the phenol by gas chromatography. P. S. Arup.

Determination of residues of fumigant mixture in cereal grain by electron-capture gas chromatography. R. Bielorai and E. Alumot (*J. agric. Fd Chem.*, 1966, **14**, 622–625).—A slightly modified previously described apparatus (cf. *J. Sci. Fd Agric.*, 1965, **16**, 594) is used for the distillation from water with added toluene of the residues in cereals. Nanogram quantities were determined in aliquots of the distilled toluene layer by a simple gas chromatographic method. Recoveries of C_2HCl_3 were 96%, of CCl_4 94%, of $CHCl_3$ 82%, and of CS_2 40–50%. (11 references.) P. S. Arup.

Vapour phase cleanup method for gas chromatographic determination of pesticide residues in plant extractives. J. J. Kim and C. W. Wilson (*J. agric. Fd Chem.*, 1966, **14**, 615–619).—The conc. plant extracts are injected into a horizontal V-shaped chromatographic tube containing a column of silicone grease on Chromosorb P, at 245°; with the use of C_6H_{14} as carrier vapour the pesticide is stripped of non-volatile or less volatile plant constituents. A C_6H_{14} vapour generator and a condenser for trapping the C_6H_{14} containing the purified pesticide are described. Average recoveries of 11 chlorinated hydrocarbon pesticides and DDT from citrus extracts and oils were 92–107%, with good reproducibility. (16 references.) P. S. Arup.

Determination of chloroacetaldehyde-2,4-dinitrophenylhydrazone in apples, peaches, and cherries. Methratta P. Thomas, H. J. Ackermann, and E. J. Kuchar (*J. agric. Fd Chem.*, 1966, **14**, 613–614).—A CH_2Cl_2 extract of this experimental fungicide (CADNP) is cleaned by passage through a column of SiO_2; after evaporation of the CH_2Cl_2 the residue is dissolved in light petroleum—EtoH; the CADNP is extracted from this solution into an aq. buffer at pH 9·4 where it is converted into the coloured glycollic aldehyde-2,4-dinitrophenylhydrazone which is measured in comparison with standards at 360 mμ. The min. detectable amount of CADNP was 0·4 μg. Recoveries were 84–101%. P. S. Arup.

Use of thin layer chromatography for detection of contaminating pesticides in commercial pesticide formulations. W. Bontoyan (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1169–1174).—The procedures of Adams and Schechter (*A.O.A.C. Mtg* 1963, Oct. 14–17, 75) and of Walker and Beroza (*J.A.O.A.C.* 1963, **46**, 250), suitably modified, were applied to the detection of contamination in pesticide formulations. Chlorinated hydrocarbons and organophosphates are particularly considered. A. A. Eldridge.

Effectiveness of some extraction procedures for pesticide residues in vegetables. J. A. Burke and M. L. Porter (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1157–1162).—Of the procedures studied for extracting field-sprayed *pp′*-TDE from kale, the method of Mills, Onley and Gaither (*ibid.*, 1963, **46**, 186), employing acetonitrile, was the most effective, recovering 99% of that removed by exhaustive extraction. The method is also effective for the extraction of parathion and diazinon. A. A. Eldridge.

Thin layer chromatography of dinitrophenol pesticides. G. Yip and S. J. Howard (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1166–1169). 4,6-Dinitro-*o*-cresol, -*o*-s-butylphenol, -*o*-s-amylphenol and -*o*-cyclohexylphenol are separated on cellulose thin-layer plates, either as the phenols or as their methyl ethers. The solvents employed for their resolution are, respectively, (*a*) mineral oil/acetic acid/ethyl ether (immobile); methanol/acetic acid/water (mobile) and (*b*) mineral oil in ethyl ether (immobile); methanol/acetonitrile/water (mobile). After spraying with $SnCl_2$ in HCl followed by *p*-dimethylaminobenzaldehyde in ethanol/*n*-butanol acidified with HCl the spots fluoresce in u.v. light. Detection limits are 0·05 to 0·3 μg. A. A. Eldridge.

Heat-labile insoluble conjugated form of 2,4-dichlorophenoxyacetic acid and 2-(2,4,5-trichlorophenoxy)propionic acid in citrus peel. W. R. Meagher (*J. agric. Fd Chem.*, 1966, **14**, 599–601).—The amount of the growth-regulating residues from peel that had been manufactured into citrus feed was ~3 times greater than that found by extraction with $COMe_2$ from the peel of the fresh fruits; the hot-air treatment during processing had obviously liberated the residues from insol. conjugates with plant constituents that could not be extracted by the usual analytical method. P. S. Arup.

Nutritional interactions in dieldrin toxicity. I. J. Tinsley (*J. agric. Fd Chem.*, 1966, **14**, 563–565).—The effects (short of gross symptoms) of dieldrin dosing (20 ppm) on rats subjected to deficiencies in essential fatty acids were studied by analyses of the liver lipins and found to aggravate the deficiency symptoms. Dieldrin toxicity

in female rats was aggravated by a riboflavin deficiency. (12 references.) P. S. ARUP.

Insecticide reactions affecting residue storage in animal tissues. J. G. Street, R. W. Chadwick, M. Wang and R. L. Philips (*J. agric. Fd Chem.*, 1966, **14**, 545–549).—The dosing of rats with DDT in addition to dieldrin or heptachlor greatly inhibited the storage of these pesticides in the adipose tissue and caused depletion of stored dieldrin. The DDT effect was also observed in sheep and pigs but not in chickens. Evidence as to the mechanism of the effect was not conclusive. P. S. ARUP.

Filth control in western Europe. E. E. A. Maes (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1176–1180).—A review of control, since 1945, of contamination of foods by insect fragments etc. A. A. ELDRIDGE.

Detection limits for some insecticides using optimally fed fruit flies (*Drosophila melanogaster*) as test organism. H. Rothert (*Dt. Lebensmittt Rdsch.*, 1967, **63**, 81–85).—Flies were cultured under standard conditions in flasks at 24° with 12 h light followed by 12 h darkness. The nutrient medium consisted of maize meal, sugar and beer yeast in agar gel. The insecticide at different levels was introduced on the nutrient plates and the time taken to record 50% death rate recorded. Stringent cleaning of the test vessels is necessary. Curves of t_{50} plotted against dose/plate are shown for a no. of insecticides and most show a sharp increase in t_{50} at a point which corresponds to the min. detectable dose. Values obtained compared with those reported by Heusmann (in brackets) are: Heptachlor, 2; chlordan, 10 (5); aldrin, 10 (5); dieldrin, 0·5 (1); Isodrin, 1; Thiodrin, 0·5; Hostatox, 5 (5); DDT, 30 (500); toxaphene, 100 (50); parathion-methyl, 0·5; trichlorfon, 5; isolan, 2 (all μg). J. B. WOOF.

Food Processing, Refrigeration

Effect of freezing and freeze-drying on the physico-chemical changes in Northwest Strawberries. A. V. Rao (*Diss. Abstr.*, B, 1967, **27**, 3983–3984).—The effects of freezing, thawing, freeze-drying, and reconstitution on the physico-chemical properties of the above fruit with particular reference to textural characteristics were investigated. After subjecting the fruit samples to varying degrees of freezing and reconstitution under standard berry to water ratio, measurements were made of the texture, colour, total solids, alcohol insol. solids, pectins, cellulose, ash, and constituents of ash were made on raw, frozen, thawed, and reconstituted berries. Conclusions are recorded. F. C. SUTTON.

Packaging

Development of extruded amylose packaging film. C. E. Mumma (*Cereal Sci. Today*, 1967, **12**, 4–7).—Satisfactory films were produced from fractionated maize starch (containing ~90% of amylose or high amylose hybrid maize starch (containing ~70% of amylose), plasticised with glycerol. The blended materials, starch, glycerol and deionised water, were processed in a laboratory extruder (described) at temp. $\gtrless$182° and $\lessgtr$121°. After extrusion the film was stretched up to five times its original length in order to improve its mechanical properties. The products gave satisfactory results in tests for mechanical strength, vapour and gas permeability, and compatibility with foods. P. S. ARUP.

Metal absorption by foods from metallic objects. II. Lead content of tinned objects. K. G. Bergner and H. Miethke (*Dt. Lebensmittt Rdsch.*, 1967, **63**, 49–51).—Lead has been determined in a number of samples using the method of Jegorow, which involves acid treatment, removal of Fe in the reduced form, extraction of the Pb with dithizone in $CHCl_3$ at pH 9·5 and measurement of the extinction of the extract at 518 and 605 mμ in a 4 cm cuvette. In all cases the level was below 0·1%. J. B. WOOF.

Analysis of organo-tin stabilisers. Thin-layer chromatographic detection of di-(2-ethylhexyl-) tin compounds and the quantitative determination of dialkyl-organotin compounds. D. Helberg (*Dt. Lebensmittt Rdsch.*, 1967, **63**, 69–71).—Qual. TLC was carried out on silica gel layers using a ring technique and a solvent system consisting of iso-octane–di-isopropylether–acetic acid (80 : 3 : 8). Three sectors were streaked with stabiliser extract in cyclohexane (**I**), **I** with 10 μg di-(2-ethylhexyl)-tindiacetate (**II**) and **II** alone respectively. Separation is complete in 60–75 min. and 5–100 μg can be loaded. For quant. work, the extract sample is loaded as a known vol. from a syringe in the form of a 1 cm streak and a standard prep. is separated alongside it. After development, the plate is sprayed with 0·012% dithizone reagent and the orange coloured zones removed. The complex is eluted with acid propanol and again treated with dithizone in the presence of borate buffer, pH 8·4. The orange-coloured complex is then estimated spectrophotometrically at 490 mμ by reference to a standard curve. J. B. WOOF.

Diffusion of phthalate plasticisers from laquered aluminium foil into fatty foods. W. Pfab (*Dt. Lebensmittt Rdsch.*, 1967, **63**, 72).—Cheese and lard were stored in two types of foil; one contained 83·2 mg/dm² dibutylphthalate and the other 92·5 mg/dm² vinyl-chloride-vinyl acetate mixed polymer with free carboxyl groups and 27·3 mg/dm² dichlorohexylphthalate. The fat was then extracted with methylene chloride and saponified. The phthalate residues were extracted from the non-saponifiable residue with butanol and cyclohexanol and estimated by gas chromatography on a column of 15% Zitrophol on Chromosorb P at 125 to 140. In lard, 0·15 and 0·05 mg/dm² were found respectively for the two foils and in cheese 0·12 and 0·13 mg/dm² were detected. J. B. WOOF.

Direct determination of organo-tin stablisers in PVC. K. G. Bergner, U. Rüdt and D. Mack (*Dt. Lebensmittt Rdsch.*, 1967, **63**, 180).—The sample to be analysed 150–200 mg is dissolved in 20 ml of warm tetrahydrofuran and precipitated with 50 ml ethanol. The ppt. is filtered off and washed with ethanol and 0·1% Pyrocatechol Violet (Merck) is added dropwise to give max. blue coloration to the combined filtrates. The blue complex is then titrated to a green end point with 0·001 M Titriplex III or Complexon III. To correct for the presence of Mg, Ca and Zn, Eriochrome black (1%) and 1 ml conc. aq. NH_3 is added to the filtrate. After titration until the red coloration changes to a clear blue, the tin is titrated as before with the Titriplex. The difference between the two is a measure of the tin content; 0·16 to 0·22% tin was found in PVP foils. J. B. WOOF.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Protein biosynthesis. M. Suscillon (*Serie biblies Commiss. Energ. atom*, 1966, No. 73, 66 pp.).—A generalised summary is presented, based on the literature, of our knowledge of the way in which specific proteins (including nucleic acids) are formed from the initial natural amino-acids received by the organism. A discussion of the genetic aspects is followed by a detailed break-down of the various stages of protein biosynthesis in order to justify commonly accepted theory of the process. Finally, the enzymic control of biosynthesis is explained. Reference is made throughout to the value of radio-isotopes for obtaining more accurate information on the genetic, energetic and enzymic phases. (118 references.) W. J. BAKER.

Some molecular controls in biology. M. P. Perutz (*Endeavour*, 1967, **26**, No. 97, 3–8).—The synthesis and activity of enzymes in micro-organisms is regulated by feedback controls. These controls are probably switched on and off by protein mol. that change their structure in response to chemical stimuli. *X*-ray analysis has shown that the four subunits of haemoglobin rearrange themselves under the stimulus of O_2. Haemoglobin is used as a model for discussing the action of the regulatory proteins. (23 references.) I. DICKINSON.

Edible cottonseed protein concentrates. J. T. Lawhon and H. S. Rao (*Cereal Sci. Today*, 1967, **12**, 40–42, 60).—Laboratory experiments on the prep. of meal free from gossypol by extraction with azeotropic mixtures of $COMe_2$–cyclohexane and of COMe-cyclohexane–water are described. A ternary system containing the solvents in the ratio 64·25 : 33·75 : 2·00 was chosen for pilot plant experiments. (16 references.) P. S. ARUP.

Binding of the carcinogenic dye 3-methyl-4-dimethylaminoazobenzol (methyl butter yellow) to protein and nucleic acid of the liver cell. E. Zimmermann and G. Walther (*Dt. Lebensmittt Rdsch.*, 1967, **63**, 78–81).—The dye was prepared with the *N*-methyl group labelled with ^{14}C and the first or second benzene rings labelled with ^{3}H. Using this radioactive prep. it was possible to determine the extent of binding to cytoplasmic and microsomal proteins and nucleic acids by incubation with rat liver homogenate, by application of the dye in vivo and in isolated perfused liver. After extraction, pptn. and purification of the proteins, a $^{14}C/^{3}H$ ratio of 0·588 was found in the cytoplasm and 0·375 in the microsomes compared with 0·602 for the dye as added. There was thus considerable demethylation of the bound mol. If dithionite was added during incubation, recovery of ^{3}H activity indicated that binding to protein occurred through the A-ring. The thermal denaturation curve of the RNA was slightly displaced as a result of dye binding. (22 references.) J. B. WOOF.

Food additive for the control of dental caries. J. H. Curtin, J. Glagolski and B. M. Smythe (*Fd Technol. Aust.*, 1967, **19**, 508–513).—The use of calcium sucrose phosphates as a food additive for the control of dental caries was studied. The substance is obtained by reaction of $POCl_3$ with an aq. solution of sucrose in the presence of lime. The product, a mixture of sucrose phosphates, is obtained as a white, non-hygroscopic, water-sol. powder. Tests have shown it to be harmless to man and animals and it has been shown to reharden softened dental enamel. A controlled trial with 1,000 children showed that toothpaste containing calcium sucrose phosphates produced a small but significant reduction in dental caries in some groups of teeth. In another carefully controlled trial involving 1,300 children it was shown that the addition of 1% calcium sucrose phosphate to all processed carbohydrate foods reduced the no. of new-decayed, missing or filled tooth surfaces in the first year of the trial by 25% compared to the control group. (23 references.) S. A. BROOKS.

Unclassified

Optimisation of production processes in the food industry with the aid of a data processing system. C. Kremkow (*Dt. Lebensmitt-Rdsch.*, 1967, **63**, 170–177).—A programme for electronic data processing systems is described in which relations between process parameters and quality or output of product can be investigated. With its help a mathematical model relating cause and effect can be established which enables production to be optimised. J. B. WOOF.

Statistical evaluation of results of investigations into food stuffs and the need for statistical analysis. J. Eisenbrand (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 265–269).—A discussion from a statistical viewpoint of the 5·5 million samples analysed per year in Germany in 10 different classes of food product. J. B. WOOF.

Comparison of various methods for the determination of moisture in foods. H. M. B. Ballschmieter (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 203–207).—The moisture content of 11 different food products was determined by the following methods: (i) vac. drying for 5 h at 70°, (ii) oven drying at 105° with and without the addition of quartz sand, (iii) drying with the Brabender moisture tester at 90°, (iv) drying under an i.r. lamp and (v) the Karl Fischer titration method. Statistical evaluation of the results relative to a standard 20 h vac. drying method showed that the Karl Fischer method was especially suitable in most cases but (iii) was good for flour, egg and butterfat powders. (iv) was found suitable for milk and cheese powders. (16 references.) J. B. WOOF.

Inactivation of bacterial spores by visible radiations. B. V. Fuller and G. Richardson (*J. appl. Bact.*, 1967, **30**, 347–353).—Exposure to visible radiation resulted in a decrease in visible count of spores of *Bacillus subtilis* and *Clostridiun welchii*. This inactivation is partially reversible if a period of storage at 370° in the dark intervenes before plating, and the presence of dextrose in the counting medium improves the recovery of light damaged spores. (11 references.) C.V.

Genetic analysis of double male and recombination deficient partial diploid strains of *Escherichia coli*. A. R. Kaney (*Diss. Abstr.*, B, 1967, **27**, 4045).—Double male strains of *E. coli* were prepared by mating two different Hfr strains, one of them having been converted to an F^- phenocopy. The double males possessed two non-homologous linkage groups, with the origins and sex factors from both parents. One, but not both, of the linkage groups was transferred to F^- recipients. The three double male strains thus prepared reverted after many generations to simple Hfrs. Partial diploid strains were prepared by mating the double males with F^- recipients carrying the marker *rec* (recombination deficient). The shorter linkage groups of the double males constituted the F-merogenotes of the partial diploids. All crossing over between the merogenote and chromosome was blocked except for a single, irreversible one occurring at the origin end of the merogenote. Some possible implications of these findings are discussed. F. C. SUTTON.

Mycotoxins and their significance in food chemistry. H-J. Rehm (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 269–272).—A review of mycotoxins, the organisms producing them and their toxicity. (23 references). J. B. WOOF.

Viscous behaviour of custard systems. K. Longrée, S. Beaver, P. Buck and J. Nowrey (*J. agric. Fd Chem.*, 1966, **14**, 653–659).—The presence of milk, egg, starch, and sugar is shown to be necessary for the production of custard of normal consistency; the cooking temp. must be $\geq 85°$. Sugar prevents protein flocculation during cooking whilst starch offers surfaces for protein-starch aggregate formation. Agitation during cooling must be carried out at slow speed, and for not too long, in order to avoid unrecoverable loss of η. P. S. ARUP.

Multiplication of yeast on prehydrolysates of olive wood. IV. Influence of pH of the medium on the inhibitory action of methanol, formaldehyde, formic and butyric acid and furfural. I. Schnabel, L. Deheza and J. M. Garrido (*Revta Ciencia apl.*, 1966, **20**, 522–533).—The influence of the potential inhibitors at pH 4·5 and 6 in presence and absence of $K_2S_2O_5$ was examined. Methanol had no effect at either pH; CH_2O inhibited only at pH 4·5 but this effect was counteracted by $K_2S_2O_5$; formic and butyric acids had no effect at pH 6·0 and a small effect at pH 4·5; furfural inhibited only at a fairly high concn. None of these materials alone appears to be responsible for the inhibition shown with aged olive wood. (17 references.) L. A. O'NEILL.

3.—SANITATION, WATER, etc.

Sporicidal properties of some halogens. C. M. Cousins and C. D. Allen (*J. appl. Bact.*, 1967, **30**, 168–174).—Comparison is made of NaOCl, Na dichloroisocyanurate (**I**), dichlorodimethyl- and dibromodimethyl-hydantoin and an iodophor as sporocides against *Bacillus subtilis* and *B. cereus*. The former organism had much the greater resistance. KBr to give ratio, available Cl : KBr = 13·4 : 8 increased the action of **I** at pH 9 but not at pH 7 or 8. The rate of sporicidal action of NaOCl (available Cl 200 ppm at pH 9) was increased by addition of NaOH (1·5–4·0%). A. G. POLLARD.

Chlorine dioxide disinfection: temperature effects. M. A. Bernade, W. B. Snow and V. P. Olivieri (*J. appl. Bact.*, 1967, **30**, 159–167).—Survival of washed *E. coli* cells following exposure to various [ClO_2] at different temp. may be expressed by a reaction model which is of first order in respect of cell concn. and of time of exposure. Bactericidal rates increase sharply with increase of [ClO_2] and/or with rise in temp. A. G. POLLARD.

Use of formaldehyde for disinfection of hospital woollen blankets in laundering. J. C. Dickinson and R. E. Wagg (*J. appl. Bact.*, 1967, **30**, 340–346).—Addition of formaldehyde (**I**) to the final or penultimate rinse of the blankets had a strong disinfecting action on micrococci and *E. coli* used as test organisms. The residual effect of **I** in the dried blankets was considerable; the objectionable odour of the laundered blankets was reduced by using **I** in the penultimate rinse only, although the disinfectant action was still notable. A. G. POLLARD.

Synergism of carbamate and organophosphate insecticides by non-insecticidal carbamates. F. W. Plapp, jun., and T. M. Valega (*J. econ. Ent.*, 1967, **60**, 1094–1102).—LD_{505} for isolan, carbaryl and malathion were determined by comparing them for three resistant and one susceptible strain of *Musca domestica*. Nearly 200 substances were evaluated as synergists. Their activity is tabulated. Considerable reductions of resistance were obtained. (28 references.) C. M. HARDWICK.

Housefly control and insecticide resistance with continued use of diazinon, ronnel and dimethoate. E. J. Hansens, H. J. Benezet and E. S. Evans, jun. (*J. econ. Ent.*, 1967, **60**, 1057–1064).—The control obtained overall a part of 5 years, in barns, is described, together with the levels of resistance and cross-resistance. Most flies reared in manure from treated barns were more resistant than adults from the same barn. Resistance decreased late in the season. Sanitation and weather affected results. C. M. HARDWICK.

Effects of application methods in the toxicity and distribution of dieldrin in houseflies. Yun-Pei Sun, C. H. Schaeffer and E. R. Johnson (*J. econ. Ent.*, 1967, **60**, 1033–1037).—Dieldrin was more toxic to house flies by injection than topical application and least by injection than topical application and least by infusion. Similar results are found for DDT but SD 11319 (3-hydroxy-cis-crotonamide dimethyl phosphate), had similar results by injection and infusion. Possible explanations are discussed. Distribution of dieldrin, its penetration into central nervous system and levels in haemolymph are described. C. M. HARDWICK.

Metabolism of methylcarbamate insecticides by the $NADPH_2$ [nicotinamide adenine dinucleotide phosphate] requiring enzyme system from houseflies. M. Tsukamoto and J. E. Casida (*Nature, Lond.*, 1967, **213**, 49–51).—Various parts of or entire bodies of adult houseflies were tested as a source of enzyme for degradation of methyl carbamate insecticides. Insecticides (24) labelled with

^{14}C, together with synergists, were investigated using six housefly strains, three of which were resistant to carbamate. A variety of co-factors, temp., times of incubation, buffer composition and pH were used. Degradation components were analysed by thin layer chromatography. The highest activity was found with the nicotinamide adenine dinucleotide phosphate ($NADPH_2$) system of the abdomen. Activity was greatest with resistant flies and was increased by the presence of $NADPH_2$. Activity resided almost entirely in the microsome fraction of abdomen homogenates. (25 references.) S. A. BROOKS.

Selective effects of certain anti-fertility compounds on the housefly as shown by reciprocal crosses and histological sectioning. J. B. Kissam, J. A. Wilson and S. B. Hays (*J. econ. Ent.*, 1967, **60**, 1130–1135).—Hydroxyurea did not affect males but females showed morphological changes in ovaries accompanied by reduced egg production and viability. Methylmethane sulphonate and tretamine affected males more than females. These results were based on the use of reciprocal crosses of treated and untreated flies and histological examination. Two different modes of action are indicated. C. M. HARDWICK.

Effect of apholate and thiotepa on nucleic acid synthesis and nucleotide ratios on housefly eggs. R. R. Painter and W. W. Kilgore (*J. Insect Physiol.*, 1967, **13**, 1105–1118).—Viable eggs laid by flies chemosterilised by apholate or thiotepa showed the normal development of deoxyribonucleic acid but some increase in deoxyribosidic components in the acid-sol. matter. In apholate-sterilised eggs there was slightly less guanylic acid than normal and there was also some unidentified substance(s) not found in normal egg ribonucleic acid. In eggs sterilised by thiotepa labelled with ^{32}P all components of the Na salt of ribonucleic acid isolated were labelled. A possible mechanism of this effect is presented. A. G. POLLARD.

Cumulative effects of substerilising dosages of apholate and metepa on laboratory populations of the housefly. P. B. Morgan, G. C. LaBreque, C. N. Smith, D. W. Meifert and C. M. Murvosh (*J. econ. Ent.*, 1964, **60**, 1064–1067).—A colony of flies fed 0·2% metepa continuously died out in the 10th generation. Feeding of 0·01% apholate caused a rapid increase in sterility reaching 69% by F_{30} generation. A sharp decline between F_{30-60} was probably due to formation of a sub-colony. Later it rose to 22%. Evidence for the transmission of genetic damage is given. C. M. HARDWICK.

Repellency of human skin-surface lipid hydrocarbons to the yellow-fever mosquito. W. A. Skinner, H. C. Tong, T. R. Pearson and H. I. Maibach (*J. econ. Ent.*, 1967, **60**, 927–929).—The separation of the lipids is described. Only the unsaturated hydrocarbon fraction was repellent when tested in a dual-part olfactometer. C. M. HARDWICK.

Effect of chemosterilant hempa on yellow-fever mosquito and its liability to induce resistance. J. A. George and A. W. A. Brown (*J. econ. Ent.*, 1967, **60**, 974–978).—Fourth instar *Aedes aegyptii* were exposed to 1280 ppm hempa and the effect on the chromosomes investigated. Broken chromosomes appeared after 48 h. Larval selection resulted in decreased % sterility at the F_5 generation. A later reversal may have been due to the inheritance of genetic defects. C. M. HARDWICK.

Relationships between some physical properties of insecticides and their intrinsic and contact toxicities to adult mosquitos. A. B. Hadaway and F. Barlow (*Bull. ent. Res.*, 1966, **56**, 569–579).—Comparison is made of the intrinsic toxicity (by topical application) and the contact toxicity (by exposure to dry deposits of a no. of insecticides) to *Anopheles stephensi* with some physical properties, e.g., solubility in hexane, partition between hexane and water, (as indicative of potential penetration of the cuticle) of groups of related carbamates and of org. P compounds. Although no consistent relationships were established there was evidence that low solubility of solid insecticides is a limiting factor in the penetration and uptake of solids in mosquitos and is associated with low contact toxicity. With solubilities sufficiently high to ensure dissolution in the wax layer of the cuticle, contact toxicity is increased by a low partition coeff. (hexane/water) of the material. A. G. POLLARD.

Ultra-low-volume aerial application of trichlorfon for control of adult mosquitoes, face flies and horn flies. F. W. Knapp. (*J econ. Ent.*, 1967, **60**, 1193).—Trichlorfon gave rapid knockdown of adult mosquitoes for 48 h of the test. Face fly reduction was poor but hornfly control was >90% for 10 days. C. M. HARDWICK.

Further tests with systemic insecticides in rabbits as toxicants for body lice and new tests for yellow-fever mosquitoes. M. M. Cole and D. L. VanNatta (*J. econ. Ent.*, 1967, **60**, 955–959).—The effectiveness of 19 compounds administered orally to rabbits for control of *Pediculus humanus humanus* and the effectiveness of 39 others against *Aedes aegyptii* is given. (13 references.) C. M. HARDWICK.

Relation of chemical structure to fish toxicity in nitrosalicylanilides and related compounds. C. R. Walker, R. J. Starkey and L. L. Marking (*U.S. Fish. Wildl. Serv.*, 1966, Res. Publ. 13.)—Relations between chemical structures of salicylanilides and benzanilides and their toxicity to rainbow trout and goldfish were evaluated in standard, static bioassays. Single and multiple substitutions of alkyl, nitro and halo groups were tested. Substitution of hydroxy at C2 accompanied by nitro at C3 or C5 on the benzoic acid moiety are basic requirements for toxic activity against fish. Halogenation at $C4^1$ markedly affects the selective toxicity of the compounds to either rainbow trout or goldfish. (13 references.) S. A. BROOKS.

4.—APPARATUS AND UNCLASSIFIED

Comparative elemental analyses of standard plant material. H. J. M. Bowen (*Analyst, Lond.*, 1967, **92**, 124–131).—Results from 29 laboratories for 40 elements in dried kale powder show that consistent values were generally obtained, by different methods, for Au, B, Ba, Br, Ca, Cl, Co, Cr, Fe, Ga, I, Mn, Mo, N, P, Rb, S, Sc and W. The sample can thus be used as standard for assessing the accuracy of analytical methods for these 19 elements in biological materials. Small differences between results were found for Cu, K, Mg, Na, P, Se, Sr and Zn, *e.g.*, flame photometry gave high values for Na; activation analysis without chemical separation was unreliable for K and Mg; atomic absorption spectrometry gave high values for Cu and Sr; polarography gave lower results than other methods for Zn. There were gross discrepancies in the results for Al, As, Hg, Ni and Ti, analogous to those reported for mammalian blood (*idem*, *U.K.A.E.R.E.*-R4196). A comparison is made of the precisions obtained by different techniques (activation, atomic absorption, colorimetry, flame photometry, polarography) used to determine Ca, Co, Cu, Fe, Mn, Mo, Na and Zn. W. J. BAKER.

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ABSTRACTS

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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.

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