JOURNAL
OF THE
SCIENCE OF FOOD AND AGRICULTURE
(INCLUDING ABSTRACTS)

Published by the Society of Chemical Industry

Volume 21 | No. 7 | July, 1970

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GROWTH OF MAIZE ENDOSPERM TISSUE *IN VITRO*

I.—Nitrogen requirement of tissue grown on solid media

By KATHLEEN R. FARRAR and L. S. GANUGAPATI

Ammonium salts can replace asparagine as the main nitrogen source for maize endosperm tissue culture. Although the acetate, oxalacetate and oxoglutarate are toxic, the succinate or citrate may be used.

Introduction

Maize endosperm will proliferate readily in tissue culture[1-3] to give a material of inoffensive flavour and texture which could be of value as a dietary supplement if it were possible to grow it economically on a large scale. The present study has shown that the asparagine or yeast extract used by earlier workers[3,4] can be entirely replaced by ammonium salts. Previous attempts to do this had not been encouraging,[5,6] although there was evidence that the two factors involved (utilisation and toxicity) could vary independently in different types of tissue culture.

Experimental and Results

Materials

The maize endosperm cultures were kindly supplied by the late Dr. J. Straus, of the University of Oregon.

Culture media

At the beginning of the work, the medium used was that of Straus (personal communication); asparagine (2 g) was dissolved in distilled water (100 ml) and added to a mixture of White's solution (100 ml), agar (9 g), sucrose (20 g), vitamin solution (5 ml), ferric citrate solution (4 ml), and distilled water (800 ml).

White's solution: Na_2SO_4 (8·0 g), $MgSO_4.7H_2O$ (21·2 g), $Ca(NO_3)_2.4H_2O$(13·9g), KNO_3(3·2g), KCl(2·6g), $NaH_2PO_4.H_2O$ (6·6 g). The first two ingredients, dissolved in distilled water (500 ml), were added slowly to distilled water (3460 ml) in which the others had been dissolved successively; finally Nitsch's solution (40 ml) was added.

Nitsch's solution: H_2SO_4 (0·5 ml), $MnSO_4.4H_2O$ (3 g), $ZnSO_4.7H_2O$ (0·5 g), H_3BO_3 (0·5 g), $CuSO_4.5H_2O$ (25 mg), $Na_2MoO_4.2H_2O$ (25 mg), $CoCl_2$ (25 mg), dissolved in distilled water (1000 ml).

Vitamin solution: glycine (450 mg), niacin (75 mg), thiamine hydrochloride (15 mg), pyridoxine hydrochloride (15 mg), calcium pantothenate (15 mg), dissolved in distilled water (300 ml).

Ferric citrate solution: ferric citrate (1·25 g) dissolved in previously boiled hot water (500 ml).

The prepared medium was adjusted to pH 6·8 by adding sodium hydroxide solution, distributed (15 ml) into 2 oz glass bottles with metal screw caps, and sterilised for 15 min at 15 lb/in^2. Cultures were grown at 27°.

Replacement of asparagine by ammonium succinate

Preliminary work had shown that some growth would occur under these conditions. The effect was studied systematically with succinate concentrations in the range 0·05–1·0%; the medium was that described above, asparagine being omitted, and replaced by varying amounts of ammonium succinate. Since all experiments involved a constant quantity of nitrogen from the White's solution, the additional nitrogen (asparagine or ammonium salt) is referred to subsequently as the 'nitrogen supplement'. 20 replicates were used for each concentration and cultures were harvested after 5 weeks.

Each inoculum was removed with a single scoop of the section lifter, in order to minimise microbial contamination and damage to the tissue. Inoculum size was therefore somewhat variable. It was found[7] that as long as inoculum size was kept within the limits of 100–200 mg, there was no significant effect on percentage yield, the parameter used as an index of the efficacy of the various nitrogen supplements.

The weight of tissue harvested was significantly correlated with inoculum size ($P < 0·01$) at very low and very high concentrations of ammonium succinate, i.e. when all the tissues were being starved by deficit or poisoned by excess of nitrogen. At optimum concentrations (0·2–0·4%), correlation coefficients were relatively low, probably because in these cases growth depended more on the biological properties of individual tissue pieces than on inoculum size.

Results for concentrations of ammonium succinate in the range 0·05–1·0% are given in Table I. Low concentrations of ammonium succinate were also tested (Table II). The experiment was repeated several times. The results of these repeat experiments are summarised in Table III.

In experiment 2 (Table III) some concentrations of ammonium succinate were compared with each other and with controls containing no ammonium succinate. The results are given in Table IV.

The effect of ammonium succinate began to be statistically significant when its concentration reached 0·025%; the medium then contains 98 mg N/l (unsupplemented medium 41 mg).

For the experiment of which the results are recorded in Table I the samples grown on 0·1% ammonium succinate were compared with those grown on the 0·05% and the asparagine media. In this experiment, the 0·1% ammonium succinate medium had given the highest percentage increase in wet weight apart from the asparagine controls. The results are shown in Table V.

The results so far showed that media supplemented with ammonium succinate (0·025–0·6%) gave significantly greater percentage increases in wet weight than did a basal medium containing only the nitrate of White's solution. The optimum concentration observed in one experiment gave an increase lower, though not significantly so, than the increase given by a 0·2% asparagine control.

After experiment 2 (Table III) which seemed to indicate that the cultures were losing vigour, some selection of the stock cultures was carried out. In Table VI, experiments from April 1966 onwards are from the new stock obtained from this selection.

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TABLE I

Increase in wet weight with ammonium succinate concentration

Nitrogen supplement	Concentration, %	Increase in wet weight, %
Ammonium succinate	0·05	492
,, ,,	0·10	590
,, ,,	0·15	518
,, ,,	0·20	477
,, ,,	0·25	474
,, ,,	0·30	439
,, ,,	0·35	454
,, ,,	0·40	319
,, ,,	0·50	267
,, ,,	0·60	215
,, ,,	0·80	137
,, ,,	1·00	85
Asparagine	0·20	725

TABLE II

Increase in wet weight with low ammonium succinate concentration

Nitrogen supplement	Concentration, %	Increase in wet weight, %
None	—	244
Ammonium succinate	0·01	381
,, ,,	0·02	482
,, ,,	0·03	556
,, ,,	0·04	544
,, ,,	0·05	644
,, ,,	0·075	616
,, ,,	0·10	620
,, ,,	0·125	649
,, ,,	0·15	744
,, ,,	0·175	644
,, ,,	0·20	622
,, ,,	0·25	856
,, ,,	0·30	808

TABLE III

Increase in wet weight with ammonium succinate concentration in repeat experiments

Nitrogen supplement	Concentration, %	Increase in wet weight, %		
		Experiment 1	Experiment 2	Experiment 3
None	—	84	162	125
Ammonium succinate	0·001	87	213	
,,	0·005	144	256	
,,	0·01	173	260	225
,,	0·025	258	335	
,,	0·05	552	393	400
,,	0·075	426		
,,	0·10	429	468	467
,,	0·150	552		
,,	0·20	578	336	547
,,	0·25	511		649
,,	0·30	556	428	378
,,	0·35	444		
,,	0·40	433	306	
,,	0·50	441		
,,	0·60	411	209	
,,	0·80	192	133	
,,	1·0	189	92	
Asparagine	0·2		532	

TABLE IV

Effect of ammonium succinate concentrations compared with each other

Media compared (% ammonium succinate)	t	Degrees of freedom	P, %
0·000 and 0·001	1·73	36	<10 > 5
0·001 and 0·01	1·55	38	<10 > 2·5
0·000 and 0·025	2·63	35	< 1
0·000 and 0·05	4·25	36	> 0·1

TABLE V

Effect of ammonium succinate–asparagine media concentrations compared

Media compared	Sample size	t	Degrees of freedom	P, %
0·1% ammonium succinate	18	1·56	35	>10
0·2% asparagine	19			
0·1% ammonium succinate	18	1·30	35	>10
0·05% ammonium succinate	19			

TABLE VI

Percentage increase in wet weight of tissues grown on 0·2% ammonium succinate and 0·2% asparagine medium

Date	% increase
Jan. 1965	477 (725)
Feb. 1965	485
Feb. 1965	515
Mar. 1965	575
May 1965	622
Oct. 1965	578
Dec. 1965	428 (532)
Dec. 1965	430
Jan. 1966	547
Apr. 1966	1217
Apr. 1966	2072
Nov. 1966	(1767)
Jan. 1967	(1868)
Feb. 1967	1305
Feb. 1967	1903
Mar. 1967	1243

Variations in growth in a number of control and other experiments using 0·2% ammonium succinate are shown in Table VI (figures in brackets indicate 0·2% asparagine medium).

Composition of tissues grown on 0·2% ammonium succinate

These results are summarised in Table VII. From these figures it can be calculated that 63% of the total nitrogen of the medium has been incorporated into the tissues, and that of this, at least 80% must have come from the ammonium salt. If all the nitrogen were converted into protein (a matter which was not investigated), the protein content would be about 25% of the dry weight.

TABLE VII

Composition of tissues grown on 0·2% ammonium succinate

Final wet weight, g	Final dry weight,		% of dry weight		
	g	%	C	H	N
5·095	0·115	2·26	42·8	6·6	3·8
3·990	0·092	2·30	42·6	6·4	4·1
4·795	0·090	1·87	42·6	6·2	3·6
4·232	0·102	2·41	43·0	6·7	4·2
4·102	0·098	2·39	42·8	6·6	3·8

TABLE VIII

Effect of ammonium acetate concentration on growth of tissues

Ammonium acetate, %	No. of samples	% increase in wet weight
0	18	196
0·001	20	233
0·01	20	261
0·02	19	288
0·03	20	179
0·04	18	129
0·05	22	60
0·075	19	0
0·10	17	−5·5

It has been found possible to maintain cultures for five years on a medium containing ammonium succinate as the only nitrogen supplement.

Experiments with solid media containing ammonium acetate

Preliminary trials with a medium containing ammonium acetate instead of succinate (concentration range 0·05–1·0%) showed that all cultures shrank, with the exception of a few at the lowest concentration. Table VIII shows the effect of 0–0·1% ammonium acetate on the growth of the tissue. At the three highest concentrations several cultures shrank.

Although media containing 0·02% or less of ammonium acetate supported more growth than the unsupplemented medium, this increase was statistically significant at the 5% level only in one case (at 0·02%). There was some evidence that cultures which had been maintained for some months on succinate media were less susceptible to ammonium acetate toxicity than others maintained on asparagine, but the effect was not very large; nor did any useful results emerge from an extensive series of experiments using mixed acetate–succinate media.[7]

Experiments with ammonium citrate

The effect of several concentrations of ammonium citrate was investigated in two experiments. The results are given in Table IX.

Both experiments gave agreement in indicating an optimum concentration around 0·1% citrate, but there were differences between the two which in some cases were statistically highly significant. They may be due either to some toxic contamination (possibly detergent) of expt. 2, or to a difference in vigour of the cultures. It is clear, however, that citrate becomes toxic at a lower concentration than succinate.

A series of experiments in which sodium citrate and succinate were compared with the ammonium salts at the same concentration,[7] showed that succinate anions had no significant effect on the growth of the tissue, but that citrate was slightly inhibitory.

TABLE IX

Effect of ammonium citrate concentration on increase in wet weight in two experiments

Ammonium citrate, %	% increase in wet weight	
	Expt. 1	Expt. 2
0		196
0·001		231
0·01		344
0·02		405
0·03		368
0·04		422
0·05		448
0·10	954	489
0·15	892	
0·20	657	446
0·30	467	415
0·40	313	195
0·60	−14	−4
0·80	−32	

TABLE X

Effect of ammonium nitrate concentration on wet weight increase

Nitrogen supplement	Concentration, %	Wet weight increase, %
Ammonium nitrate	0·2	172
Ammonium nitrate	0·1	497
Ammonium nitrate	0·05	655
Ammonium succinate	0·2	1243

Experiments with ammonium oxoglutarate, oxalacetate, and nitrate

To avoid decomposition during heat sterilisation, ammonium oxoglutarate and oxalacetate were Seitz-filtered before being added at 0·2% concentration to the sterile medium. Both were inhibitory, causing shrinkage of the cultures.

The results of a few experiments using ammonium nitrate are summarised in Table X.

Discussion

This work was undertaken in the hope of finding a cheaper source of nitrogen for the growth of maize endosperm tissue than the asparagine of Straus's medium. The first choice, ammonium succinate, proved to be a fortunate one; it supported growth nearly as well as asparagine. Ammonium succinate, however, is by no means the cheapest ammonium salt, and other salts were tested, with somewhat surprising results.

One of the difficulties in using ammonia as a nitrogen source is that of finding a suitable acid with which to neutralise it. Mineral acids which are not metabolised will remain after the removal of ammonia, leaving the medium excessively acid. However, not all of the acids known to be metabolised can be used to provide the required anions.

It is known that nitrate can be utilised by plant tissue cultures,[5] and there is some evidence[8] that when nitrate is present, ammonia can be utilised over a greater range of pH. However, in the present work, it was found that ammonium nitrate was toxic at concentrations with a total nitrogen content equivalent to that of an ammonium succinate medium giving optimum growth. Whether or not this is due to differential rates of metabolism of the two ions giving rise to

acidity, it seems clear that ammonium nitrate had a toxicity for some tissue cultures which cannot be accounted for in terms of its constituent ions. At least one other set of workers has come to a similar conclusion, using an entirely different plant tissue.[9]

Ammonium citrate was less effective as a nitrogen source than the succinate, and experiments using the sodium salts of these acids showed that citrate was slightly inhibitory at the same concentration (0·1%) at which succinate had no significant effect on growth. Ammonium acetate, oxoglutarate, and oxalacetate were definitely toxic, so that the ability of anions to be removed through the citric acid cycle does not appear to be a factor necessarily reducing their toxicity.

The toxicity of ammonium acetate was at first sight surprising, since acetate is thought of as a universal 'building block' in biosynthesis. There are reports, however, that acetate in peat soils is phytotoxic,[10] that 0·1% sodium acetate inhibited the growth of guayule seedlings,[11] and that a number of organic acids (including acetic) suppressed the growth of sugar cane, although hydrochloric acid controls did not, and lactic, malic, and succinic acids were stimulants.[12] It has also been found that 0·2% ammonium acetate inhibited the growth of maize embryos.[7] A thorough study of the effect of organic acids (as their sodium salts) on various kinds of plant tissue cultures[13] has given results broadly in agreement with this work; acetate was inhibitory to all tissues tested at 0·06%, citrate much less so, while succinate was tolerated by one tissue even at 1%. The reason for this rather widespread toxicity of acetate is not known, although it may be significant that it is inhibitory to the enzyme glutamate decarboxylase at concentrations at which succinate is ineffective.[14]

Acknowledgments

The authors are indebted to the late Dr. J. Straus for the gift of cultures and for much advice, to Professor A. J. Birch for facilities and encouragement, and to the Agricultural Research Council for a grant (to L.S.G.).

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Received 8 January, 1970;
amended manuscript 9 March, 1970

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MILK REPLACERS FOR PIGLETS*

By M. J. MANNERS

Introduction

The sow farrows approximately twice a year. Her litters consist of about 11 live pigs, of which she rears 8 or 9. The sow cannot rear more pigs than she has functional teats, because piglets all suckle at once and there is no milk for a 'second sitting'. If artificial rearing were simple and straightforward, it would be possible to attempt to increase litter size by selective breeding or by hormone treatment. Extra-large litters of piglets could be helped over the short period when they must have colostrum by allowing them to suckle in relays once every two hours before going into artificial rearing batteries.

Under natural conditions, the stimulus of weaning is needed to cause the sow to come into oestrus. The earlier the sow is weaned, the sooner she can be got 'in pig' again and the greater will be her output of piglets in a given period. The cost of maintaining breeding sows rises only slowly with increased output of piglets. If one sow can be the mother of more piglets because of the shorter breeding cycle associated with early weaning, the general genetic quality of the breeding herd can be higher, since more rigorous selection of sows can be practised.

In theory, artificial rearing should reduce the cost of pig production for various reasons; (i) it should be possible to eliminate most of the mortality associated with the normal suckling period (where 25% loss is regularly accepted on a national scale). Artificial rearing has proved that almost all live-born pigs can be reared, and, thus, that normal pre-weaning losses are not due to anatomical or physiological deficiencies of a proportion of all newborn piglets; (ii) feeding the piglet via the sow is energetically inefficient, although it may be economically sound as the sow can digest cheaper foods than the piglet can; (iii) litters of very large size can be aimed for; (iv) over a given period of time, more piglets can be obtained from a fixed number of sows if early weaning is practised; (v) it should be easier to achieve uniformity in size of 8 week-old pigs; and (vi) disease control should be easier.

A fuller discussion of the advantages and disadvantages of early weaning has been given by Braude.[1]

If, by some reasonably cheap hormone treatment, or otherwise, it could be made certain that sows would come into oestrus and conceive during the suckling period, some of the arguments in favour of artificial rearing of piglets would no longer have any force. Nevertheless, natural rearing would still entail the disadvantage of heavy pre-weaning mortality in suckled piglets, the energetic inefficiency of feeding the piglets via the sow, and lack of control over disease transmission from sow to offspring.

In the literature on milk replacer diets for artificial rearing of piglets, success has been claimed for mixtures differing very widely in composition. It is unlikely that many of the diets used to date are especially suitable to the requirements of the very young piglet. If they were, artificial rearing of piglets would be taken up by the pig industry very rapidly. While there have been sporadic attempts to popularise artificial rearing in the last 20 years, there is still little enthusiasm for the technique. This is probably because the physiological requirements of the piglet reared artificially are not fully understood. Studies of digestive physiology should be given priority, rather than simple comparisons of diets based on measurements of growth and gross economy of food conversion.

Artificial feeding

There are several excellent reviews[1–4] on piglet nutrition which can be consulted for summaries of work which has already been done.

Piglets should thrive best when fed small feeds at regular intervals. On the sow in early lactation, they receive about 24 feeds a day, evenly distributed throughout the 24 hours.[5] However, under farm conditions when pigs are reared artificially, two or three feeds only are given, and these during normal working hours. This predisposes to overloading of the stomach of the greedier members of a litter, and the results are often disastrous. In groups, it is best to feed piglets on dry meal or pellets in self-feeders. The dryness of the meal feed imposes a limit on intake, and the piglets are less likely to develop digestive upsets under such feeding arrangements. At the National Institute for Research in Dairying, two methods of feeding homogenised cow's milk to 2-day weaned piglets were compared: twice-daily *versus* 24-times-daily.[6] Some improvement in rate of gain and efficiency of feed conversion was observed on the hourly compared with twice-daily feeding. However, it was noted that the high water content of the milk appeared to limit feed intake on this diet. Now that electronically controlled, automatic feeding arrangements are becoming more common, the dispensing of measured small quantities of feed every hour will be widely applied to piglet rearing, and will reduce the hazard of the procedure.

At Bristol, an automatic feeder is being developed on different lines to the Shinfield machine. However, all the research over the past 14 years on the comparison of diets of varying composition has been done under a twice-daily feeding regime. Feeding takes place at 9 a.m. and 4 p.m. This has imposed considerable stress on the piglets that have been reared, but the diets and system of management have been adapted in ways which minimise stress.

Bulkiness of diets

Some of the diets that have been used required about 3 g of water/g of diet to mix to a gruel-like consistency. Others needed much less water to achieve the same consistency. No success has been obtained with the former types of diet and as little water as possible is now added to make a drinkable gruel. 1 g of water (or less) to every 1 g of diet is the ratio used, and the fact that piglets can be reared with twice-daily feeding may be linked closely to this limitation on bulk intake that has been imposed. Drinking water is provided, in addition, in a separate compartment of the feeding trough.

Level of feed intake

Another very important factor in determining success or otherwise of twice-daily feeding is dry matter intake. *Ad lib.*

* Presented at a Symposium on 'Milk Replacers for Young Farm Animals', organised by the Agriculture Group, on 21 October, 1969

feeding of gruels under conditions of twice-daily feeding would be likely to lead to gastro-intestinal troubles and mortality. Diets are fed at the rate of about $0{\cdot}24\ W^{0{\cdot}75}$ g/feed (where W = weight of pig in g). This is a restricted scale of feeding, arrived at by trial and error. It works well under these conditions and with this type of diet.

Since a very young piglet has a potential for very rapid growth, and since its digestive system is quite immature, care in formulation of its diet is of importance in attempts to rear piglets artificially, and most of the author's research has been directed towards improving diets for rearing piglets weaned at 2 days of age. To minimise stress, the diet should contain neither too much nor too little of any nutrient. This is a council of perfection as the optimum levels of nutrients are not known in every case. However, the piglet's metabolism will be put under strain if dietary imbalances are presented to its digestive system. In addition to care in diet formulation, it should be emphasised that environmental temperature, type of housing, number of animals in a pen, frequency of feeding, hygiene – in fact all aspects of management – are important, and no aspect of the piglet's well-being should be neglected if piglets are to be reared successfully on artificial diets.

pH of the gut

Investigations of the whole problem of pH in the gut of the piglet would be very rewarding in practical terms, since a great deal is likely to depend on the reaction of the gut contents. The results of some of these have already been published.

pH in the gut of older pigs

pH values for older pigs are shown in Figs 1 and 2. These both illustrate that a wide range of values may be expected at all points in the gut. In Fig. 1, Møllgaard's[7] data are for 'pigs under normal conditions of feeding'. Samples were taken from tubes inserted into the various parts of the gut. Sampling times after feeding were ½ h, ¾ h, 3 h and 5 h for stomach, duodenum, jejunum and ileum respectively. Kvasnitskii's[8] data were from 6-month old pigs fed a range of protein levels. Gastric pH was slightly lower in pigs fed a high protein ration. The high protein ration also influenced pH in the first half of the small intestine, causing it to be lower than on the low protein ration. The data of Chachulowa *et al.*[9] were for caecal contents on a range of diets. Møllgaard's data show clearly the difference between the pH of the outer liquid in the stomach and that of the inner food mass. Møllgaard remarked on the low pH found in the duodenum. For the jejunum and ileum, there was fairly close agreement between the values reported by Møllgaard and those given by Kvasnitskii. It appeared that there was a continual rise in pH of contents as food passed down the small intestine. It was not until the digesta reached the latter half of the small intestine that pH became neutral.

pH in the gut of suckled pigs

Fig. 2 shows data of Williams Smith & Jones[10] for suckled piglets alongside those for weaned pigs and it can be seen that, while pH in the stomach contents tended to fall after weaning, pH in the distal small intestine tended to rise. There was fairly good agreement between the range of values found by Williams Smith & Jones for weaned pigs and those summarised in Fig. 1 from data from abroad.

pH in the gut of suckled pigs was also studied by Walker[11] and his data bear out the observation of Williams Smith & Jones that pH values of gut contents of suckled piglets showed little tendency to change with age during the suckling period. In stomach contents, although mean pH was 3·4, there was very considerable variation between piglets. The mean pH in the duodenum was 6·5, and in the rest of the small intestine 6·4, the contents of the former showing much more variation in pH than those of the latter.

Although, for most of the suckling period, pH in the gut of the piglet did not change with age, values for stomach contents were an exception to this rule in the results of Williams Smith & Jones.[10] Here, pH in the first day of life differed markedly from that in the stomach of older piglets, in that the pH was much higher. Williams Smith & Jones commented on the difficulty of arriving at a typical pH value of stomach contents because of the variation in pH between the contents of different parts. They made an incision in the middle of the greater curvature of the stomach and took the first 20 ml of contents to emerge, for pH measurement.

pH in the gut of piglets reared artificially

Results published by Hartman *et al.*[12] provide a comparison between pH in the gut of piglets reared artificially and that in the gut of suckled piglets. These workers compared 8 litters of suckled piglets with 8 litters which were weaned at one week of age to self-fed dry rations. For the first week after weaning, a 40% dried skim-milk ration was

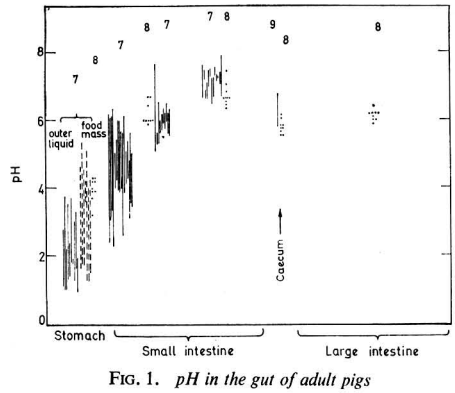

FIG. 1. *pH in the gut of adult pigs*
Numbers refer to references

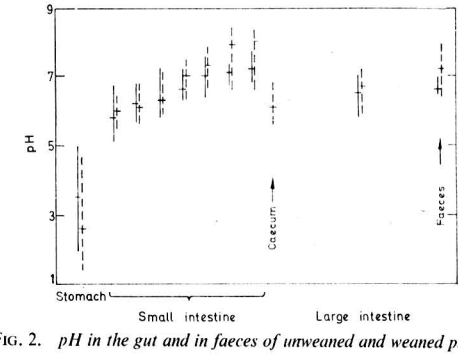

FIG. 2. *pH in the gut and in faeces of unweaned and weaned pigs*[10]
—— Range for unweaned pigs; — — — range for weaned pigs.
Horizontal lines: median

fed, followed by a 20% protein maize–soyabean meal starter diet to 5 weeks of age. From 5 weeks onwards, an 18% protein maize–soyabean meal diet was fed. From 5 weeks onwards, pH of stomach contents was lower in the piglets reared artificially than in the sow-reared piglets. In the small intestine, the piglets reared artificially showed distinctly lower pH values than the sow-reared piglets as soon as they were changed on to the early-weaning diets. In the anterior, middle and posterior thirds of the small intestine of piglets reared artificially, pH was 5·8–6·0, 5·7–6·2 and 5·9–6·4, respectively. In sow-reared piglets, over the same period, pH in the same three sections of the small intestine was 6·2–6·5, 6·4–6·8 and 6·6–7·0, respectively.

McCrea & Manners have collected a small number of pH measurements on gut contents of piglets reared artificially. The piglets were killed 2½ h after the last feed by injection of pentobarbitone sodium. The body cavity was opened and the measurements were made by inserting pH electrodes into the gut of the piglet at a number of points before dissecting out the gut. The points at which measurements were made were labelled with marked clamps. The gut was dissected out later to determine the positions at which the measurements had been taken. pH values were then plotted against distance along the intestine (expressed as percentage of total length). The individual points were joined on the graph, and the values at standard distances along the gut were then read off. For the stomach, the electrodes were plunged about 2½ cm into the food mass at standard points, one in the cardiac area, one in the fundus, and one in the pyloric area. It was felt that this procedure, which involved the minimum disturbance of the gut before the measurements were taken, was preferable to squeezing out contents before taking measurements. Table I gives details of the diets fed and the methods of feeding, both of which changed as the piglets grew older. The Table gives mean values and their standard errors for pH in the gut of the piglets killed at the various ages studied. Despite a change from individual feeding in cages to group feeding in floor pens after 4 weeks of age, there was little difference in pH in the gut between the 3 age groups studied while the piglets were still receiving the casein–glucose–dried milk diet. At 5 weeks of age, there was greater variability in pH in the stomach and in the distal part of the small intestine than at earlier ages. This might have been due to irregularity in feeding times under *ad lib.* feeding in contrast to the standard interval between the last feed and the times of killing of the younger piglets. In the four 7-week old piglets on the 'weaner' diet, pH of stomach contents was considerably lower than that in the younger piglets studied, the values obtained being more typical of those to be expected in adult pigs. In the small intestine, pH in the duodenum showed an upswing compared with that in the same part of the intestine in younger piglets. pH in the caecum and in the large intestine was at a lower level than had been observed in the younger piglets in the series. Obviously, there are various complications in comparing the data from the piglets in this series of observations. Both the type of diet and the method of feeding may have influenced the values collected, in addition to any change in output of digestive secretions which may accompany an increase in age of the piglet. Nevertheless, the results of these measurements show the approximate pH that can be expected under a fairly conventional system of artificial rearing.

Factors affecting gastric pH measurement

The measurement of gastric pH of the living animal in its normal environment can be made by means of an electrode inserted into the stomach via a cannula. Maner *et al.*[13] used this method to measure gastric pH before feeding and at

TABLE I

Mean values with their standard errors for pH in the gut of piglets reared artificially on different diets and at different ages (Manners & McCrea, unpublished data)

Portion of gut	Casein–glucose–dried milk diets			'Weaner'-type diet (barley meal, fish-meal, soyabean meal)
	3 one-week old and 2 two-week old piglets*	5 three-week old and 3 four-week old piglets*	4 five-week old piglets†	4 seven-week old piglets†
Stomach:				
Cardiac	5·24 ± 0·17	4·98 ± 0·16	5·25 ± 0·10	3·86 ± 0·67
Fundic	4·75 ± 0·39	4·50 ± 0·20	4·05 ± 0·51	2·23 ± 0·29
Pyloric	5·12 ± 0·25	4·38 ± 0·24	4·14 ± 0·42	3·11 ± 0·29
Small intestine (% of length):				
5	5·29 ± 0·11	5·22 ± 0·09	5·15 ± 0·05	5·69 ± 0·06
25	5·27 ± 0·08	5·48 ± 0·14	5·50 ± 0·16	5·39 ± 0·20
50	5·58 ± 0·18	5·74 ± 0·19	5·87 ± 0·22	6·07 ± 0·24
75	6·06 ± 0·20	6·18 ± 0·16	6·54 ± 0·44	6·04 ± 0·17
95	5·61 ± 0·09	6·58 ± 0·11	6·62 ± 0·47	6·47 ± 0·12
Caecum	6·45 ± 0·07	6·61 ± 0·12	6·41 ± 0·17	5·51 ± 0·11
Large intestine (% of length):				
10	6·62 ± 0·10	6·62 ± 0·10	6·62 ± 0·13	5·59 ± 0·09
50	6·43 ± 0·10	6·68 ± 0·07	6·82 ± 0·09	5·78 ± 0·06
90	6·48 ± 0·17	6·48 ± 0·08	6·49 ± 0·17	6·23 ± 0·20

* Diets fed as gruels according to a limited scale of feeding; piglets individually caged
† Diets fed dry *ad libitum* in self feeders; piglets in groups in floor pens

intervals of up to 4 h thereafter. At 4 and at 8 weeks of age the pre-feeding pH averaged 1·6 –1·8. At 4 weeks of age, when diets were fed in liquid form, pH 1 hour after feeding was 3·2 on casein–glucose–lard diets, compared with 5·0 on diets in which isolated soyabean protein replaced the casein. At 2 h after feeding, pH had fallen to 1·7 on the casein diet and to 4·2 on the soyabean protein diet. At 4 h after feeding, pH had fallen further on both diets to 1·1 and 1·3, respectively. On different diets, containing much less protein and fat, fed dry to pigs of 8 weeks of age, pH 1 hour after feeding was 1·7 on the casein diet and 2·5 on the soyabean protein diet. By 2 h after feeding, pH was 1·7 and 1·9, respectively, on the two diets. The gastric pH values found were thus much lower than those reported by Hartman *et al.*[12] and by the present author, but Maner *et al.* were presumably measuring the pH of the outer layers of stomach contents in closest contact with the gastric secretions, and in this area pH would be lower (as demonstrated by Møllgaard's figures given earlier). Maner *et al.* reported that, in pigs which gave a mean stomach pH value of 1·6 just before slaughter, the mixed stomach contents from the same animals after slaughter had a pH of 3·6. They regarded this difference as due to contamination with saliva and/or duodenal contents. This was to a large extent likely to have been due to the influence of the higher pH of the central mass of the gastric contents.

Thus, there appear to be two types of pH measurement which may be made on stomach contents. The first is a record of the pH of mixed contents, and this indicates the extent to which gastric acid secretion has modified the buffering power of the diet and associated salivary and gastric secretions. The second is a record of pH from the outer layers of the food mass such as would probably be recorded by a pH endoradiosonde[14] or by a pH electrode introduced via a gastric cannula. Using the latter technique, Wallin[15] reported that when the electrodes became surrounded by a food mass, a much higher pH was recorded than that found when the glass electrode was repositioned.

Acid-buffering effect of various diets

The contents of the stomach of the piglet have considerable acid-buffering power both on normal diets and when the piglets suckle the sow. Kvasnitskii[8] found that 1 g of sow's milk combined with 6–8 mg of pure hydrochloric acid. The acid-buffering power of a variety of diets is important in connexion with the development of stomach acidity. Weighed portions of various diets were taken and mixed with hot deionised water. After allowing the mixtures to stand for ½ h, they were stirred and titrated with hydrochloric acid by adding fixed quantities of acid once a minute and recording pH changes on a moving chart. This procedure was necessary because of the rapid buffering of acid as it was added to the slurry. Typical changes in pH observed on two types of diet are shown in Fig. 3. One diet was a milk replacer such as was fed to 2-day old piglets, and the other was a diet that was fed to pigs when they had reached 20 lb liveweight. The latter diet required far less acid to lower its pH than did the protein-rich milk replacer. Thus, when piglets are young and particularly deficient in acid secretion, diets are fed which require large amounts of hydrochloric acid to acidify them. Later, when acid secretion is becoming stronger, diets which need less acid to achieve low pH values in the stomach are fed. From Fig. 3, pH can be seen to have reached some degree of stability 1 min after adding the acid. By joining the points on the graph corresponding to 1 min after each addition of

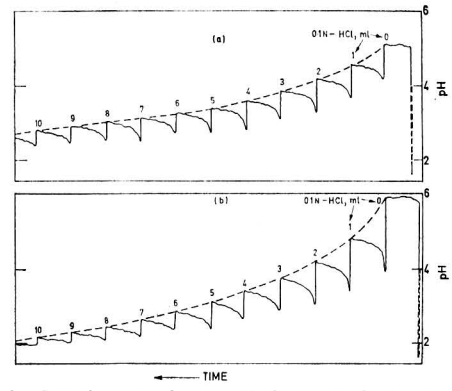

FIG. 3. *Recorder trace showing pH changes in slurries of two different types of diet when successive, fixed quantities of hydrochloric acid were added at 1 min intervals*

(a) Diet A (18·2 g dry matter): 43·7% glucose; 30·8% casein; 15·0% dried skim milk; 5·0% maize oil; 5·5% minerals and vitamins
(b) 'Weaner'-type diet (17·2 g dry matter): 62% barley meal; 12% white fishmeal; 10% flaked maize; 10% sucrose; 5% ex soyabean meal; 1% minerals and vitamins

acid, it was possible to plot a curve showing the pH resulting from the addition of increasing amounts of acid. A similar procedure was carried out on the supernatant liquid resulting from centrifuging a slurry of diet and water, to see how much of the buffering power came from the soluble material and thereby deduce how much buffering power was the property of the less soluble part of the diet. Fig. 4 shows chart records for pH changes from the supernatant liquid from the same two diets. Again, the amount of acid required to achieve a given pH was calculated, as in the previous example. Fig. 5 (a) shows the resultant curves showing the amounts of acid needed to achieve a given pH for slurries of five different diets that were investigated. Two commercial diets needed the most acid to acidify them to pH 2. Two early-weaning diets needed less acid. The 'weaner'-type ration, based on barley, fishmeal and soyabean meal, required least. The titration curves for the supernatant liquid from the same five diets are also shown in Fig. 5 (b).

Effect of gastric acidity on bacterial multiplication

Williams Smith & Jones,[10] commenting on the higher pH in the stomach contents of suckled piglets on the first day of life than subsequently, observed that the whole alimentary tract was flooded with bacteria in the first day or so of life and that this was probably due to the pH of the food in the stomach at that time being such as to permit rapid multiplication of ingested bacteria, which then passed unharmed into the small intestine and continued to multiply there. The lower pH value of the stomach contents after the first day of life was sufficient to curb the multiplication of all ingested bacteria except the lactobacilli, which continued to proliferate in the stomach of pigs of all ages and formed the principal inhabitant of the upper alimentary tract. These authors also found that when alkaline substances were added to pig food to preserve a high pH during the time the food was in the stomach, bacteria whose multiplication was normally limited were found to proliferate and pass into the small intestine with very little decrease in numbers.

Drasar *et al.*[16] have presented convincing evidence from human patients that the bacterial flora of the small intestine is derived from the flora of the stomach contents, as opposed

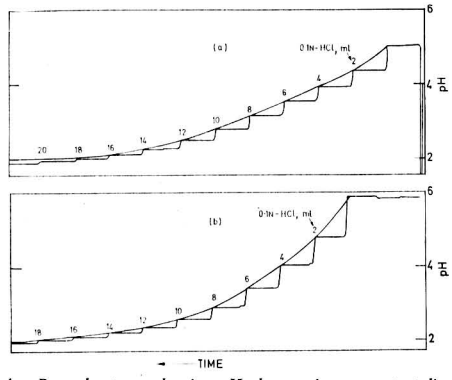

FIG. 4. *Recorder trace showing pH changes in supernatant liquid from slurries of two different types of diet when successive, fixed quantities of hydrochloric acid were added at 1 min intervals*
(a) Casein-glucose-dried milk diet; (b) 'Weaner'-type diet

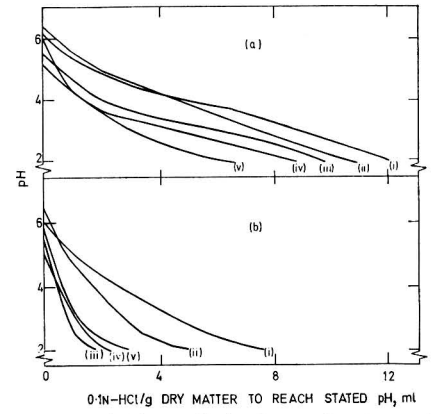

FIG. 5. *Effect of additions of hydrochloric acid on the pH of 5 diets*
(a) Slurry of diet and water; (b) supernatant after centrifuging slurry of diet and water
(i) and (ii): Commercial diets for emergency early weaning; (iii) and (iv) glucose–casein–dried milk diets for 2-day weaning; (v) barley–fishmeal–soyabean meal diet for 5-week weaning

to the theory of Gorbach[17] of retrograde spread up the intestine. Drasar *et al.*[16] found that bacterial colonisation of the fasting stomach was only found in achlorhydric persons. In normal people after fasting, the stomach was sterile in most cases, yeasts being present occasionally. In normal persons after a meal, bacteria could be isolated from samples of gastric juice, but the total number of bacteria isolated fell with time until, 60 min after the meal, samples were sterile. The lower the pH of the sample, the smaller the number of bacteria isolated from it. Thus, there would appear to be a wave of bacteria passing down the intestine after each meal in normal adult humans. For the majority of the time, however, the material leaving the stomach would be likely to be virtually sterile. In achlorhydric persons, on the other hand, since the contents of the stomach are never sterile, a fairly constant flow of bacteria through the small intestine would be expected. If these results are justified as being indicative of what might be likely to happen in the gut of the very young pig, it would seem likely that the small intestine of the piglet reared artificially must be exposed to a constant flow of micro-organisms in the efflux from the stomach until the period is reached when the secretion of hydrochloric acid begins to assume mature proportions. Twice-daily feeding would be likely to aggravate this situation since the rate of stomach emptying is greatest when the stomach is full.[8,18] Thus, under conditions of deficiency of hydrochloric acid secretion, the greater the amount of food consumed at any one meal, the less likely it is that pH will fall to levels capable of inhibiting bacterial growth, and the more likely it is that a considerable part of the contents will pass from the stomach to the small intestine soon after feeding.

Since the pH in the stomach of piglets studied by the author remained high up to 5 weeks of age there is some justification in presuming that there was little restriction on the survival or multiplication of micro-organisms within the stomach contents. Consequently these would move into the lower gut as the stomach emptied. The ability to rear piglets probably depends largely on the two factors mentioned earlier aimed at minimising the loading of the gut, namely care in formulation of diets so that when mixed with water they are as low in bulk as possible and, the use of a limited scale of feeding. Early on in the experimental work it was found that if piglets were allowed to eat as much as they wished under twice-daily feeding of liquid diets, after a time certain animals started to scour, then others became ill, and soon there was a major disease problem. It seems likely that feeding small quantities of feed once-hourly would allow the limited capacity of the piglet's stomach to secrete hydrochloric acid to have maximum effect, both on bacterial multiplication and on peptic proteolysis.

Cranwell *et al.*[19] found that the time of onset of hydrochloric acid secretion varied between pigs, and also depended on the type of environment. In a clean environment, hydrochloric acid secretion was evident within the first week of life, whereas in two pigs in a conventional environment, secretion of hydrochloric acid was not apparent until they were about 30 days old. They found indications of an inverse relationship between hydrochloric acid secretion and the amount of lactic acid formed by bacterial fermentation in the stomach. In the period before the onset of hydrochloric acid secretion, the production of lactic acid was governed by the frequency of suckling. From this, the secretion of hydrochloric acid appears to be delayed in the situation where hydrochloric acid secretion would be particularly valuable in inhibiting the passage of organisms into the lower gut.

Kenworthy & Allen[20] studied the structure of the mucosa of the small intestine of initially germ-free piglets reared under varying degrees of bacterial contamination. In a mono-contaminated piglet, villi were long and slender and the microvilli of the epithelial cells were very long. In multi-contaminated animals, mucosal damage was evident. Villi were stunted and deformed. The microvilli were about one third of the length of those in the mono-contaminated piglets. The epithelial cells showed other changes in shape and structure, depending on the degree of bacterial contamination of the piglets. Thus, it would appear that, apart from the danger of deaths in young piglets arising from colonisation of the alimentary tract by certain types of bacteria, there is ample scope for less significant, but possible very debilitating, damage to the absorptive mucosa when piglets are reared in conventional surroundings. The importance of the lack of gastric acidity is made more obvious by such experimental work. The gut of the piglet is clearly likely to be very susceptible to colonisation by harmful bacteria, should they exist in numbers in its environment.

Effect of gastric pH on proteolytic activity

The possible effect of high gastric pH on proteolytic activity of stomach contents was discussed by Kvasnitskii.[8] He made measurements of proteolytic activity by placing sticks of egg albumin in the stomach of pigs of various ages (via a cannula) and, after a period of time, noting the reduction in size of the sticks. He found no activity towards this substrate before three weeks of age, variable time of onset of activity from one animal to another, traces of activity between the third and sixth weeks of life and substantial levels of activity in the 7th and 8th weeks of life. After weaning, digestive activity towards egg albumin rose even more and by the 3rd month of life was equivalent to that in the adult pig. Pepsin is now known to show two pH optima, one at pH 1·5–2·0 and another at about pH 3·3–3·8.[21,22] Egg albumin is only digested at the lower pH range, whereas most proteins (e.g. casein) are digested at both pH optima. Thus, the pig's stomach could well have had proteolytic activity towards proteins which Kvasnitskii's method of assay would not have measured. In fact, he mentioned that, on acidification, gastric contents of very young piglets showed proteolytic activity towards Mett's sticks, thus demonstrating the presence of pepsin. Kvasnitskii rarely found free acidity in stomach contents of suckled piglets. He stressed the great importance of the lack of free acidity on the development of the gut flora, and in connexion with gastro-intestinal upsets and illnesses of piglets.

Kvasnitskii reported that proteolytic activity of pancreatic secretion was high in young pigs at 20 days of age, but decreased considerably with age. The changes which he found to occur in proteolytic activity of the pancreatic juice were the opposite of the developmental change which he had found in digestive activity of the gastric juice, and compensated for the inactivity of the latter towards protein in early life. On the other hand, Braude *et al.*[23] studying suckled piglets, found proteolytic activity of pancreas tissue and of intestinal contents towards casein at pH 8·4 increased with age over the period 4–36 days. Similarly, Hartman *et al.*[12] found that proteinase activity of pancreatic tissue and of intestinal ingesta rose over the first 7 weeks of the suckling period. Braude *et al.*[23] made the suggestion that the hard milk clot which formed in the stomach contents of suckled piglets 2–3 weeks old, and the retention of the clot, may be a necessary mechanism to allow efficient digestion by small amounts of proteolytic enzymes secreted during the first 2–3 weeks of life. Later, they found the clot became soft and dispersed again, as it was in very young piglets.

It is now known that there are several forms of pepsin secreted by the stomach of the pig, in addition to some pepsins having more than one pH optimum. It has been found that crystalline swine pepsin can be separated into two components, one of which digests proteins with pH optima close to those found with swine fundic mucosa, and the other with lower pH maxima close to those found with pyloric mucosa.[24] It has been suggested that the two proteolytic pH maxima shown by certain pepsins are caused by two sorts of active centre, each of which attacks maximally, at a different pH, a different type of substrate. For substrates where pepsin shows two pH optima, the optima are not fixed but are affected by type of substrate and by substrate concentration.[22]

Taylor[21] concluded that there was no physiological reason why intragastric proteolysis should not begin at as high as pH 4·5 and proceed swiftly at pH values above 3·5. On this basis, it can, perhaps, be expected that in the sow-reared piglet considerable peptic proteolysis occurs, but in the piglet reared artificially under certain conditions peptic proteolysis is likely to be very limited in extent.

Significance of pH changes in the gut

The pH of the contents of the small intestine is likely to be equally relevant to the efficiency of digestion in the very young piglet through any effect that pH may have on the activity of the various enzymes involved in the hydrolysis of proteins, carbohydrates and fats. Possibly the low pH observed might be such as to restrict the activity of enzymes and thus be a partial explanation of the difficulty of rearing piglets artificially on a commercial scale. It is unfortunate that there are few data on pH in the small intestine of piglets reared artificially and it is possible that both the results of the present author and those of Hartman *et al.* are atypical. However, there is clearly a need for further measurement of pH in order to discover whether artificial rearing does, in some way, depress intestinal pH to lower levels than would be found in sow-reared piglets.

Studies of the effect of pH on the activity of digestive enzymes other than pepsin generally show a fairly narrow range of optimum pH and a rapid falling off in rate of hydrolysis of substrate on either side of the peak. The enzymes used in such studies may have gone through involved separation procedures and may no longer show the same pH optima that they would in whatever combination they might exist in the gut of the living animal. Also the type of buffer or the type of substrate used in the assay often affects the pH optimum observed. However, even fairly crude enzyme extracts show peaked pH–activity curves, so whatever the optimum pH for an enzyme may be *in vivo*, it is fairly certain that, if widely differing pH levels are compared, the activity of the enzyme is likely to be lower at one pH than at the other.

The pH optima for the main porcine digestive enzymes are given in Tables II and III. The mucosal enzymes, with exceptions, show pH activity optima nearer to the pH levels found in the small intestine of the piglet than do most of the pancreatic enzymes. In the sow-reared piglet, pH in the small intestine appears to be just about optimum for the activity of the carbohydrases. However, in the piglet reared artificially, there is less certainty that these enzymes could be expected to be fully active. Proteolytic and lipolytic enzymes in the mucosa require a more alkaline pH *in vitro* for optimum activity than is found in sow-reared piglets or piglets reared artificially. At a pH of 6·0, DiNella *et al.*[30] found negligible mucosal lipase activity. Mucosal dipeptidase activity at pH 6·0 varied between the enzymes studied by Josefsson & Lindberg[29] from negligible activity to approximately one-quarter of maximum activity. In this connexion, it is noteworthy that most difficulties have arisen over the supply of fat and protein in diets for piglets reared artificially and, apart from the inadvisability of feeding sucrose to very young piglets, carbohydrates have been found to be relatively problem-free as dietary ingredients.

In contrast to the position with regard to mucosal enzymes, the pH optima of most pancreatic enzymes are so much higher than the pH levels observed in the gut of piglets reared artificially that activity of many of these enzymes may be severely reduced in the piglet reared artificially, and possibly sub-optimal in the sow-reared piglet. Cholesterol esterase and α-amylase showed the lowest *in vitro* pH optima, but even these are well above the pH that was found in the small

TABLE II

***In vitro* pH optima for hydrolases of porcine intestinal mucosa**

Enzyme	Optimum pH	Substrate	Reference
Invertase	6·5	Sucrose	Dahlqvist[25]
Maltases	6·5–7·5	Maltose	Dahlqvist[26]
Lactase	6·0	Lactose	Dahlqvist[27]
Enterokinase	5·2–6·0	Trypsinogen	Neurath & Schwert[28]
Dipeptidases	7·4–7·9	Various dipeptides	Josefsson & Lindberg[29]
Lipase	9·0	Olive oil	DiNella *et al.*[30]

TABLE III

***In vitro* pH optima for porcine pancreatic hydrolases**

Enzyme	Optimum pH	Substrate	Reference
Trypsin	8·2–9·2* 7·3–8·6* 7·8–8·8*	Haemoglobin Gelatin Casein	Northrop *et al.*[31]
Chymotrypsin	7·0–9·0 7·8	Proteins Synthetic substrates	Desnuelle[32]
Chymotrypsin	7·0–9·0*	Casein	Northrop *et al.*[31]
Carboxypeptidase A_2	7·0–8·0	Hippuryl-L-phenylalanine	Folk & Schirmer[33]
Carboxypeptidase B	7·9–8·0	Hippuryl-L-arginine	Wolff *et al.*[34]
Elastase	8·8	Elastin	Lewis *et al.*[35]
Ribonuclease	7·5	Yeast RNA	Yamasaki *et al.*[36]
α-Amylase	6·9	—	Fischer & Bernfeld[37]
Lipase	7·6–8·2	Olive oil	Aumaitre & Rérat[38]
Cholesterol esterase	6·6	Cholesterol oleate	Yamamoto *et al.*[39]
Phospholipase A	7·9–8·4	Egg yolk	Haas *et al.*[40]

* Approximate (read from graph)

intestine of the piglet reared artificially. Cholesterol esterase[9] maintained considerable activity *in vitro* between pH 4·0 and 5·0. α-Amylase,[37] on the other hand, showed rapid loss of activity below pH 6·0, *in vitro*. The proteolytic enzymes of pancreatic juice required a pH in the region of pH 8·0 for maximum activity *in vitro*.[31–36] They showed some activity *in vitro* at pH 6·0, varying from one enzyme to another. Aumaitre & Rérat[38] found that, at pH 6·8, lipase activity *in vitro* was reduced to one-third of its activity at the pH optimum at 7·6–8·2. These authors did not report on activity of the enzyme below pH 6·8.

It is unfortunate that so much enzyme assay work has been done without regard to the pH prevailing in the gut of the piglet. Lundh & Borgström[41] commented on the difference between pH optima of pancreatic enzymes and the actual pH values which they recorded along the length of the human small intestine, the latter values being similar to those found by Walker[11] and by Williams Smith & Jones[10] in the small intestine of the sow-reared pig.

In addition to the possible effect of pH within the gut on activity of digestive enzymes, pH may have other effects. For example, *in vitro* studies by Freeman,[42] intended to simulate conditions within the gut of the pig, showed that the distribution of fatty acids between micellar and oil phases was markedly affected by pH. Below a pH of 6·0, there was limited distribution of fatty acids in the micellar phase, and impairment of fatty acid absorption might be expected under such conditions in the gut. The partition of fatty acids in the micellar phase increased rapidly as pH increased above 6·2.

Another factor which may be relevant to much work done in the past on enzyme levels in pancreas, pancreatic juice, intestinal tissue and contents is that assay has often been carried out on material obtained from sow-reared piglets. The enzyme concentrations found may not represent the levels likely to be found in piglets reared artificially. An exception to the general trend was the work of Hartman *et al.*,[12] where material from sow-reared piglets and from piglets reared artificially was compared with interesting results. While tributyrinase activity of pancreatic tissue from sow-reared piglets was high throughout the first 8 weeks of life, that from piglets reared artificially showed a considerable drop after weaning at one week of age, and remained depressed for several weeks. In the same study, proteinase activity of pancreatic tissue did not rise as rapidly in the piglets reared artificially as it did in the sow-reared piglets.

It seems that further comparisons between digestive physiology of sow-reared piglets and piglets reared artificially would be likely to reveal interesting contrasts which, in turn, might well point the way towards the solution of many of the problems of artificial rearing.

Acknowledgment

The author wishes to thank Dr. D. E. Kidder of the Department of Veterinary Medicine for supplying references required in the compilation of Tables II and III.

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Received 15 January, 1970

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MILK REPLACERS IN THE ARTIFICIAL REARING OF LAMBS*

By J. B. OWEN and D. A. R. DAVIES

In this country the need for artificial lamb rearing methods arises mainly from the development of more prolific ewes such as the Finnish Landrace, and the breed being developed at Cambridge, which produces litters of 3 or 4 lambs, and from lambing more frequently than once per year. Allowing the ewe to rear 3 or 4 lambs naturally usually results in higher lamb losses, poorer lamb growth and a heavier strain on the ewe than when only 1 or 2 lambs are reared, with the potential benefits of increased prolificacy negated. There is therefore a substantial incentive to investigate and use methods of rearing the extra lambs independently of their dams so that the high prolificacy may be fully exploited.

In Mediterranean countries the use of artificial rearing methods is also receiving attention. There, many of the sheep are kept for producing milk and replacing natural suckling would substantially increase the amount of milk available and would allow intensification of dairy sheep along dual purpose lines.

The first essential in artificial rearing is to provide a suitable milk which will promote efficient and healthy growth, and then it must be used in a system which is economically viable on a commercial scale.

The use of natural ewes' milk is plainly prohibited but it does provide a reference point from which replacers can be evaluated. Some work has been carried out on this such as that reported by Pinot & Teissier.[1] They showed that milk replacers based on skim milk with added tallow and full cream cows' milk both produced satisfactory results, but neither was as efficiently utilised as ewes' milk. Large & Penning[2] also showed that lambs given a reconstituted powder based on skim milk with 30% added fat of mixed animal and vegetable origin performed as well as those given full cream dried milk and Cunningham *et al.*[3] indicated the superiority of skim milk plus added fat (coconut oil) over ordinary cows' milk, as a feed for young lambs.

In all of the above work the aim was to produce a milk replacer similar in gross composition to ewes' milk and the ewe milk replacers available commercially have been formulated with this end in view (i.e. liquid containing 18–20% solids, with 30% of the solids fat). The techniques involved in preparation are similar to those used in the preparation of calf milk replacers.

The product has given successful results but on occasion, there have been digestive disorders and abomasal bloat which, together with a lower digestibility of the nutrients, suggest that improvements in formulation could be achieved. Improvements would give better utilisation of the food and higher growth rates; it may not, however, be essential to aim for maximum growth at this stage, because of the lamb's ability to show compensatory growth later in its life.

At Cambridge the present authors have embarked on a systematic evaluation of the complex of several major interacting factors: milk replacer composition, milk replacer allowance, and solid food composition.

A recent experiment involved 72 Suffolk cross lambs in a factorially designed experiment with twofold replication. The treatments were all combinations of the following: 3 fat levels, 40%, 30% and 20% of the dry matter in the milk powder; 3 protein: energy ratios, high, medium and low expressed as 59, 46 and 38 g crude protein/Mcal gross energy, respectively (these ratios were calculated to include the range which has been reported in natural ewes' milk); 2 amounts of milk powder, 9 kg and 5 kg; 2 levels of crude protein in the concentrate first offered, 16% and 25%.

The experiment covered the period from 2 days of age until the lambs were 17·5 kg live weight. This allowed the lambs to receive their dam's colostrum in the pre-experimental period, and ensured that all lambs received one of the two concentrate rations for at least two weeks after weaning. It has already been established by Miller[4] that larger lambs show no response to crude protein levels above 18%, so that continuing the experiment beyong 17·5 kg was not considered.

* Presented at a Symposium on 'Milk Replacers for Young Farm Animals', organised by the Agriculture Group, on 21 October, 1969

TABLE I

Effect of milk composition on growth to 21 days, kg/day

Protein: energy ratio	Fat, %			Mean
	40	30	20	
High	0·140	0·203	0·234	0·192
Medium	0·175	0·257	0·220	0·218
Low	0·171	0·231	0·187	0·196
Mean	0·162	0·231	0·214	0·202

S.E. Within Table ± 0·0163
Marginal means ± 0·0094

TABLE II

Effect of milk composition on growth to 17·5 kg, kg/day

Protein: energy ratio	Fat, %			Mean
	40	30	20	
High	0·191	0·213	0·248	0·218
Medium	0·234	0·260	0·256	0·250
Low	0·232	0·258	0·236	0·242
Mean	0·219	0·244	0·247	0·237

S.E. Within Table ± 0·0125
Marginal means ± 0·0072

Effect of milk composition on growth performance to 3 weeks of age of these lambs is shown in Table I. Lambs receiving diets containing 40% fat grew less well than those receiving the 30% and 20% fat level. The difference was mainly the result of low milk intakes, but was also due to lower fat digestibility and increased digestive upsets and scouring. Type of fat is probably important; in the above experiment a mixture of tallow, coconut oil and butterfat was used, whereas in other work better tolerance to high levels of inclusion have been indicated using butterfat only.

Table II shows the performance of lambs from 2 days old to 17·5 kg live weight. Again over this period lambs receiving the 40% fat milk replacer grew less well, but the differences were reduced, because of compensatory growth in the post-weaning phase.

It is also clear that growth was reduced when the ratio of protein to energy was high; the medium ratio gave the best results, although not significantly so at each fat level. The source of protein may be important; in this experiment it was necessary at the higher protein levels to include a source of protein (egg albumin) other than that from skim milk.

In Table III are shown the effects of milk allowance and solid food composition. The latter has little effect on growth but the experiment affords a striking confirmation of the ability of the lamb to grow as well on the smaller milk allowance as on the larger. This has been a consistent finding in this and previous experiments at Cambridge, and at present prices, the relative costs of milk replacers and weaner concentrates strongly favour using an early weaning system with minimal milk replacer consumption.

One of the naturally occurring variables which could lead to modifications of recommendations for the latter system, is the initial weight of the lamb. The effects of this on performance was investigated in a factorial experiment involving 24 lambs. Two birth weight ranges, 4·5–5·5 kg and 2·5–3·5 kg, were looked at in combination with 2 milk powder allowances, 9 kg and 5 kg.

The results (Table IV) confirm other experimental work that initial weight of the lamb is relatively unimportant as a growth rate determinant. Consequently milk allowances need not be adjusted to allow for this. Neither does it seem necessary to adjust milk allowance to give a constant weaning weight. It appears that with lambs live weight is less important than age in governing the lamb's ability to cope with solid food. In all experiments solid food consumption occurred first when lambs were about 21 days old. If the milk allowance is removed at this stage concentrate consumption increases rapidly; however, it remains low if milk feeding continues even if the daily allowance is well below appetite levels. On such a regime lambs grow less quickly than their weaned counterparts and because of this an abrupt weaning procedure appears advantageous.

TABLE III

Effect of milk allowance and solid food composition on growth (kg/day) to 21 days, and to 17·5 kg live weight

	21 days	17·5 kg live weight
Milk allowance:		
9 kg Powder	0·209	0·238
5 kg Powder	0·195	0·236
S.E.	0·0077	0·0059
Solid food composition:		
25% Protein	0·201	0·231
16% Protein	0·203	0·242
S.E.	0·0077	0·0059

TABLE IV

Effect of initial live weight and milk allowance on lamb performance

	Large lambs		Small lambs		S.E.
	9 kg	5 kg	9 kg	5 kg	
Initial live weight, kg	4·70	4·43	2·51	2·60	0·213
Average live weight gain to 15 kg, kg/day	0·252	0·208	0·224	0·219	0·0140
Total pellet consumption to 15 kg, kg	7·28	12·52	8·67	12·77	1·777

To summarise, there is an increasing need for milk replacers to be used in lamb rearing in this and other countries. Replacers containing 20–30% added fat can be used successfully but further improvements in composition, giving fewer digestive disorders and improved efficiency of utilisation should be achieved. It is clear that at present milk replacers can be used in economic lamb rearing only by adopting an early weaning system. In such a system weaning would occur at 3–4 weeks of age, the lambs having consumed no more than 5 kg of milk powder.

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Received 16 January, 1970;
amended manuscript 22 June, 1970

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CARBOHYDRATES IN MILK REPLACERS FOR CALVES*

By A. W. A. BURT and SHEILA M. IRVINE

The lack of appropriate intestinal enzymes apparently limits the use of other carbohydrates to replace glucose and lactose in milk replacers.

The replacement of 20% lactose or glucose by wheat flour slightly reduced the digestibility of the organic constituents of the diet and a further slight reduction occurred when wheat flour was replaced by precooked maize starch. The relative digestibilities of these materials were: glucose, 100; lactose, 93·5; wheat flour, 71·5, and cooked maize starch, 62·5%.

Addition of a mixture of amylases and maltase reduced the digestibility of a milk replacer containing cooked potato starch.

Invertase addition immediately prior to feeding substantially improved the digestibility of a milk replacer containing 25% sucrose.

Introduction

Dried milk products are used extensively in the production of commercial milk replacers for rearing calves. Dried skim milk is the major source of protein, and dried whey is often also included. Both these substances contain very substantial amounts of lactose and therefore provides in addition to protein, a substantial proportion of the carbohydrate of milk replacers.

The amount of fat which is included in the formulation varies between 5 and 20% and is usually appreciably less than that provided by whole milk. Depending on the relative proportion of skim milk, dried whey and added fat used, there is, therefore, a variable amount of formula 'space' which can be occupied by additional carbohydrate.

The type of carbohydrate used for this purpose and its level of inclusion depends primarily upon: (a) the ability of the calf to digest the particular carbohydrate source; (b) the effect of the carbohydrate on the incidence of diarrhoea; (c) its effect on the physical properties of the product, particularly on its behaviour when reconstituted with water; and (d) its cost and availability.

In practice, choices are usually made to achieve a balance of advantages between these factors, in particular (a) and (b) are extremely important, but have to be achieved within the limitations of (c) and (d).

This paper deals firstly with some of the limitations upon the use of carbohydrates imposed by the intestinal enzyme content in the calf, secondly with some comparisons of the digestibility of milk replacers containing different sugars and starches and thirdly with some effects of adding enzymes to milk replacers containing starch and sucrose.

Enzymic limitations on the use of carbohydrate

The ruminant is perfectly capable of digesting a wide variety of carbohydrates when these are fed in the dry state and the rumen is fully functional. Microbiological attack in this organ is not relevant to the digestion of milk replacers fed as liquids, since these largely pass directly to the abomasum owing to the closure of the oesophageal groove in the calf. In any event, milk replacers are often fed to calves which have not developed significant rumen function.

Numerous studies have been carried out on the intestinal enzymes involved in the digestion of carbohydrates by the calf using both direct and indirect methods. Indirect methods commonly include the ingestion or the intestinal infusion of standard carbohydrate loads in the fasting animal followed by the determination of changes in blood sugar levels (glucose and/or total reducing sugars) during the postprandial period.[1–3] More recently data have become available from direct enzyme assays on extracts of the small intestine and pancreas.[4–7]

Utilisation of lactose correlates with the high levels of lactase activity present in the intestinal mucosa, although this activity declines somewhat after the first month of life.[7] The ability to utilise sucrose is apparently absent, sucrases have not been detected and sucrose infusion has not produced appreciable change in blood sugar level at any age. Intestinal maltase activity is initially very weak and remains so, and pancreatic maltase while present, is also relatively weak and does not increase after the 4th day of life. However, the calf pancreas does show amylase activity which increases very markedly with age, particularly after the first 2 months of life.[7] A noticeable feature of the studies of enzyme activity so far completed is that while age effects are apparent and considerable in some instances, there appears to be no evidence of adaptation to diet. Whether the 3-month old calf was weaned on to dry food 2 months previously and had therefore developed a fully functional rumen or whether it was maintained entirely on liquid milk replacer apparently had no appreciable effect on enzyme levels,[7] although the nature of the carbohydrates reaching the duodenum in the two cases must have been significantly different. Nor is there any apparent adaptive response to the previous feeding of milk substitutes containing substantial amounts of starch.[8] It might therefore be concluded from the studies of intestinal enzyme levels and responses in blood sugar to carbohydrate infusion, that lactose and glucose are the carbohydrates of choice for milk replacers. Likewise it could be concluded that starch is of no benefit to the young calf and is only utilised to a very limited extent by the older calf, while sucrose has no value whatsoever. However, studies of the responses of the whole animal to changes in diet lead to some modification of these conclusions, as shown below. The assay procedures and the data available do not yet lend themselves to any degree of precision in quantifying enzyme activity in relation to the rate of intake and passage of carbohydrate through the intestine.

Digestion of sugars and starches

A substantial proportion of the many digestibility measurements and feeding experiments reported in the literature are

* Presented at a Symposium on 'Milk Replacers for Young Farm Animals', organised by the Agriculture Group on 21 October, 1969

not relevant to the practical formulation of milk replacers, because the level of inclusion of the carbohydrates tested was much higher than can be reasonably achieved in practice or because of pecularities of the basic diet.

In a recent experiment at Colworth, glucose, lactose, wheat flour and precooked maize starch, included at 20% in a practical milk replacer formulation (Table I) were compared. These diets were fed to 8 Ayrshire bull calves in a digestibility trial using a 4 × 4 latin-square design with 4 collection periods of 8 days duration. Feeding experimental diets started on the 5th day; the first collection period began on day 7 and the last collection period finished on day 51 after birth. The diets used are shown in Table I, their analysis in Table II and the digestibility and N retention and faeces score data in Table III.

The replacement of lactose by glucose marginally increased the digestibility of all constituents, but none of the differences were statistically significant. Wheat flour depressed the digestibility of dry matter, water, organic matter and N-free extractives (*NFE*) compared with glucose and lactoses and most of the differences were statistically significant. Cooked maize starch had a further adverse effect and produced a significant reduction in the digestibility of *NFE* compared with wheat flour.

Faecal moisture content was increased by lactose compared to glucose and was increased further by wheat flour and cooked maize starch. Visual faecal scores were similarly affected, although it should be noted that the slightly higher moisture content of the faeces from calves fed wheat flour compared with lactose, was not associated with any decrease in faecal score. From this experiment the relative digestibilities of the different carbohydrates can be assessed; thus, if glucose is assumed to be 100% digestible, then the digestibility of the dry matter of the other carbohydrates was: lactose, 93·5%; wheat flour, 71·5%; and cooked maize starch 62·5%. In this experiment glucose was somewhat superior to lactose, but it must be remembered that the glucose diet still contained 33% lactose compared with 53% in the lactose diet. Although, under normal conditions, the calf has adequate intestinal lactase it may be that the inclusion of 53% lactose represented the dietary level at which the enzymic capacity available for digestion was slightly surpassed.

It has long been known that excessive intakes of glucose or lactose can cause diarrhoea. Rojas *et al.*[9] induced this condition very rapidly by adding sufficient lactose to double the intake of this sugar provided by skim milk. Mathieu & de Tugny[10] reported that glucose included in partly skimmed milk at levels up to approximately 20% of the dry matter had little effect upon the incidence of diarrhoea, but the incidence substantially increased at 30–45% glucose. Walker & Faichney[11] suggested 9 g hexose equiv./kg liveweight/day as the level of intake beyond which diarrhoea is likely to be induced in the calf and lamb. Roy[12] suggested that this limit might be raised to 12g/kg/day in the presence of a fat intake of at least 5·5 g/kg/day. In the experiment reported above hexose intake averaged 8·8 g/kg liveweight/day on the glucose and lactose treatments and the fat intake was 2·4 g/kg/day. The results therefore suggest that there may be slight differential effects of glucose and lactose on the incidence of diarrhoea at levels close to the critical hexose intake.

The second major point illustrated by this particular experiment is the lack of response of the calf to pretreated cereal starches. Precooked maize starch was inferior to raw wheat flour in several respects.

Other work supports the proposition that cooking cereal starches does not improve their availability to the calf.[13,14] Mathieu & Thivend[14] fed manioc, maize or expanded maize, or

TABLE I

Relative effects of lactose, glucose, wheat flour and cooked maize starch

% in diet

	Diet			
	1	2	3	4
Lactose	20			
Glucose		20		
Wheat flour			20	
Cooked maize starch				20
Fat–skim milk mixture	72	72	72	72
Whey powder	6	6	6	6
Vitamins and minerals	2	2	2	2

TABLE II

Analysis of the diets

	Lactose	Glucose	Wheat flour	Cooked maize starch
Moisture	3·7	5·5	6·4	6·3
Oil	14·5	14·5	15·7	15·0
Crude protein	21·8	22·0	23·9	22·1
Ash	6·0	5·8	5·9	6·1
Organic matter	90·3	88·7	87·7	87·6
NFE	54·0	52·2	48·0	50·4

TABLE III

Apparent digestibility, %

	Lactose	Glucose	Wheat flour	Cooked maize starch	Significant Difference (P = 0·05)
Dry matter	93·7	95·0	89·3	87·5	3·7
Water	95·3	97·2	91·8	87·4	5·0
Fat	85·6	88·6	86·3	83·5	10·9
N	88·8	90·8	81·4	79·7	6·6
Organic matter	94·3	95·4	90·4	88·2	3·5
NFE	98·8	99·0	95·6	93·3	1·9
Ash	85·2	87·1	80·5	77·2	9·4
Faeces score (visual)	2·4	2·9	2·4	2·1	0·5
Faecal moisture, %	82·9	79·6	84·6	86·5	
N retention, g/day	12·2	11·1	10·0	10·5	2·5

potato flake at 2 levels of inclusion as isocaloric replacements of fat in whole milk. Whole milk (roughly 12% total solids, comprising 29·2% fat and 70·8% skimmed milk solids) was compared with a low level of starch (13·5% total solids containing 18·5% fat, 18·5% starch and 63% skimmed milk solids) and with a high starch intake (16·5% total solids, containing 3% fat, 45·5% starch and 51·5% skim milk solids). This system of feeding achieved intakes isocaloric with whole milk, but inevitably confounded the level of starch inclusion with the level of dry matter in the liquid diet and the percentage of fat in that dry matter, as increase in the rate of starch inclusion was associated with a higher concentration of dry matter in the diet and with a lower fat content in that dry matter.

At the lower level of starch inclusion, the digestibility of dietary organic matter was generally slightly depressed compared with whole milk but performance was very similar. Expanded starches increased the incidence of diarrhoea compared with raw starches. Uncooked maize was generally most digestible and raw potato least digestible and there was a substantial effect of level of inclusion (Table IV).

This consistent lack of response to precooking cereal starches and the incerased incidence of diarrhoea often associated with these materials is somewhat suprising as it might be expected that the breakdown of starch granules brought about by cooking would make them more susceptible to enzymic attack.

Addition of enzymes to milk replacers containing starch

The use of added enzymes to improve the utilisation of starch in milk replacers has given surprisingly poor results. Raven & Robinson[13] obtained no response from the addition of malt enzymes to a milk replacer containing 47·5% expanded maize starch.

However, Henschel *et al.*[15] obtained a response in blood sugar from the addition of amyloglucosidases to small amounts of cooked starch introduced through a cannula into the upper small intestine. The addition of amyloglucosidase to diets containing cooked maize starch substantially reduced the incidence of diarrhoea.[16]

Some years ago the effect of adding fungal maltase, amylases and amyloglucosidase to a milk replacer containing cooked potato starch was tested. The enzymes were effective at acid pH and were shown to degrade potato starch *in vitro*, but an *in vivo* experiment with 6 calves showed that the enzyme supplements had an adverse effect upon the digestibility of most of the dietary constituents and upon the incidence of diarrhoea (Table V). This effect increased progressively with the amount of enzyme added. The relatively high incidence of diarrhoea on the control diet is in agreement with the findings of Mathieu & Thivend[14] on diets containing potato flake.

The reasons for the increased incidence of diarrhoea and lower digestibility following the addition of the enzyme preparation are not immediately apparent.

Sucrose and enzyme supplementation

There are many published reports which describe the absence of sucrase activity in the intestine of the calf[7] and ingestion of substantial quantities of sucrose has resulted in diarrhoea and death in many instances.[17] In one case, the digestibility of sucrose in a partly skimmed milk with 69 g/l of added sucrose was over 80% but the calves receiving this material died rapidly following extremely severe diarrhoea.[18] Although fructose has been reported to cause diarrhoea and to fail to raise blood sugar,[3] invert sugar has given satisfactory results.[17] Velu *et al.*[17] reported that the blood reducing sugar response to a fructose load (4·4 g/kg liveweight) was approximately one-third of the response to glucose in 1-week old calves.

The effect of adding invertase (B.D.H. liquid concentrate) to milk replacers (Table VI) containing 25% sucrose was therefore examined using 6 Ayrshire calves in a changeover trial. The invertase was added to the reconstituted liquid immediately before feeding to the calf.

The control diet with no invertase addition produced severe diarrhoea but the calves survived the prefeeding and 8-day collection periods. The digestibility of the dietary fractions was correspondingly low and the faeces voided daily contained over 1200 g water (Table VII). The addition of either level of invertase concentrate substantially increased the digestibility of the diet. The output of water in faeces was diminished by 600–900 g/day and there was a corresponding reduction in the incidence of diarrhoea and the output of

TABLE IV

Comparison of starches from Mathieu & Thivend[14]

Starch source	Organic matter digestibility, %	
	Low starch intake (18·5% starch)	High starch intake (45·5% starch)
Manioc	93·8	80·0
Maize	96·6	82·8
Expanded maize	94·8	79·6
Potato	90·6	76·1
Potato flake	95·7	77·7
Whole milk	98·5	

TABLE V

Use of enzymes to improve starch utilisation by calves

Diet: dried skim milk, 38·0%; dried buttermilk, 13·0%; dried whey, 6·7%; ground potato flakes, 25·0%; bleached palm oil, 13·5%; soyabean lecithin, 1·5%; minerals and vitamins, 2·3%
Enzyme addition: 0, 0·25 and 0·5% for diets 1, 2 and 3 respectively

	Digestibility			
	1	2	3	S.D. ($P = 0·05$)
Dry matter	80·1	77·8	75·4	9·3
Water	76·5	73·4	63·4	15·4
Organic matter	81·0	78·2	76·4	9·6
NFE	84·6	86·4	84·7	8·1
N	72·1	64·2	59·0	14·6
Days diarrhoea, %	41	58	79	36

TABLE VI

Sucrose diet

	%
Dried skim milk	39·5
Dried buttermilk	17·0
Dried whey	5·0
Palm oil	10·5
Soyabean lecithin	1·0
Crude sugar	25·0
Minerals and vitamins	2·0

TABLE VII

Sucrose as a carbohydrate source and the effects of enzyme addition

	Apparent digestibility, %			Significant Difference (P = 0·05)
	Control	+ Invertase (1)	+ Invertase (2)	
Dry matter	81·5	93·1	91·3	5·5
Water	69·5	91·9	90·5	11·6
Organic matter	81·9	93·6	91·7	5·4
N	76·3	85·6	82·3	7·9
NFE	84·3	97·7	96·0	5·2
Faecal water output, g/day	1221	275	588	—
Urine water output, g/day	2100	3081	3044	479
% days diarrhoea	88	18	14	36
N retention, g/day	8·1	9·9	9·7	1·6
Invertase concentrate added, ml/10 g sucrose	0	0·2	0·05	

water in urine increased. N retention was significantly increased. The lower rate of invertase addition gave a slight reduction in the digestibility of the diet compared with the higher rate of addition suggesting that the low rate may have been slightly sub-optimal.

These results indicate that suitable invertase sources might be combined with sucrose as an alternative potential carbohydrate source in milk replacers.

Discussion

Despite considerable work recommendations for the use of carbohydrates in milk replacers are not clearcut. Although the pre-ruminant calf is poorly equipped enzymically to deal with the digestion of starch, appreciable quantities may be included in milk replacers without producing notable reductions in the digestibility of the diet. Paradoxically, precooking starches enhances diarrhoea when it might be expected to improve the digestive action of the very limited amounts of maltase and amylase in the intestine. It is probable that the action of the microbial population of the lower gut in combination with effects of different materials upon rate of passage through the intestine may be responsible for some of these effects. A substantial proportion of the digestion of raw starch may be due to bacterial action and the effect of precooking starches may be to make them so susceptible to bacterial attack that the high rate of production of organic acids results in malabsorption.

While glucose and lactose are generally regarded as the preferred carbohydrates, the results quoted show that it is possible to make beneficial use of other carbohydrates in practical diets. Glucose and lactose themselves are capable of producing diarrhoea if sufficient quantities are ingested and the critical levels are not too dissimilar from those commonly achieved in some practical feeding situations. However, this situation may be modified by the effects of fat inclusion and possibly by some differential effect of lactose and glucose. Walker & Faichney[11] concluded that the absorptive capacity of the young ruminant for hexose could be more readily overloaded than that of other species. The reasons for this are still not readily apparent.

It is probable that future studies of the rate of passage and site, rate and mode of digestion of these materials in the intestine of the calf will throw further light on these problems, as has happened with proteins. Here, effects of substituting soyabean for milk protein[19] and heating milk protein[12] upon the rate of passage of material from the abomasum have already been demonstrated.

The results presented here stress the need for precise definition of the level of inclusion of the carbohydrate and of the nature of the remainder of the diet, before statements about carbohydrate utilisation by the calf achieve any useful degree of precision.

The results obtained from the addition of invertase to diets containing sucrose suggest that the latter may serve as a suitable source of carbohydrate replacement in milk replacers if economic conditions permit its inclusion with a suitable source of invertase.

While the practical interest in the use of carbohydrates in milk replacers has declined over the last few years, as the percentages of fat in these materials has risen, the situation may alter when replacements for milk protein such as soyabean protein are made more effective and come into more extensive use. These generally contain much less carbohydrate than dried skim milk and there will therefore be a need for increased levels of other readily available carbohydrates to replace the lactose of dried skim milk.

The solutions to the problems posed will no doubt arise from the combined effects of improved enzyme technology and better knowledge of the gastro-intestinal physiology of the young calf.

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Received 16 January, 1970

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PROTEIN IN MILK REPLACERS FOR CALVES*

By J. H. B. ROY

Introduction

The importance of the protein fraction of milk replacer diets lies not only in its major contribution to the growth of the calf, but also to the fact that minor aberrations in protein quality and changes in concentration may have a profound influence on the health of the calf. Moreover, the relatively high cost of milk replacers lies in the necessity, at the present time, of using milk protein as the sole or major source of protein.

Before considering the composition of milk replacers in terms of protein, it is necessary to consider the requirements for protein of the pre-ruminant calf given various planes of nutrition.

Requirement of protein

The requirement of available protein (R_{AP}), estimated by the factorial method,[1] is obtained from the equation:

$$R_{AP} = \frac{1}{(BV)} 6{\cdot}25\,(E + G)$$

where BV is the biological value of protein expressed as a coefficient, E is the endogenous urinary N excretion (g/day) and G is the amount of N retained (g/day). In terms of apparently digested crude protein (R_{ADP}) the requirement is:

$$R_{ADP} = 6{\cdot}25\left[\frac{1}{(BV)}(E + G + M.D) - M.D\right]$$

where M is the metabolic faecal N excretion (g/kg dry matter ingested) and D is the dry matter intake in kg/day required to supply the energy needs of the calf.

To convert from available protein requirement, which is independent of the dry matter intake of the calf, to digestible crude protein, it is only necessary to add on the factor $6{\cdot}25\left[\left(\frac{M.D}{BV}\right) - M.D\right]$ to the available protein requirement.

The metabolic faecal N, the inevitable loss of nitrogen in the faeces arising from the digestive juices, bacterial residues and epithelial cells that become eroded during the passage of food through the digestive tract, is much lower in the pre-ruminant than in the ruminant calf. With pre-ruminant calves given whole milk or a good quality milk substitute the value is about 1·9 g N/kg dry matter intake.[2] When allowance is made for this loss, the true digestibility of milk protein in whole milk is about 100%. Moreover, because of the higher efficiency of digestion and energy utilisation in the pre-ruminant calf, owing to the absence of rumen fermentation, a lower amount of dry matter intake is required to achieve the same growth rate and N retention than that required by the ruminant calf. As a result of these two factors, the digestible protein requirements are not greatly above the available protein requirements in the calf receiving only liquid diets.

Of the protein that is truly digested, only a proportion is retained in the body, the remainder being degraded and excreted in the urine. The proportion retained after allowing for the endogenous urinary N excretion is represented by the biological value, which reaches its maximum when the amino acids supplied by the protein are in exactly the correct proportions for the requirement of the calf. However, the maximum biological value will only be reached when the diet being given to the calf is limiting in protein, i.e. when there is an excess of energy and of all other nutrients, except protein. Under these conditions the biological value of milk protein is about 80% or even as high as 90%.[3,4]

The inevitable loss of nitrogen in the urine by calves on an N-free diet, as a result of metabolic processes in the tissues, is called the endogenous urinary N excretion. The endogenous urinary N expressed per unit of body weight, like the basal metabolism, declines with age. For pre-ruminant calves, the average value is between 63 and 82 mg N/kg live weight.[2,5-7]

The amount of N stored for each kg gain in weight is generally between 26 and 34 g, but possibly due to differences in carcass composition, especially in the degree of

* Presented at a Symposium on 'Milk Replacers for Young Farm Animals', organised by the Agriculture Group on 21 October, 1969

hydration of the tissues, it may be higher for calves gaining weight slowly and also for calves gaining weight very rapidly if the diet contains a high concentration of protein.

The absolute maximum amount of N stored each day appears to be a characteristic of the mature weight of the breed, and also of its muscle : bone ratio.[8] Pre-ruminant calves soon reach a peak of N retention and this remains practically constant with age, but obviously declines in relation to metabolic body size.

TABLE I

Effect of diet and age on N retention by pre-ruminant calves

	Diet		
	29% protein/ 21% fat	26% protein/ 29% fat	19% protein/ 28% fat
No. of calves	12	12	12
N retention, g/day			
4 weeks	25·5	25·8	18·8
10 weeks	23·8	26·5	15·7
N retention, g/kg$^{0.73}$/day			
4 weeks	1·29	1·27	1·03
10 weeks	0·84	0·91	0·61

Tables I and II give the results of some experiments which show the effect of diet, age and breed on N retention in calves. Increasing the energy content of a milk replacer diet by increasing the concentration of fat from 1 to 29% had no effect on N retention, whereas reducing the protein concentration to 19% protein from 26–29% protein had a marked effect in reducing N retention. This suggested that 19% protein in the dry matter is a sufficiently low content for protein to be the limiting factor for growth.[9,10]

As shown in Table II, the Friesian breed retains considerably more N than the Ayrshire. This breed difference is further illustrated in the results of an experiment[8] given in Tables III and IV, where N metabolism of the Jersey, Ayrshire and Friesian breeds was studied at two levels of intake when calves were of the same live weight or at the same percentage of mature weight. At the same weight, N digestibility was somewhat higher for the Friesian than for the Ayrshire and Jersey. N retention was in increasing order of magnitude as the size of the breed increased. However, the effect of breed was in part confounded by the fact that the larger breed was naturally younger at the same weight than the smaller breed. For all three breeds the *ad lib.* intake of diet resulted in a N retention 6 g greater than that produced by the restricted level of feeding.

TABLE II

Effect of diet, breed and age on N retention by pre-ruminant calves

	Diet		Age, weeks		Breed	
	36% protein/ 1% fat	30% protein/ 21% fat	4	10	Friesian	Ayrshire
No. of calves	12	6	18	18	9	9
N retention, g/day	28·7	27·5	27·6	28·9	35·9	20·6
N retention, g/kg$^{0.73}$/day	1·17	1·19	1·34	0·98	1·31	1·01

TABLE III

Comparison of Jersey, Ayrshire and Friesian breeds in utilisation of a milk replacer at a given live weight

	Jersey		Ayrshire		Friesian	
	Restricted	*Ad lib.*	Restricted	*Ad lib.*	Restricted	*Ad lib.*
No. of calves	10	10	10	10	10	10
Live weight, kg	65	65	65	68	66	68
Age, days	69	62	47	38	33	35
N digestibility, %	94·5	93·8	94·1	94·1	95·1	95·1
N retention, g/day	22·8	26·4	28·1	37·5	33·0	38·5
Adjusted N retention, g/day*	23·2	29·6	28·2	34·7	32·5	38·2

* Adjusted for difference between means in apparently digested N within restricted and *ad lib.* levels of intake

TABLE IV

Comparison of Jersey, Ayrshire and Friesian breeds in utilisation of a milk replacer at 15% mature weight

	Jersey		Ayrshire		Friesian	
	Restricted	*Ad lib.*	Restricted	*Ad lib.*	Restricted	*Ad lib.*
Live weight, kg	65	65	85	87	99	99
Age, days	69	62	70	54	64	59
N digestibility, %	94·5	93·8	93·5	93·9	95·7	95·9
N retention, g/kg$^{0.73}$/day	1·08	1·25	0·96	1·27	1·24	1·39
Adjusted N retention, g/kg$^{0.73}$/day*	1·02	1·25	1·02	1·23	1·24	1·44

* Adjusted for differences between means in apparently digested N within restricted and *ad lib.* levels of intake

At the same percentage of mature weight, which at the low percentage of 15 is practically synonymous with age, N digestibility was significantly higher for the Friesian than for the other two breeds. Similarly, N retention, even after adjustment for differences in apparently digested N, was identical for the Jersey and Ayrshire breed, when based on metabolic body size, but about 20% higher for the Friesian at both restricted and *ad lib.* levels of intake. The fact that calcium retention per unit of metabolic body size (W kg$^{0.73}$) was similar for all three breeds, suggests a higher muscle : bone ratio for the Friesian breed, and a higher efficiency both of digestion and retention of absorbed N by the Friesian.

Based on the evidence obtained above, the requirements for available and digestible protein may be calculated for various weights of calf gaining weight at different rates. These are given in Table V. To estimate the minimum protein content of a milk replacer, it is necessary also to know the energy requirements of the calves and the concentration of energy in the dry matter of the milk replacer. Based on a requirement of 52 kcal digestible energy/kg live weight for maintenance[11] and 3000 kcal digestible energy/kg gain in weight,[2,4,11] and with a milk substitute containing 5·0 kcal digestible energy/g dry matter, the minimum crude protein content required in milk replacers has been calculated and is given in Table VI. From this Table the following points are evident. First, the greater the weight gain required, the higher must be the protein content of the diet, and secondly, the heavier the calf, the lower the protein content required for a given weight gain. This is in keeping with the results of van Weerden & van Hellemond[12] who found that, with veal calves, lowering the protein content from 25 to 23% at 5 weeks and from 23 to 20% at 9 weeks of age had no very marked effect on growth and feed conversion. Blaxter & Wood[11] showed that, when whole milk, in which about 26% of the calories are derived from protein, was given at a level to supply the energy requirements for maintenance, as little as 7% of the calories are necessary as protein, whereas for a gain of 1 kg/day the value is about 22%. Their values were calculated assuming that milk protein was completely digested and retained in the body so that the actual percentages required are somewhat higher. The requirements are also in keeping with the finding mentioned earlier that 19% crude protein in the dry matter of a milk replacer diet will not support maximum growth in calves when the diet is given *ad lib.* after the colostrum-feeding period.

Digestion of milk protein

The young calf secretes two proteolytic enzymes, rennin and pepsin in the abomasum. Originally it was thought that the milk-fed calf secreted only rennin and that the secretion of pepsin did not become marked until the calf ate solid food,[13] or at least until 4 weeks of age.[14] However, further studies[15] have suggested that the young calf may secrete either rennin or pepsin or both and that the exact pattern is not predictable from the age of the animal or the nature of its diet. It is not known whether differences between individuals in the nature of the enzymes secreted affect the digestion of protein. Most calves however show a transition from a secretion containing predominantly rennin during the first 2 weeks of life to one containing predominantly pepsin at 8 weeks of age, but a few calves secreted mainly pepsin from early life. For clotting by rennin, the optimum pH is 6·5, whereas the optimum pH

TABLE V

Daily requirement* of the pre-ruminant calf for available and apparently digestible crude protein, g

Live weight, kg	Maintenance		Maintenance + 0·5 kg gain/day		Maintenance + 1·0 kg gain/day		Maintenance + 1·5 kg gain/day	
	Available	Digestible	Available	Digestible	Available	Digestible	Available	Digestible
20	15	15	130	130	245	250	—	—
40	20	25	140	140	255	260	—	—
60	30	30	145	150	265	270	380	385
80	35	40	155	155	270	275	385	390
100	40	45	160	165	275	280	395	400
120	45	50	165	170	280	285	400	405
140	55	55	170	175	290	295	405	410

* For the calculation of requirements, the following factors have been used: endogenous urinary N, 184·4 mg/$W^{0.73}$; N retention, 30 g N/kg gain in weight; biological value, 80%; metabolic faecal N, 1·9 g/kg dry matter intake

TABLE VI

Minimum crude protein content (%) required in milk replacer diets for calves

Live weight, kg	Maintenance		Maintenance + 0·5 kg gain/day		Maintenance + 1·0 kg gain/day		Maintenance + 1·5 kg gain/day	
	Air dry basis (96% dry matter)	Dry matter	Air dry basis (96% dry matter)	Dry matter	Air dry basis (96% dry matter)	Dry matter	Air dry basis (96% dry matter)	Dry matter
20	6·5	6·5	26·5	27·5	—	—	—	—
40	5·5	6·0	20·0	21·0	26·0	27·0	—	—
60	5·0	5·0	16·5	17·0	22·5	23·5	26·0	27·0
80	4·5	5·0	14·0	14·5	19·5	20·5	23·0	24·0
100	4·5	4·5	12·5	13·0	17·0	17·5	21·0	22·0
120	4·0	4·5	11·0	11·5	16·0	16·5	19·0	20·0
140	4·0	4·0	10·0	10·5	14·5	15·0	18·0	18·5

for proteolytic activity was found to be 4 for rennin and 2 for pepsin. Moreover the proteolytic activity of pepsin is much higher than that of rennin at their optimum pH values.

After the ingestion of milk by the calf, clotting takes place within 1–10 min. There is an immediate release of a large amount of non-protein N into the duodenum, possibly glycopeptide released from *k*-casein by the action of rennin before coagulation takes place.[16,17] During the first 30 min after feeding there is a rapid increase in protein output, and thereafter the release of protein N is linear, and for the first 3–4 h after feeding it would appear to be largely whey protein that escapes digestion in the abomasum. After this time, partly digested casein is released as a result of disintegration of the curd.[18]

Before consideration is given to the replacement of milk protein by cheaper sources of protein, the marked differences between the digestion of milk protein from various processed milks must be borne in mind. Processing of milk that results in a marked denaturation of the whey proteins is detrimental to the calf.[7, 19–27] Table VII shows the effect of various processing treatments on the denaturation of whey proteins (as measured by non-casein N as a % of total N). Associated with the denaturation of the whey proteins are a reduction of ionisable calcium, release of SH groups, poor clotting ability by rennet and reduced digestibility, but no decline in biological value, of the protein.

TABLE VII

Effect of heat treatment of milk on the calf

Treatment of milk	Non-casein N as % of total	and effect on calf
None	25	No demonstrable effect
Holder pasteurised (63 °C for 30 min)	23	
Spray-dried skim (preheating temperature 77 °C for 15 sec)	22	
Spray-dried skim (preheating temperature 74 °C for at least 30 min)	15	Detrimental, especially during the first 3 weeks of life
Roller-dried skim (110 °C)	13	
Ultra-high temp. sterilised (135 °C for 1–3 sec)	11	

The detrimental effect of such poor quality diets may be seen in three ways: in the absence of an exciting infective agent, weight gain will be reduced by as much as 30% in the first 3 weeks of life; the rate of build-up of 'infection' when large numbers of susceptible calves are passed successively through a calf house will be greater;[28] and the incidence of diarrhoea and mortality will be higher once the infection has built-up as indicated by the dominance of one or two strains of *Escherichia coli*.[29]

In experiments comparing milk replacers containing two spray-dried skim milk powders, one of which had been 'severely' preheated at 74° for 30 min with the result that 50% of the whey proteins had been denatured (milk A) and the other 'mildly' preheated at 77° for 15 sec with only about 10% of the whey proteins denatured (milk B), very marked differences were found in the passage of protein and non-protein N into the duodenum.[18]

The pyloric outflow from milk B during the first 3 h after feeding had the appearance of clear whey, whereas that from milk A was milky in appearance and full of small pieces of clot. It was clear that large amounts of casein in milk A were escaping digestion in the abomasum. If it is assumed that the whey protein N largely escapes digestion in the abomasum, then in the case of the 'mildly' heat-treated milk only some 6–11% of the ingested casein would appear to pass undigested into the duodenum during the first 6½ h after feeding. As some of the protein N is clearly of endogenous origin, this percentage is probably much smaller. With the 'severely' heat-treated milk, between 33–39% of the ingested casein passes out undigested during the same period. This is illustrated in Table VIII.

The escape of casein from the abomasum without proteolysis would appear to be more important than any difference in the rate of proteolysis of the casein that remains in the abomasum, expecially at low levels of intake. This is indicated in Table IX, where the rate of proteolysis of casein that remained in the abomasum is very similar for the two milks at the low level of intake but more markedly different at the high level. This similarity in rate of proteolysis occurs in spite of the fact that the pH of the abomasal contents tends to be higher for the 'severely' heat-treated milk.

It thus seems that the detrimental effect of heat treatment of milk can be associated with qualitative and quanttiative changes in the bacterial flora due to the passage of abnormal amounts of protein in the chyme. It is well known that, in man, the dominance of the saccharo-proteolytic flora, consisting mainly of Enterobacteriaceae, including *E. coli* and clostridia, is associated with abnormal amounts of peptides and proteins in the chyme.[30]

TABLE VIII

Effect of heat treatment of milk on passage of undigested protein into the duodenum (assuming whey protein N, when undenatured, escapes digestion in the abomasum)

Milk A = 'severely' preheated skim milk; milk B = 'mildly' preheated skim milk

	Volume of milk ingested/feed, l	Total protein N intake, g	Total undenatured whey protein N intake, g	Protein N passage into duodenum in 6·5 h, g	Protein N passage as % of protein N intake	Undigested casein protein N (including endogenous protein N), g	Undigested casein protein N as % of casein protein N intake
Milk B	2·5	13·1	2·2	2·8	21	0·6	6
	3·5	18·3	3·0	4·7	26	1·7	11
Milk A	2·5	13·4	1·2	6·0	45	4·8	39
	3·5	18·7	1·7	7·3	39	5·6	33

TABLE IX

Effect of heat treatment of milk on rate of proteolysis of casein

Milk A = 'severely' preheated skim milk; milk B = 'mildly' preheated skim milk

	Volume of milk ingested/feed, l	Total casein N intake, g	Non-protein N intake, g	Non-protein N passage into duodenum in 6·5 h, g	Non-protein N from proteolysis (including endogenous secretions), g	Loss from abomasum of undigested casein N in 6·5 h, g	Casein N intake degraded in 6·5 h, %	Casein N intake that remained in abomasum degraded in 6·5 h, %
Milk B	2·5	10·9	0·9	4·1	3·2	0·6	29	31
	3·5	15·3	1·3	8·5	7·2	1·7	47	53
Milk A	2·5	12·2	1·0	3·4	2·4	4·8	20	32
	3·5	17·0	1·3	6·3	5·0	5·6	29	44

Even with diets containing milk protein that has received no heat treatment or a 'mild' heat treatment, an excessive concentration of protein in the diet will result in diarrhoea. Indeed, a linear inverse relationship exists between the N concentration in faecal organic matter and the faecal dry matter content.[20]

Substitution of milk protein by alternative sources of protein

The substitution of expensive milk protein by vegetable protein in the diet of pre-ruminant calves has not been very successful. Most experiments have been done with soyabean protein, which has a reasonably well-balanced amino acid composition. In general, the cruder the product, the worse have been the results. Ground cooked soyabeans in place of skim milk gave little weight gain over a 100-day period, but purified soyabean protein gave much better results.[31] Recently it has been claimed[32,33] that a 71% protein soyabean flour providing 86% of the protein of a milk replacer gave as good results as whole milk, but the level of feeding was such that the Friesian calves given whole milk gained weight at 0·20 kg/day and the soyabean fed calves at 0·33 kg/day. It was suggested that the good performance on this soyabean diet was due to its low content of trypsin inhibitor, normally present in the crude product. When a soyabean flour containing 50% protein was used, growth was negligible, either owing to diarrhoea or the presence of a trypsin inhibitor. The poor performance on the 50% protein soyabean diet was reflected in a marked decrease in the volume of pancreatic secretion and its enzyme concentration compared with that of calves given skim milk. From even more recent experiments, it has been suggested[34,35] that acid or alkali treatment (at pH 4 or 10·6) of soyabean flour markedly improves its utilisation by the calf. Milk substitute diets containing these treated soyabean flours as the sole source of protein have resulted in growth rates of up to 0·57 kg/day in the first 8 weeks of life. This increased growth rate did not appear to be associated with differences in digestion in the abomasum, since in a further experiment[36] untreated, acid and alkali-treated soyabean flour all gave similar results in relation to volume and pH of pyloric outflow and of rate of passage of dry matter and total N. However, no measurement was made of the proportion of the total N that was undigested protein. For all three soyabean flours, passage of dry matter and total N was much faster than for milk, and the pH declined more rapidly after feeding milk than after soyabean flours.

Even isolated soyabean proteins, ADM assay protein C-1 (Archer–Daniels Co., Cincinnati, Ohio, U.S.A.) and α-protein, supplemented with methionine, which are considerably more expensive than soyabean flour, have resulted in values for digestibility of N of only 72–75% and for nitrogen retention of 14–30% of ingested N.[15] There is also the problem that anti-soyabean protein antibodies were found in calves given a 'synthetic milk' containing 10% soyabean meal.[37]

The use of fish flour has also been investigated. In one experiment[38] up to 40% of the protein of a milk replacer was supplied by fish flour without reducing weight gain and feed conversion, but the level of feeding was low and the protein content of the diet was about 23%. As the calves gained weight at 0·38 kg/day, for which a much lower protein content would have been adequate, it is perhaps not surprising that the marked difference in digestibility of 80% for fish flour compared to 90% for milk protein was not shown up in the performance of the calves. When fish flour supplied 60% or more of the protein of the diet, a marked depression in performance was found. In a more recent experiment[39] in which 2–3 day old calves were given defatted fish flour contributing 14–42% of the protein, the majority of the calves had diarrhoea. When a milk substitute containing 5–15% defatted fish flour was fed to 2-week old calves, the incidence of diarrhoea was still high, although no difference was discernible in the growth to 6 weeks of age; the lowest incidence of diarrhoea occurred with the lowest rate of inclusion of fish flour.

Conclusions

At the present time, it would appear that there is no satisfactory complete substitute for milk protein in milk replacer diets, and, even with milk protein, care must be taken in processing milk to ensure that the minimum alteration occurs as this may affect its digestibility especially in the very young calf. It is possible that acid or alkali treatment of soyabean flour may result in a product that can completely replace milk protein, but considerably more work will have to be done at high levels of feeding and with large numbers of calves before it can be used commercially for the complete replacement of milk protein in milk replacer diets used directly after the colostrum-feeding period.

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Received 16 January, 1970

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FAT IN MILK REPLACERS FOR CALVES*

By A. M. RAVEN

Introduction

It is now over 20 years since experiments were first carried out in the U.S.A. on the possibility of developing satisfactory milk replacers for calves by combining separated milk with cheap vegetable or animal fats, but it is only in the last few years that the use of high-fat milk replacers has been realised commercially on farms in the U.K. One of the main reasons for this apparent slowness in development was that much of the early work was carried out before the significance of fat stabilisation and the importance of vitamin E in the nutrition of the calf had been fully realised and disappointing results were often obtained, particularly with unsaturated vegetable oils such as unhydrogenated cottonseed and soyabean oils. A further reason for some disappointing results seems to have been a lack of appreciation of the need for adequate emulsification and/or homogenisation of the fat in the milk replacer. More recent experiments have established the types of fat which can be included in milk replacers and also provided information on methods of inclusion to give the most satisfactory results in terms of animal performance.

In the digestion of milk replacers it should be appreciated that when whole milk or a liquid milk replacer is fed to young calves a sucking mechanism operates and the milk by-passes the rumen and reticulum, by closure of the oesophageal groove, and enters directly into the abomasum or true stomach. The milk or milk replacer thus avoids fermentation in the rumen and is enzymically digested in the abomasum and intestines. Although digestion by the milk-fed calf is therefore similar in some respects to that of simple-stomached animals it is essential to recognise that important qualitative and quantitative differences between the calf and other species of farm animal do occur. For example the liquid-fed calf shows poor ability to digest starch[1–5] and little improvement due to adaptation in more prolonged feeding experiments,[6] compared with a rapid development of ability to utilise starch by the young pig[7] and a high efficiency of utilisation by the young chick.[8] The liquid-fed calf also shows poor ability to digest vegetable proteins[9,10] compared with the ability of the young pig[7] and chick.[8] With regard to fat, reported digestibilities of 83–93% for certain animal and vegetable fats other than butterfat in milk replacers fed to calves[2,11–20] show that the young calf is inherently capable of adequately digesting a number of different fats as alternatives to butterfat. Although in this ability to digest fats the young calf shows some similarity to pigs and poultry, the differences in digestion and absorption of food constituents which are known to occur between pre-ruminant calves and other animal species are sufficient to emphasise the need for caution in any attempt to apply results obtained with other species to the calf.

* Presented at a Symposium on 'Milk Replacers for Young Farm Animals', organised by the Agriculture Group on 21 October, 1969

Incorporation of fat into milk replacers

Fat can be incorporated into milk replacers either by melting the fat and mixing it with separated milk powder or by homogenising the fat with liquid separated milk followed by drying to obtain a fatted milk powder. The inclusion of one or more emulsifying agents, such as lecithin, in the fat can be used to improve its digestibility. The effects of different methods of incorporation on the apparent digestibility of various fats have been studied in a number of experiments by Raven & Robinson[18] and the main results obtained are shown in Table I.

The diets containing blended fat were prepared by melting the fat and then mixing it with spray-dried separated milk powder to form mixtures containing the amounts of fat shown in the Table, which are similar to the amount of butterfat present in the dry matter of whole milk. The diets containing homogenised fat were made by homogenising sufficient of the fat with liquid separated milk followed by spray-drying to produce 'filled' milk powders containing the required level of fat. Lecithin, when added, was included in the fat prior to preparation of the 'filled' milk powders, at a level of 3·3% in the fats of experiments H and J and 3·0% in the tallow of experiment L. All the fats were stabilised prior to use and the 'filled' milk powders were supplemented with trace minerals, antibiotics and vitamins A, D_3 and E before feeding. The results clearly indicate that method of incorporation of fat into the diet has a marked effect on its subsequent digestibility by calves. Butterfat when melted and then blended with separated milk powder had a relatively poor digestibility of only 71·8% compared with 95·2% for the butterfat of whole milk, and 97·4% for butterfat homogenised with liquid separated milk in a more recent experiment.[20] The need for homogenisation to achieve maximum digestibility of fat in filled-milk diets was confirmed by the results with tallow containing lecithin when homogenisation resulted in substantially higher digestibility than blending the fat with separated milk powder. Microscopic examination of the fat globules in the two reconstituted milk diets indicated that with homogenised tallow the globules were 2 – 4 μm in diameter whereas with blended tallow the majority were 5 – 10 μm with an appreciable fraction 10 – 20 μm in diameter. Although the inclusion of lecithin is most beneficial in the absence of homogenisation its inclusion in homogenised fat has been found to be associated with slightly higher digestibility of both palm-kernel oil[18] and tallow.[17] It therefore seems desirable to include one or more effective emulsifying agents in order to ensure maximum digestibility of the fat. In studies of growth rate by the young calf Hodgson & Murdoch[21] reported a higher rate of gain on a diet containing 10% of homogenised lard as compared with physically blended lard, and Roy *et al.*[22] reported that mechanical homogenisation of margarine (17% in the dry matter of the diet) significantly increased growth rate compared with that obtained when the margarine

was only emulsified with lecithin. In addition to its value as an emulsifying agent in improving the digestibility of fat there are a number of other reasons which help to justify the inclusion of lecithin in milk replacers, e.g. its nutritional value as a source of energy and choline.

Effect of other dietary constituents on the digestibility of fat

In a study of factors which influence fat digestibility Olsen[23] found that factors which tended to increase or decrease the incidence of diarrhoea had a marked effect upon lipid digestion, e.g. overfeeding of milk to calves at early ages resulted in an increase in diarrhoea and a decrease in lipid utilisation. Since the digestion of fat and protein are to some extent related by clotting in the abomasum, which gives a clot consisting of a matrix of casein containing fat globules, it is of interest to consider the possible influence of protein on fat digestibility.

The effect of level of separated milk protein in the diet on the digestibility of butterfat has been studied in a 6 × 6 latin-square experiment with six calves each about 3 – 4 days old at the start of the experiment. Each of the six diets compared contained 20% of butterfat plus lecithin but had crude protein contents ranging from 12·7 up to 27·6% of the dry matter as shown in Table II. The diet with the highest content of protein consisted of spray-dried separated milk powder (51%), high-fat milk powder (45%) and glucose (4%), plus supplements of trace minerals, antibiotics and vitamins A, D_3, E and the B complex. Butterfat was used as the source of fat, and the high-fat milk powder was prepared by homogenising the requisite quantities of stabilised butterfat containing 3·3% of added lecithin and liquid separated milk to produce a 'filled' milk containing 45% of fat in the dry matter, followed by spray-drying. Amendments to the level of protein in the diets were made by interchange of separated milk protein and glucose, with additional lactose and minerals being used as required to maintain similar levels of these constituents in all the diets. The diets were reconstituted with water at the time of feeding, which was on a twice daily basis.

The results shown in Table II indicate that differences in mean digestibility of butterfat on the various diets were small and non-significant, and there was no evidence of any significant trend in digestibility with increase in level of crude protein. It is evident therefore that digestibility of the butterfat was unaffected by level of separated milk protein in the diet within the range studied of 12·7 up to 27·6%. It is also of interest that progressive substitution of glucose by separated milk protein did not affect the digestibility of organic matter, and since increase in level of protein did not increase faecal excretion of nitrogen it may be concluded that the true digestibility of the milk protein was very close to 100%.

In addition to level of separated milk protein the effect of source of protein on the digestibility of fat has been studied in an experiment of similar design using milk replacers containing different sources of animal protein. One milk replacer contained only separated milk protein and consisted of spray-dried separated milk powder (45%), high-fat milk powder containing tallow and lecithin (30%), glucose (25%), plus supplements of trace minerals, antibiotics and vitamins A, D_3, E and the B complex. The high-fat milk powder was prepared by homogenising the requisite quantities of stabilised tallow containing 3·3% of added lecithin and liquid separated milk to produce a 'filled' milk containing 50% of fat in the dry matter followed by spray-drying. The inclusion of 30% of this high-fat milk powder in each milk replacer therefore provided 15% of tallow in each of the replacers. Different forms of animal protein were provided by replacing 30% of separated milk powder, which supplied half of the total protein in the control milk replacer (MR7) by the following sources of protein, coupled with adjustments to the level of glucose to maintain the overall composition of the diets: MR 8 – 38% of delactosed whey (27·8% crude protein on a dry matter

TABLE I

Mean digestibility of fat in milk replacers

Diet	Experiment	Mean liveweight of calves,* kg	Total fat in diet, % of dry matter	Apparent digestibility of fat, %
Whole milk	H	44·0(3)	28·1	95·2
Blended butterfat	H	47·6(4)	28·2	71·8
Blended butterfat + lecithin	H	48·1(4)	27·4	88·3
Homogenised palm-kernel oil	J	43·1(4)	26·6	94·1
Homogenised palm-kernel oil + lecithin	J	42·6(4)	26·0	96·3
Blended tallow + lecithin	L	39·0(6)	25·0	70·7
Homogenised tallow + lecithin	L	39·5(6)	25·1	86·8

* The figures in parenthesis indicate the number of calves given each treatment

TABLE II

Chemical composition of the milk replacers and mean digestibility results

	MR 1	MR 2	MR 3	MR 4	MR 5	MR 6
Chemical composition of the diets:						
Fat, % of dry matter	21·0	22·0	21·6	21·6	22·1	22·6
Crude protein, % of dry matter	12·7	15·4	18·8	21·7	24·5	27·6
Apparent digestibility:						
Organic matter, %	95·4	95·5	95·3	94·6	95·6	95·5
Fat, %	93·5	94·1	95·4	90·2	92·3	92·2

basis); MR 9 – 12% of dried blood (90·0% crude protein); MR 11 – 15% of white fish meal (72·3% crude protein); MR 10 – 13·5% of meat meal (79·5% crude protein); and MR 12 – 13·5% of meat meal supplemented with a proteolytic enzyme supplement. Each diet was fed twice daily, the required quantity of milk replacer being reconstituted with water at the time of feeding.

The results given in Table III show that the use of the alternative sources of protein to separated milk protein all led to some reduction in digestibility of protein, the most marked reductions occurring with dried blood and meat meal, and it is evident that the digestibilities of the fat in the diets concerned were appreciably lower than on the solely milk protein treatment. In fact a significant correlation coefficient of + 0·529 ($P < 0·001$, $n = 34$) was obtained between the percentage digestibilities of crude protein and fat. One notable exception to this general trend which reduced the degree of correlation, was the slightly higher digestibility of fat obtained on the whey protein diet as compared with the solely milk protein diet. This is particularly surprising since the whey protein diet contained about twice the mineral content of the other diets, and this factor would be likely to lower the digestibility of the fat.

With the mineral constituents of milk replacers, as retentions of calcium, phosphorus and magnesium by calves on high-fat milk replacers are generally lower than on whole milk[20] it is reasonable to consider providing higher levels of these minerals in milk replacers in order to achieve similar retentions in g/day. Cheng *et al.*[24] have shown however that reductions in dietary calcium and magnesium increase the digestibility of triglycerides by the rat, and more recently in experiments with human patients Drenick[25] has shown that oral administration of soap-forming agents, such as calcium chloride, aluminium hydroxide and magnesium sulphates significantly increases faecal fat excretion. In the case of calves, Raven & Robinson[26] found that doubling the intakes of calcium and phosphorus in a milk replacer containing 28% of tallow, by the inclusion of 4% of dicalcium phosphate, reduced the mean digestibility of the total lipid by 1–5-week old calves from 86·0 to 82·0%. In considering the need to provide adequate levels of mineral elements in milk replacers to meet the nutritional requirements of the calf it is clearly desirable to avoid excesses not only because of interactions between mineral elements but also because excess of soap-forming elements can lead to reduced absorption of dietary fat.

Nutritive value of different fats in milk replacers

In considering the nutritive value of different fats it is evident from the many growth studies, which have been made using milk replacers containing different fats, that the inclusion of certain vegetable oils has resulted in poor growth and other adverse effects. The particular fats concerned were cottonseed oil,[27,28] corn oil[27,29] and soyabean oil.[27,30–34] Although in some reports the fat was not homogenised and there was no indication of supplementation of the diet with vitamin E, the results of Jarvis & Waugh[28] with cottonseed oil, Adams *et al.*[29] with corn oil and Jacobson & Cannon,[30] Wiese *et al.*[31] and Jacobson *et al.*[32] with soyabean oil all showed that even with homogenisation and supplementation with vitamin E growth was poor. Other adverse factors recorded by these workers included unthriftiness, scouring, poor food utilisation, increased susceptibility to disease and high mortality. In most of these experiments hydrogenated forms of cottonseed oil, corn oil and soyabean oil were found to give much better growth, similar to that on diets containing butterfat. Studies with coconut oil, which contains a relatively high proportion of short-chain saturated fatty acids, have also given satisfactory rates of growth.[16] With regard to animal fats, growth studies with tallow[16,35] and with lard[29,31,36,37] have given rates of gain similar to or somewhat lower than obtained with diets containing butterfat.

On the basis that certain fats can be included in milk replacers without giving rise to detrimental effects on growth it is relevant to consider the efficiency with which such fats are utilised, and Table IV summarises the digestibilities which have been reported for different fats. The values for butterfat were obtained with whole milk or 'filled' milks prepared by a process involving homogenisation, and similarly the values for the other fats all relate, as far as can be established, to homogenised fats. The results indicate that butterfat has a uniformly high mean digestibility of 96%, compared with mean values of 90 and 87% for lard and tallow, respectively. Palm oil and palm-kernel oil have mean digestibilities of about 89% but coconut oil has a higher digestibility of 94%. The single value of 83·4% for hydrogenated coconut oil is surprisingly low and may be due to the use of a purified basal diet based on glucose and casein instead of separated milk, particularly as the value for lard obtained in the same study also appears to be low.

As the main purpose of including fat is to bring about a substantial increase in the energy value of milk replacers it is of interest to consider the results of a recent experiment in which the energy value of a control low-fat diet was altered by the provision of additional lactose and by the inclusion of fats known to differ in digestibility. Six Friesian bull calves were allocated to a 6 × 6 latin-square experiment when about 3–4 days old, and balance trials carried out to compare the six diets are shown in Table V.

The only difference between separated milk diets MR 13 and MR 14 was in level of feeding. Diet MR 13 was fed to

TABLE III

Chemical composition of the milk replacers and mean digestibility results

	MR 7	MR 8	MR 9	MR 10	MR 11	MR 12
Chemical composition of the diets:						
Total ash, % of dry matter	5·7	11·6	5·3	5·8	5·5	5·5
Fat, % of dry matter	15·3	15·6	17·0	17·4	17·2	17·2
Crude protein, % of dry matter	20·1	20·8	20·8	21·6	20·7	20·7
Apparent digestibility:						
Organic matter, %	93·0	92·6	89·8	90·7	90·3	89·4
Fat, %	78·6	79·9	68·3	73·6	74·0	70·2
Crude protein, %	86·2	83·4	77·9	83·8	79·0	77·5

TABLE IV

Digestibility of different fats in milk and milk replacers for calves

Fat	Reference	Digestibility of fat, %	Mean digestibility of fat, %
Butterfat	*	95·6	96
	2	96·0	
	†	96·2	
	13	98·9	
	15	97·2	
	16	92	
	19	97	
Lard	11	91·7	90
	12	84·0	
	19	93	
Tallow	16	86	87
	18	86·8	
	19	87	
Palm oil	13	91·2	91
Palm oil (hydrogenated)	2	86·7	87
Palm-kernel oil	15	87·2	87
Palm-kernel oil (hydrogenated)	15	87·7	91
	18	95·2	
Coconut oil	14	94·9	
	16	93	
Coconut oil (hydrogenated))	12	83·4	83

* Blaxter, K. L., & Wood, W. A., *Br. J. Nutr.*, 1952, **6**, 1

† Grimes, C. W., & Gardner, V. E., *J. Dairy Sci.*, 1959, **42**, 919

give the same intake of dry matter (283·5 g/feed) as diets MR 15, MR 16, MR 17 and MR 18. Diet MR 14 was fed at 80% of this level so as to provide the same intake of separated milk powder, and therefore nitrogen, as diets MR 15, MR 16, MR 17 and MR 18. Each diet was fed twice daily, the required quantity of replacer being reconstituted with warm water immediately before feeding to give liquid milk replacers containing 12·4% of dry matter with the exception of MR 14; in this case, the lower quantity of dry matter provided led to a lower percentage of dry matter in the reconstituted diet.

It is evident from the results given in Table VI that the inclusion of 20% of tallow in diet MR 17 did not lead to any appreciable reduction in fat digestibility compared with the 10% level of inclusion in diet MR 16. Olsen[23] has also reported that calves can digest equally well the lipid of 10 or 20% (dry matter basis) animal fat milk replacers, but he found there was some decrease in digestibility when the content of fat was increased to 30%. The digestibility of the tallow in diets MR 16 and MR 17 was sufficiently high to maintain the metabolisable energy values of these milk replacers at just over 90% of their gross energy values, and the higher digestibility of the butterfat effected an increase in the metabolisability of diet MR 18. The inclusion of fat clearly led to substantial increases in metabolisable energy value per kg dry matter of the milk replacers, the values for the 10% tallow, 20% tallow, and 20% butterfat diets being 12·2, 23·1 and 26·6% higher than the value of 3·94 Mcal/kg dry matter for the separated milk diet with the same intake of dry matter and the diet with additional lactose in place of fat.

Turning to nitrogen retention it should be noted that the digestibilities of crude protein were similar for all the dietary treatments and the percentage retentions of nitrogen therefore indicate differences in efficiency of utilisation of absorbed nitrogen. Although diet MR 14 provided a similar intake

TABLE V

Percentage composition of the milk replacers

Ingredient	MR 13	MR 14	MR 15	MR 16	MR 17	MR 18
Spray-dried separated milk powder	100	100	80	70	60	60
Lactose	—	—	20	10	—	—
High-fat milk powder T*	—	—	—	20	40	—
High-fat milk powder B*	—	—	—	—	—	40
Antibiotic, vitamin and trace mineral supplements	+	+	+	+	+	+

* High-fat milk powder T was prepared by homogenising the requisite quantities of stabilised tallow containing 3·3% of added lecithin and liquid separated milk to produce a filled milk containing 50% of fat in the dry matter, followed by spray drying High-fat milk powder B was prepared in the same way but contained butterfat in place of tallow

TABLE VI

Chemical composition of the milk replacers and mean digestibility and balance results

	MR 13	MR 14	MR 15	MR 16	MR 17	MR 18
Chemical composition of the milk replacers:						
Fat, % of dry matter	2·0	2·0	1·6	12·1	21·8	22·1
Crude protein, % of dry matter	35·2	35·2	29·3	28·5	28·3	29·1
Digestibility and balance results:						
Apparent digestibility of fat, %	79·7	73·0	79·4	87·6	86·4	97·4
Apparent digestibility of crude protein, %	94·3	92·6	93·5	93·3	94·0	95·0
Metabolisable energy of milk replacer, Mcal/kg dry matter	3·93	3·91	3·94	4·42	4·85	4·99
Metabolisable energy of milk replacer, Mcal/100 Mcal gross energy	90·9	90·2	91·9	91·4	90·3	94·4
Nitrogen intake, g/day	31·9	25·5	26·6	25·9	25·7	26·4
Nitrogen retention, g/day	8·89	4·49	8·71	10·24	11·16	12·86
Nitrogen retention, % of intake	27·8	17·6	32·8	39·6	43·5	48·7

of nitrogen to the intakes on diets MR 15, MR 16, MR 17 and MR 18 it is evident that lack of sufficient dietary energy resulted in very poor retention on diet MR 14. The feeding of additional lactose in diet MR 15 led to a substantial increase in nitrogen retention from 17·6 to 32·8% of intake and the further increases in energy intake on diets MR 16 and MR 17, brought about by the inclusion of 10 and 20% of tallow in place of the lactose, led to further improvements in the efficiency of nitrogen retention. The higher digestibility of butterfat compared with tallow resulted in the metabolisable energy intake on diet MR 18 being higher than that on diet MR 17 and this further increase in energy intake was also accompanied by an improvement in efficiency of nitrogen retention.

Level of fat in milk replacers

As the main purpose of including fat in a milk replacer is to serve as a concentrated source of energy the optimum level to include is largely determined by the economic value of fat as a source of energy in competition with suitable carbohydrates and the need for an increase in energy concentration of the diet. With regard to suitable carbohydrates it is known that only lactose and glucose are well utilised by the young calf. Starch is less well utilised and only small quantities can be tolerated. A satisfactory replacer should avoid having too high a proportion of sugars or of mineral matter since excess of either of these components increases the possibility of scouring. The effects on incidence of diarrhoea of adding minerals simulating whey ash, animal tallow or both to a basal diet of non-fat milk solids were reported by Bush *et al.*[38] They found that added minerals increased the overall incidence of diarrhoea whereas animal tallow decreased its incidence in comparison with diets without added fat.

In considering the optimum level of fat to include it should be appreciated that fats generally used, e.g. tallow, are lower in digestibility than butterfat of whole milk and that undigested fat can exert detrimental effects on the nutritive value of milk replacers, e.g. by reducing the absorption of other nutrients. Also, it is important to distinguish between feeding for veal production and feeding to produce mature beef or dairy herd replacements. For veal production the calf is only given milk or liquid milk replacer and the inclusion of fat leads to a marked increase in energy value which undoubtedly brings about substantial improvements in rate of growth, efficiency of feed conversion and quality of the finished carcass.[16] With regard to level of added fat Warner *et al.*[16] found that 25% of homogenised tallow gave a higher rate of liveweight gain and better carcase quality than 12½% of homogenised tallow. In contrast to veal production, when it is intended to rear calves to maturity they are usually offered dry concentrates and roughages during the time they are fed milk replacer. In this situation where supplementary feeding of dry concentrate is being provided on an *ad libitum* basis there is some evidence that increasing level of fat in the milk replacer can lead to reduced consumption of the dry concentrate.[39] This reduction in concentrate intake could result from either voluntary curtailment of energy intake or through fat getting into the rumen in sufficient quantities to depress food intake, in a manner similar to that which has been reported to occur when 10% of certain fats are included in dry concentrate mixtures for cattle.[40] It seems likely that in some circumstances part of the fat in a high-fat milk replacer could gain entrance to the rumen, either through seepage back from the abomasum or through inefficient functioning of the oesophageal groove mechanism. In either case any depression of voluntary intake of dry concentrate would reduce energy intake and therefore lead to a less than full response to the use of fat in the milk replacer.

In general, as Blaxter[41] showed with calves receiving milk diets, lack of energy is more likely to be a limiting factor for growth than lack of protein. Since whole milk contains about 26% of fat in the dry matter, the amount of fat in milk replacers seems likely to increase towards this level as ways are found to improve the utilisation of alternative fats to butterfat, with higher levels being used for veal production than for conventional rearing.

Digestion of fat in milk replacers by the young calf

Although the digestion of liquid diets by the pre-ruminant animal has been reviewed recently by Porter,[42] it is relevant to briefly consider the process of digestion and absorption of fat before discussing possible reasons why substitute fats are generally of lower digestibility than butterfat in liquid diets fed to calves. In describing the course of digestion Porter[42] stated that during feeding milk clots rapidly as it enters the abomasum and the pH of the abomasal contents increases from 1 to 2 before feeding to about pH 6 immediately after feeding, and then decreases slowly to pre-feeding values. The consumption of food is accompanied by secretion of a lipolytic enzyme known as pre-gastric esterase, which appears to act in the abomasum in a manner similar to that of pancreatic lipase in the small intestine. Although Siewert & Otterby[43] have reported the accumulation of small quantities of free fatty acids in abomasal contents the quantitative significance of pre-gastric esterase in fat utilisation has still to be determined. Porter[42] pointed out that as digestion in the abomasum proceeds the clot breaks up and the chyme passing into the duodenum changes 3–4 h after feeding from a whey-like fluid containing mainly carbohydrate and soluble nitrogenous compounds to a thicker material containing protein and fat. Feeding leads to a rise in the pH of duodenal contents to a value of about pH 4 but has little effect in the distal small intestine and there is a gradual increase towards the ileum where values lie within the range pH 7–8.

Reviews by Senior[44] and Hoffmann[45] of lipid digestion by non-ruminant animals have indicated that the absorption of long-chain fatty acids from the small intestine is dependent upon their being solubilised in bile salt micelles. In comparing solubilisation in the non-ruminant with that in the ruminant Garton[46] recently pointed out that it is promoted by the presence of monoglycerides, derived from the action of pancreatic lipase on triglycerides, and by partial ionisation of fatty acids which occurs at a pH of about 6·5 in the upper jejunum of the non-ruminant. In the case of the ruminant, hydrolysis of glycerides and hydrogenation of unsaturated fatty acids in the rumen results in the lipids which pass through the omasum and abomasum to the small intestine being largely composed of free fatty acids, together with the structural lipids of the micro-organisms which accompany the digesta. Since micellar solubilisation of long-chain fatty acids is presumably also necessary for their absorption from the jejunum of the ruminant, the absence of monoglyceride and the fact that absorption can take place at a pH much lower than 6·5 necessitates some other mechanism for solubilisation. With regard to this mechanism Garton[46] pointed out that pancreatic juice provides phospholipase which converts biliary lecithin to lysolecithin, and Freeman[47] has shown a marked effect of lysolecithin in increasing the solubilisation of stearic acid. Garton[46] stated that although

this effect could account for solubilisation of long-chain fatty acids at about pH 6·5 or greater when they are partly ionised, it does not explain the solubilisation which takes place before fatty acids are absorbed from the upper jejunum where much lower pH values prevail. He suggested that some other biliary constitutent such as intact lecithin may be of significance in promoting the formation of a micellar solution with conjugated bile salts. It seems likely that in the case of pre-ruminant calf both monoglycerides and lysolecithin are important aids to solubilisation but their relative importance has still to be ascertained.

The partition of fatty acids between lymph and portal blood of animals depends on chain length. Whereas long-chain fatty acids appear in the lymph those with less than 12 carbon atoms appear primarily in the portal blood. The absorption of lipid by calves given whole milk has been studied by Shannon & Lascelles[48] using a lymphatico-venous shunt for the collection of thoracic duct lymph. When the calves were fed twice daily the mean hourly flow of lymph and output of neutral lipid, free fatty acid and phospholipid showed little change with time after feeding, compared with a definite pattern of change after feeding on a once-daily basis. The results demonstrated that lipid is absorbed over a long period in the milk-fed calf, presumably owing to slow release from the abomasum into the small intestine.

The steps concerned in digestion and absorption of fat by the liquid-fed calf can be considered as transport, emulsification, hydrolysis, solubilisation and absorption. Differential effects during any one or more of these steps may thus give rise to differences in efficiency of absorption of fat.

Differences in nutritive value between various fats

In considering possible reasons for the differences in nutritive value which exist between fats it is useful to compare butterfat and tallow, because the latter fat is frequently the most economic fat available for use in milk replacers and also because its mean digestibility of 87% is appreciably less than that of 96% for butterfat. The fatty acid composition of the butterfat and tallow used in the experiment described earlier and the apparent digestibilities of the principal long-chain fatty acids present in the fats have been determined and are given in Table VII.

Stearic and palmitic acids were the least well absorbed fatty acids, and the digestibilities of some fatty acids in tallow were lower than those of the same acids in butterfat. This difference in digestibility of individual acids in tallow and butterfat was much more pronounced with the saturated acids, and the differences show a marked increase with increase in chain length. The main reason for the overall difference in digestibility between the tallow and the butterfat was the appreciable difference in digestibility of the long-chain fatty acids, notably palmitic and stearic acids, in the two fats. Other contributory causes were the higher percentage of the least well absorbed acid (stearic acid) in tallow which can be considered as taking the place of acids of higher digestibility in butterfat, and the significantly lower digestibility of oleic acid in tallow than in butterfat. The fact that the digestibilities of palmitic and stearic acids were very similar on both the 10 and 20% tallow diets indicates that the total intakes of palmitic and stearic acids were not the determining factor. The main possibilities which could account for the differences in digestibility obtained appear to be differences in triglyceride structure, differences in relative proportions of individual fatty acids, differences in provision of amphiphiles which promote micellar solubilisation and possibly enzyme specificity with regard to different fats.

The effect of triglyceride structure has been examined in a recent experiment by Brown & Raven (unpublished results) in which batches of butterfat and tallow were used to prepare a number of different types of fat which were included in milk replacers by homogenisation with liquid separated milk followed by spray-drying to produce 'filled' milk powders containing about 20% of fat (dry matter basis). The fats compared were butterfat, interesterified butterfat, tallow, interesterified tallow, interesterified supplemented tallow and tallow admixed with supplementary triglycerides. The interesterified supplemented tallow was prepared by interesterification of a mixture of 80% tallow/10% glyceryl trimyristate/10% glyceryl trioleate, whereas the tallow admixed with supplementary triglycerides was prepared by simply mixing 80% tallow, 10% glyceryl trimyristate and 10% glyceryl trioleate. Preliminary results show that the overall digestibility of the butterfat was not reduced by interesterification, and therefore indicate that the high digestibility of butterfat is not dependent on any specific triglyceride structure. Although interesterification of the tallow was associated with rather higher digestibility, the digestibilities of the modified forms of tallow were all appreciably less than those of the two types of butterfat.

TABLE VII

Apparent digestibility of fatty acids in tallow and butterfat diets

Fatty acid	MR 16 (10% tallow diet)		MR 17 (20% tallow diet)		MR 18 (20% butterfat diet)	
	Amount present in dietary fatty acids, wt. %	Apparent digestibility, %	Amount present in dietary fatty acids, wt. %	Apparent digestibility, %	Amount present in dietary fatty acids, wt. %	Apparent digestibility, %
12 : 0	3·1	97·3	1·3	96·7	6·2	99·5
14 : 0	4·2	93·0	3·7	92·2	9·6	98·8
14 : 1	1·3	99·7	0·9	98·8	1·3	99·8
16 : 0	24·8	82·6	25·4	81·8	23·1	96·8
16 : 1	3·3	96·5	3·4	97·4	2·4	97·4
18 : 0	20·2	75·2	20·3	73·8	14·8	94·7
18 : 1	32·7	95·9	34·8	95·2	28·6	99·0
18 : 2	4·2	98·3	4·3	98·6	4·6	99·2

With regard to fatty acid composition the main differences between tallow and butterfat which may help to bring about the differences in digestibility of individual fatty acids are the higher proportion of stearic acid and the virtual absence of short-chain fatty acids in tallow as compared with butterfat. For instance Gunstone[49] cites a figure of 25% by wt. of stearic acid in the depot fat of cattle compared with 10% in cow's milk fat, and a total content of over 8% of butyric, caproic, caprylic and capric acid in cow's milk fat. As a result of investigations of factors which influence fat absorption in the chick, Young[50] concluded that stearic and palmitic acids are not only poorly absorbed but also decrease the absorption of each other. Young[50] stated that this effect becomes more pronounced as the amount of stearic acid is increased and that it is desirable not only to limit the amount of saturated fatty acid present in a dietary fat but also the amount of stearic acid in relation to the palmitic. He also concluded that the unsaturated fatty acids, particularly oleic acid, improve the absorption of the saturated fatty acids. More recently Freeman *et al.*[51] have studied the digestibilities of lard, coconut oil, soyabean oil and interesterified forms of these fats by the young pig. No evidence was found that the glyceride structure of any of the fats examined had a significant effect on fat digestibility and they concluded that the digestibilities of individual fatty acids were influenced both by their level of inclusion in the fat and by the other component fatty acids.

Freeman[47] has studied the behaviour of fatty acids in dilute bile salt solution and the effect of certain amphiphiles on the partition of fatty acid between equilibriated oil and micellar phases. He found that palmitic and stearic acids behave in bile salt solution as typical non-polar solutes, i.e. the critical micellar concentrations of sodium glycodeoxycholate for these acids were high and their saturation ratios low. Each of these factors could be expected to limit their absorption. Apart from the solubility of fatty acids *per se* Freeman[47] concluded that the most important factor which influences the micellar capacity of fatty acids such as palmitic and stearic acid is the type of amphiphile present in the dispersion. Amphiphiles present in the intestinal lumen may be derived from the diet, e.g. breakdown of dietary fat to give monoglycerides and free fatty acids, or through the action of phospholipase in pancreatic juice on biliary lecithin. Freeman's[47] results showed that oleic acid and 1-mono-olein were both useful in increasing micellar solubilisation of stearic acid but lysolecithin was found to be more effective. In fact the net effect of lysolecithin was a total capacity to solubilise stearic acid in the micellar phase of a biphasic system that was about twice that of 1-mono-olein.

The very short-chain acids which are present to an appreciable extent in butterfat are very well absorbed since digestibility of fatty acids increases markedly with reduction in chain length. Some evidence that they may also have an important effect on overall fat digestibility has been provided by Calloway & Kurtz[52] who found that modification of an hydrogenated lard by 'butyration to the extent of one fatty acid equivalent' markedly improved the digestibility by rats of the remaining part of the lard triglycerides. Simple admixture of tributyrin with the hydrogenerated lard had no effect, however, which suggests that although triglyceride structure of a particular fat may not appreciably influence its digestibility, the addition of specific fatty acids to improve fat absorption may require interesterification for the acids to be effective.

Feeding systems

A consideration of milk replacers for calves would not be complete without some consideration of when to wean off liquid milk replacer onto dry foods when the object is to raise calves for the production of beef as or replacements for the dairy herd in contrast to the specialised production of veal. Dry feeding leads to a rapid development of the rumen and thereby permits the use of a range of foods which are cheaper than milk replacers but which require a functional rumen for their effective utilisation. The principal foods concerned are cereals, vegetable protein concentrates and roughages. However, although a functional rumen enables such foods to be used, the biochemical processes which occur in the rumen penalise the efficiency of utilisation of foods which the calf could digest efficiently by gastric digestion. For example, glucose undergoing fermentation in the rumen suffers loss of energy in the form of combustible gases and heat of fermentation, and the volatile fatty acids produced as a result of fermentation are utilised by the animal with lower efficiency than is glucose absorbed as such from the intestines. The magnitude of the difference was examined by Armstrong *et al.*[53] who found that the increase in energy retained/100 kcal glucose given was 61·6 kcal when glucose was administered by continuous infusion into the abomasum compared with only 42·3 kcal when administered by infusion into the rumen. Proteins in the rumen undergo considerable degradation to ammonia and resynthesis into bacterial protein so that the amino acid composition and biological value of crude protein leaving the rumen is reasonably constant and largely determined by that of bacterial and protozoal protein. The biological value of most sources of protein fed to ruminants is therefore about 70,[54] which, although higher than that of poor quality proteins, is much less than that of milk protein fed to other animals. With regard to fat, levels of up to 5% can be used and a high digestibility obtained for the added fat. For instance, the digestibilities of tallow and palm-kernel oil included in dry concentrate mixtures at a level of 5% were found to be 87·6 and 97·1% respectively without roughage being fed, and 90·5 and 96·2% respectively when hay was provided so that the proportion of concentrates to hay was 2 to 1.[55] However, although levels of up to 5% of added fat can be used in dry concentrates, levels of 10% and greater have been found to depress food intake and be associated with much lower digestibility of the added fat.[40]

One possible development in the future which could improve efficiency of food utilisation is extended liquid feeding of part of the diet in conjunction with dry fed concentrates and roughages. The aim of such a system would be to supplement the nutrients made available to the calf as a result of biochemical processes in the rumen with other nutrients fed as solutions or suspensions in water to enable them to by-pass the rumen and enter directly into the abomasum. The effect of feeding certain concentrate mixtures as suspensions in water compared with dry feeding has been studied by Raven & Robinson[26,56] using high and low fat concentrate mixtures fed in conjunction with hay. In these studies the effect of system of feeding on the mode of absorption of the carbohydrates was examined by measuring the changes in reducing sugars of the blood following a normal meal which contained lactose, and also one containing in addition 200 g of glucose. All the calves used showed substantial rises following wet feeding compared with much smaller changes after dry feeding, supporting the view that liquid feeding enabled a considerable proportion of the food

to pass directly into the abomasum whereas dry feeding was associated with entry into the rumen. Further confirmation of this view was provided by the results for the digestibility of crude protein and fat. Whereas dry feeding was associated with higher digestibility of crude protein, the digestibility of the fat in the high fat diet (31·3% fat in the concentrate dry matter) showed a substantial reduction from 82·7 to 67·4% with the change from liquid to dry feeding and then recovered to 82·1% on return to liquid feeding. Although the amounts of energy provided by the digested components of each of the diets were similar with liquid and dry feeding the retention of nitrogen was much higher with liquid feeding than with dry feeding. Since retention of nitrogen was also much higher on the high-fat diet than on the low-fat diet, with each method of feeding, it was clear that lack of available dietary energy was an important factor limiting retention. The more efficient nitrogen retention on each diet when fed liquid rather than dry would therefore seem to have resulted from a more efficient use of dietary energy in the former case. Recent studies with young lambs by Ørskov & Benzie[57,58] have provided useful information regarding the conditions necessary for efficient by-passing of the rumen. They found that chemical stimuli are of minor importance in determining closure of the oesophageal groove, and that the response could be obtained with lambs accustomed to sucking fluid from a teat but not when the liquid was administered as a drench. Although they concluded that the oesophageal groove response is produced by sucking there is a need for further clarification concerning the precise function of the teat and the relative efficiency of feeding from a bucket compared with through a teat.

In general the greatest promise at present with extended liquid feeding seems to lie with amino acids or proteins which could serve as supplements to improve the biological value of protein made available to the calf, and with higher levels of fat than are possible in dry concentrates. However, one can also speculate that if a means can be found to enable efficient utilisation of starch fed in this way, it would be possible to think in terms of most of the concentrate part of the diet being fed to by-pass the rumen, and a consequent revolution in current feeding practices with ruminants.

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Received 16 January, 1970

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NUTRITIVE VALUE OF MEAT MEALS
I.—Possible growth depressant factors*

By J. ATKINSON† and K. J. CARPENTER

When fed as supplements in a well-balanced diet for the rat, a commercial meat-meal sample and various heat-damaged laboratory preparations including pure tendon stimulated growth, thus providing contradictory evidence to previous reports of possible toxicity in such materials. An increase in dietary ash level, similar to that brought about by addition of meat meal, caused no growth depression in rats given, as their sole source of protein, gut preparations low in ash. Freeze-dried tendon had as low a value as oven-dried tendon in sole protein experiments in which rats lost weight and died. Meat meal itself supported slow growth and no further response was obtained with supplements of methionine, cystine, tryptophan and lysine.

Introduction

In Britain, the term 'meat meal' is confined officially to materials containing not less than 55% crude protein (N × 6·25) (Fertilisers and Feeding Stuffs Act, 1926, 4th Schedule, Part II), and materials containing between 40 and 55% crude protein are described as 'meat-and-bone meal'. This division has not been universally adopted, and in the present paper the term meat meal will refer to meals with any crude protein value between 40 and 65%.

Previous reports have shown meat meals to be of relatively low quality as a source of animal protein. The 'gross protein value' test[1] of the relative value for chicks of protein concentrates in supplementing a cereal-based mixture primarily deficient in lysine, has been applied to meat meals by many previous workers.[2–5] These authors all found meat meals to be of approximately half the value of fishmeal samples, when fed at levels contributing the same quantity of protein. When fed as sole proteins, meat meals were again found to be of low quality for chicks,[6–8] for pigs[9] and for rats.[10,11]

A low nutritive value of any dietary component may result from the presence of toxic agents, provision of any nutrient(s), in such excess that they are themselves growth-depressant, and/or lack of any essential nutrient(s). It has been particularly suggested that the heating of tendons may produce toxic factors[12] possibly by way of Maillard reactions, and it has been reported that feeding 'amino-hexoses' produced by heating glycine and glucose together, caused growth depression in rats.[13] It has also been suggested that the low growth of chicks on high-ash meat meals is caused by an excess of calcium.[14] It was found that the addition of bone meal to a starch–soyabean meal diet depressed growth only to the same extent as occurred on the addition of an equivalent amount of calcium from calcium carbonate plus phosphate.[12]

In this study, examples of materials present in the mixtures used in meat meal manufacture were taken, e.g. gut, tendon and ossein. Striated muscle was studied to see the effects of processing on a material of high nutritive value initially. The commercial and laboratory-prepared samples were fed to rats either as supplements to 'casein-plus-amino acids' or as the sole protein source in the diet.

Experimental

Test materials

Commercial samples

Meat meals were obtained direct from three English factories. The raw materials available to these factories are firstly the gut, removed at the slaughter house, then the bones coming after dissection of the joints and finally the 'greaves', the residues from pre-cooking some internal tissues for the recovery of edible fat. Some whole, condemned carcasses are also processed. Bone, gut, etc. are deliberately mixed to form charges for the cookers, and individual batches of the final products are again mixed to minimise the variability which could result from using more than one raw material. In all three factories, charges of raw material were first cooked and dried in a vessel fitted with a steam jacket. In two of the factories, the vessel was sealed for a preliminary cooking period, so that the material was first autoclaved; after this the pressure was released and the material dried. In the remaining factory, the cooking and drying were not separated in this way. The conditions during the processing of the present samples are shown in Table I. The materials were then fat-extracted using a hydrocarbon solvent, in some cases after a preliminary centrifugation. The raw materials used and the analyses of the final products are also shown in Table I.

The fish meal used for comparison, X759, was a 'White fish meal' (Provimi 66 Brand).

Laboratory-prepared samples

The following model materials were used:

(a) *psoas major* muscle (fillet steak) of cattle—this has an extremely low collagen content and its protein is almost entirely of muscle origin;

(b) suspensory ligament and extensor and flexor tendons from the metacarpal area of cattle legs—these were separated from adhering muscle and the protein was very largely collagen;

(c) complete alimentary tract (gut) of cattle from the base of the oesophagus to the anus—this was washed free of all contents, and was then mainly rumen by weight;

(d) mixtures of (a) and (b) containing 60% of meat protein and 40% tendon protein. One mixture, so prepared and then oven-dried, was given the code X618.

The samples were then either: freeze-dried (*FD*), oven dried (*OD*) at 90° for 44 h (conditions chosen to imitate the processing of Ferrando *et al.*[12]), or autoclaved (*AOD*) at 110° for 5 h and then dried in an oven set at 160° with an inter-

* This work formed part of a thesis submitted by one of the authors (J.A.) to the University of Cambridge in partial fulfilment of the requirements for the degree of Ph.D. Some of these results have been communicated in a preliminary form (Atkinson & Carpenter[23])

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mittent air flow, until the temperature of the material was 100° (by thermistor probe placed inside the material – approximately 18 h were required).

Most of the materials so produced were fat-extracted (*FE*) with light petroleum ('Laboratory-grade 40–60°' Harrington Brothers, Ltd.) in a Soxhlet apparatus and spread out on trays at room temperature to allow the residual solvent to evaporate. The various batches of material were then given 'X' code numbers.

Cod fillets, as previously described,[16] were taken for comparison as examples of another high-quality animal protein material either freeze-dried (sample 23), damaged by severe heat alone (sample 35), or by moderate heat in the presence of glucose (sample 25).

Feeding experiments

Diets

The basal forms of the test diets are shown in Table II. The mineral mixtures were based on that of Hawk & Oser.[18] Mineral mix 1B contributed the following as % of the basal diet: calcium citrate $4H_2O$, 1·2; calcium tetrahydrogen diorthophosphate H_2O, 0·5; calcium carbonate, 0·27; dipotassium hydrogen orthophosphate, 0·9; potassium chloride, 0·5; sodium chloride, 0·3; basic magnesium carbonate $3H_2O$, 0·14; magnesium sulphate H_2O, 0·16; manganese sulphate $4H_2O$, 0·02; basic zinc carbonate, 0·002; ferric citrate $5H_2O$, 0·06; copper sulphate $5H_2O$, 0·004; sodium fluoride, 0·0005; potassium aluminium sulphate $24H_2O$, 0·004; potassium iodide, 0·0002. Mineral mixture 1A was the same as the above except that it contributed only 0·0007% manganese sulphate to the diet. Mineral mix 1C was the same as 1B except that all calcium salts were left out and dipotassium hydrogen orthophosphate was replaced by potassium carbonate. Mineral mix 1D was the same as 1C except that the level of basic zinc carbonate was raised to 0·016% of diet.

TABLE I

Preparation and composition of the commercial samples of meat meal

Factory	Sample code	Raw materials* Bone	Greaves	Gut	Processing conditions: Internal pressure, lb/in² (time held, h)	Steam pressure in external jacket, lb/in²	Total cooking time, h	Dry matter, %	Crude protein (N × 6·25), %	Ash, %	Ether extract, %
Meat meals:											
1	X746	+++	+++	+	None	60	½–1	86·6	46·0	28·4	2·4
1	X747	++	+++	+				87·1	50·6	27·9	3·2
2	X748	++	++	++	10 (½ h)	80	2½	95·9	64·1	21·6	5·7
3	X749	+	++	+++	40 (½ h)	80–100	2½	89·6	53·3	24·2	1·5
3	X750	—	+	+++	or more)			92·4	63·1	22·2	2·3
2 & 3	X804	(Mixture of equal parts of X748, X749 and X750)						92·5	59·8	22·7	3·1
Fish meal:											
—	X759	—	—	—	—	—	—	92·2	63·9	23·4	4·2

* The number of + signs is intended to represent the relative quantities of the different raw materials used, as explained in the text

TABLE II

Composition of basal test diets (%) and experimental procedure

	Supplementary protein series			Sole protein series				
Expt. no:	26 (i)	36	44	26 (ii)	27	30	33	43
Casein	13·6*	14·5**	14·5**	0	0	0	0	0
Arachis oil	9·6	10	10	9·6	0	10	10	10
Potato starch	9·6	10	10	9·6	10	10	10	10
Mineral mix	3·8 (1A)	4 (1B)	2·1 (1D)	3·8 (1A)	4 (1B)	4 (1B)	4 (1B)	2·1 (1D)
Steamed bone flour	0	0	6·0	0	0	0	0	5·0
Vitamin mix	0·9 (1A)	1 (1B)	1 (1B)	0·9 (1A)	1 (1A)	1 (1B)	1 (1B)	1 (1B)
Tallow	0	0	0	0	6·9	0	0	0
Sucrose	*ad* 100	*ad* 100	*ad* 100	*ad* 100	*ad* 100	*ad* 100	10	10
Maize starch	0	0	0	0	0	0	*ad* 100	*ad* 100
Type of rat†	W	W	S–D	W	W	W	W	S–D
Rats per cage‡	1M, 1F	1M	1M	1M, 1F	1M	1M	1M, 1F	1M, 1F
Cages per treatment	4	5	4	6	8	6	4	4
Experimental period, days	21	21	24	24	22	21	10	10

* plus L-histidine HCl, 0·04; L-lysine HCl, 0·10; DL-tryptophan, 0·03; DL-methionine, 0·32; L-threonine, 0·005% of diet

** plus L-histidine HCl, 0·04; L-lysine HCl, 0·10; L-tryptophan, 0·03; L-phenylalanine, 0·14; DL-methionine, 0·34; L-threonine, 0·005% of diet

† W, Wistar; S-D, Sprague–Dawley from specific-pathogen-free colony[17]

‡ M, Male; F, female

When mineral mixes 1C and 1D were used, the calcium and phosphorus contributions came from steamed bone flour, test supplement ash, or a combination of both.

The vitamin mix 1B was based on that of Chapman *et al.*[19] and contributed (mg/100 g diet) thiamine hydrochloride, 0·6; riboflavin, 1·0; pyridoxine, 0·4; calcium pantothenate, 4·0; niacin, 4·0; inositol, 25·0; *p*-aminobenzoic acid, 10·0; menaphthone, 0·5; biotin, 0·02; folic acid, 0·2; cyanocobalamin, 0·01; choline chloride, 200; and α-tocopheryl acetate, 12; also vitamin A, 250 I.U. and vitamin D_3, 62·5 I.U./100 g diet. Vitamin mix 1A differed from 1B in contributing only 0·002 mg cyanocobalamin and 10 mg α-tocopheryl acetate/100 g diet.

The test diets were formulated as below.

Supplementary protein series

The 'casein-plus-amino-acids' used in the basal diet for experiment 26 (i) was intended to be a well-balanced source of protein for the rat; freeze-dried and oven-dried tendon were added at a 12·7% crude protein level at the expense of sucrose. In experiment 36, various laboratory preparations were fed as supplements to a modified diet with additional essential amino acids and were added at a 13% crude protein level. In experiment 44, a meat-meal sample (X804) was added at a 13% crude protein level as a supplement to the diet further modified so that the addition could be made at the expense of sucrose and steamed bone flour to keep the dietary ash level constant.

Sole protein series

In experiment 26 (ii), freeze-dried and oven-dried tendon were fed as sole proteins at a 12·7% crude protein level, again added at the expense of sucrose. Various meat and tendon mixtures were fed at a 13% crude protein level in experiment 27 and were added at the expense of sucrose and tallow, to keep the total fat content of the diets constant. A heated 'meat-and-tendon' mixture was supplemented with a mixture of essential amino acids (as shown in Table IV). It was calculated that with these additions all the requirements of the rat for essential amino acids[20] would be met even if half of all the amino acids present in freeze-dried meat and tendon had been made unavailable by the processing.

In experiment 30, freeze-dried and damaged cod fillet preparations were fed at a 10% crude protein level and were added at the expense of sucrose. Amino acid supplements were added to the latter to investigate whether depressed growth could be prevented in this way.

In experiment 43 a meat-meal sample (X804) was added at a 10% crude protein level at the expense of maize starch and steamed bone flour. Amino acid supplements were fed in further treatments as shown in Table VI. These were calculated to bring the level of the amino acids in question up to those required by the rat, on the assumption that the crude protein of the meal itself contained lysine 3·8, tryptophan 0·2, cystine 1·5 and methionine 1·0 g/16 g N in available form.[31]

In experiment 33, high-quality and low-quality gut preparations were fed at a 10% crude protein level, with and without supplementary steamed bone flour or kaolin, all added at the expense of corn starch, as shown in Table VII.

Design of feeding experiments

The rats were purchased as weanlings for use in both series of experiments. After 5 days on preliminary diet, the rats reached 45–50 g body weight and at this stage extremes, on the basis of performance during the preliminary period, were eliminated and the remainder were stratified and randomised on to the test diets as set out in Table II.

The animals were kept in wire mesh cages with raised floors. All diets were fed in the dry form, *ad libitum*. Carcass analysis for N was carried out on the rats from experiments 33 and 43.[21] The NPU_{10} (carc.) values, abbreviated to *NPU* (net protein utilisation) for the remainder of the paper, were calculated according to Bender & Doell's revised equation '3':[22]

$$NPU = \frac{\text{Net N gain of test group}}{\text{N intake of test group—N intake of 'protein-free' group}} \times 100$$

Results

In Table III are shown the results of the experiments in which test materials were added to diets which already contained a reasonably good protein source. Gross dissection of the carcasses and histological examination of the livers, revealed nothing abnormal. Although the control diet sup-

TABLE III

Mean performance of rats in supplementary protein experiments

Supplement to casein-plus-amino acids	Expt. no. 26		36		44	
	*WG**	*EFC**	*WG*	*EFC*	*WG*	*EFC*
None	3·26	0·39	3·43	0·32	2·93**	0·31
X599: *FD* tendon	3·43**	0·40	—	—	—	—
X600: *OD* tendon	4·17	0·49	(4·66)†	(0·47)†	—	—
X724: *FD/FE* meat	—	—	4·33	0·48	—	—
X760: *OD* meat	—	—	4·18	0·53	—	—
X751: *AOD/FE* gut	—	—	4·48	0·44	—	—
Cod 25	—	—	4·58	0·46	—	—
X804: Meat meal	—	—	—	—	5·72	0·44
S.E.	±0·24	±0·02	—	±0·04	±0·25	±0·02

* *WG*, live weight gain (g/rat/day); *EFC*, efficiency of food conversion (weight gain, g/food eaten, g)

** Some of the rats on these treatments lost weight for no apparent reason

† A different batch, X767, was used in this experiment

ported a good rate of gain – approximately 3 g or more, per day, the addition of the test materials in every case further stimulated food consumption, and weight gains. These experiments indicate, therefore, that there is nothing in the test materials that positively inhibits good performance.

In Table IV are given the results of feeding test materials at a 13% sole protein level. It is seen that even the freeze-dried preparation of tendon X599 failed to support growth, and there is no evidence that the oven-drying used to produce X600 was responsible for the equally bad results with this material.

The results with the tendon preparation are in contrast to those obtained with the meat samples X614 and X622. The performance of rats fed *OD* meat was significantly lower than that of rats fed *FD* meat. Heating meat in the presence of tendon to give sample X618 led to no further damage over and above that caused by the heating of the two components separately. Addition of a mixture of the essential amino acids to the same material increased growth significantly but not up to the level found with *FD* meat.

Table V shows the results of experiment 30, in which an attempt was made to increase the value of two damaged cod preparations. Supplementation of cod 35 with amino acids increased growth up to that found on *FD* cod fillets – cod 23. However, supplementation of cod 25 that had been mildly heated in the presence of glucose, did not produce the expected increase in growth. Adding glutamic acid at the same nitrogen level as that introduced by the addition of the essential amino acids gave no increase in performance over the corresponding controls. A completely synthetic amino acid diet, equivalent in amino acid levels to that of a *FD* cod fillet diet, gave equivalent performance to the latter.

In Table VI are given the results of experiment 43, in which the effect of supplementation of a meat meal with the amino acids thought at the time to be most limiting was studied. It is clear that none of the combinations of amino acid supplements stimulated the performance of the rats significantly.

The results of experiment 33 are summarised in Table VII. It is seen that adding to gut, a low-ash material, calcium equivalent to what would be contributed by a high-ash meat meal, did not alter the *NPU* value for rats tested under the standard conditions – i.e. with the test material in a quantity supplying 10% protein. This was the case both

TABLE IV

Mean performance of rats in sole protein experiments

Protein source	Weight change, g/rat/day	Mortality*	*EFC*
Experiment 26 (ii)			
X599: *FD* tendon	−0·17	4/12	—
X600: *OD* tendon	−0·28	3/12	—
Experiment 27			
X614: *FD* meat	+4·42	—	0·35
X622: *OD* meat	+3·17	—	0·28
60% X614 protein + 40% X599 protein (i.e. both *FD*)	+2·98	—	0·25
60% X622 protein + 40% X600 protein (i.e. both *OD*)	+1·38	—	0·18
X618: 60% protein from *FD* meat and 40% from *FD* tendon mixed and then *OD*	+1·59	—	0·19
X618 + amino acids**	+2·83	—	0·27
S.E. (for expt. 27)	±0·26	—	±0·01

* The first death occurred on the 18th day

** L-histidine HCl, 0·17; L-lysine HCl, 0·50; L-tryptophan, 0·02; L-phenylalanine, 0·61; DL-methionine, 0·41; L-threonine, 0·29; L-leucine, 0·44; L-isoleucine, 0·28; L-valine, 0·45% of diet

TABLE V

Mean performance of rats receiving heat-damaged cod protein with supplementary amino acids (Experiment 30)

Supplement	Weight change, g/rat/day	Mortality†	*EFC*
A. Cod 23: *FD* cod fillets	+2·75	0/6	0·30
B. All amino acids of Cod 23* at overall nitrogen level of 1·6% of diet	+2·91	0/6	0·33
C. Cod 35 (i.e. heated with 14% H_2O, 116°c for 27 h)	+0·63	0/6	0·09
E. Cod 35 + 40% of essential amino acids added in (B)	+2·88	0/6	0·30
F. Cod 35 + L-glutamic acid to give equal nitrogen addition to that of essential amino acids added to (B)	+0·82	0/6	0·12
D. Cod 25 (i.e. heated with 14% H_2O and 10% glucose, 85°c for 27 h)	−0·32	2/6	—
G. Cod 25 + 55% of essential amino acids added in (B)	+0·71	0/6	0·11
H. Cod 35 + L-glutamic acid to give equal nitrogen addition to that of 55% essential amino acids added in (B)	−0·55	2/6	—
S.E.	±0·14**	—	±0·02

* Using published amino acid analysis[23]

** Does not apply to treatments D and H on which some rats died

† The first death occurred on the 15th day

TABLE VI

Mean performance of rats receiving meat meal X804 with different amino acid supplements (Experiment 43)

Amino acid supplement	*NPR**	Estimated *NPU***
Nil	1·68	29
0·4% L-cystine + 0·4% L-methionine	1·60	28
0·4% L-cystine + 0·13% L-tryptophan	1·48	26
0·4% L-cystine + 0·75% L-lysine HCl	1·70	30
0·4% L-methionine + 0·13% L-tryptophan	1·99	34
0·4% L-methionine + 0·75% L-lysine HCl	1·86	32
0·13% L-tryptophan + 0·75% L-lysine HCl	1·47	26
0·4% L-cystine + 0·4% L-methionine + 0·13% L-tryptophan + 0·75% L-lysine HCl	1·47	26
	S.E. ±0·19	

* *NPR* (Net protein retention) $= \frac{\text{Weight change of test group} + \text{Weight loss of protein-free group}}{\text{Protein eaten by test group}}$

** *NPU* (Net protein utilisation) estimated from the function '15·5 (*NPR*) + 3·3'[22]

TABLE VII

Net protein utilisation (*NPU*) of rats receiving gut protein with and without high levels of mineral supplements (Experiment 33)

Protein source	Mineral ingredients, %			Calculated calcium level, %	*NPU*
	Mineral mix*	Steamed bone flour	Kaolin		
X752: *FD/FE* gut	4 (1B)	0	0	0·6	70
X752: *FD/FE* gut	2·1 (1C)	4	0	1·3	75
X752: *FD/FE* gut	4 (1B)	0	4	0·6	71
X751: *AOD/FE* gut	4 (1B)	0	0	0·6	25
X751: *AOD/FE* gut	2·1 (1C)	4	0	1·3	24
X751: *AOD/FE* gut	4 (1B)	0	4	0·6	10
					S.E. ± 3·6

* Code numbers, given in parenthesis in each case, are explained in the text

with the freeze-dried gut that was of high quality, and with the autoclaved, oven-dried material of low quality. With kaolin added as an inert mineral, there was again no effect on the *NPU* value obtained with the high-quality material, but with the damaged material there was a significant drop. Of course, with *NPU* values below 30 all the rats on the damaged material were losing weight.

Discussion

When fed as supplements to a 'casein-plus-amino acids' basal diet, none of the supplements tested caused growth depression. All gave, in fact, a significant increase over the basal diet, which was formulated to be a complete, well-balanced diet for the rat. This, together with work already published,[23] refuted suggestions[12] that toxic compounds were formed during the heating of tendon preparations (and possibly during meat meal processing) and that these were responsible for the poor performance of rats fed diets including tendon as the sole source of protein. It has also been found that rats performed equally badly on either heated or raw collagen, for which their very low tryptophan content provides an explanation.[24]

Others have proposed that the low growth on meat meals could be due to a depression in growth caused by an excessive dietary calcium level, resulting from the addition of high-ash meat meal to the diet.[8,14] It is claimed here that levels of calcium up to twice the rat requirement do not depress growth. Higher levels are unlikely in commercial rations for pigs when meat meals (or indeed, fishmeals) are included. In a subsequent paper[31] data showing a close parallel between the rat and pig performance on similar diets will be presented. When diets, including meat meal or high quality supplement, are equilibriated to constant ash level, then large differences in their growth-promoting ability are still seen. With a damaged gut preparation (X751), addition of kaolin caused a significant growth depression. *NPU* values of this order are variable as slight variations in the carcass weight and nitrogen percentage used for calculation have a large effect.

What of other possible causes of growth depression in meat meals? With regard to bacterial contamination, it is generally thought that if there is complete separation of unprocessed offal and finished product, adequate factory cleansing, and a high enough rendering temperature, then generally no problems of bacterial toxicity will arise. In the course of the present authors' experiments, meat meals have been fed to 'specific pathogen-free' rats, and no signs of bacterial disease were seen.

Oxidative rancidity has also been put forward as a cause of toxicity in meat meals. It was found to be impossible to construct a diet, using an oxidised meat-meal sample, which would be of high enough peroxide value to cause growth depression.[25] With the present use in England of solvent extractions, the addition of antioxidants to the meat meals, and inclusion of stabilised vitamin supplements in the feed, fat oxidation in meat meals is not a nutritional problem.

Since toxic factors and ash levels do not appear to be limiting growth, there remains the explanation of poor amino acid balance of the protein. However, amino acid supplementation of a meat-meal sample has not increased its net protein retention (*NPR*) value. This has been reported before,[26] although on addition of many amino acids at a higher dietary protein level, more success has been achieved.[27-29] It has been suggested[30] that with gelatin the presence of large amounts of non-essential amino acids may increase the requirement for essential amino acids. The problem of increasing performance of meat-meal diets by amino acid supplementation, is a complex one and the protein quality of meat meals will be considered in detail in a further paper.[31]

Acknowledgments

One of the authors (J.A.) is indebted to the Pig Industry Development Authority, London, for a grant to carry out the work.

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Received 11 February, 1970

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NUTRITIVE VALUE OF MEAT MEALS
II.*—Influence of raw materials and processing on protein quality

By J. ATKINSON† and K. J. CARPENTER

Commercial meat meals have shown low net protein utilisation values with rats of about 32, which is only 40% of the corresponding value for freeze-dried meat or gut. Biological assays for methionine and tryptophan, based on the weight gains of chicks, have shown a similar inferiority of meat meals despite 81–87% digestibility of their N. It is concluded that the lysine, methionine and tryptophan of the meat meals are approximately 80% available and that dilution of muscle protein with tendon and ossein in the raw material is at least as important as processing damage in reducing the quality of the products. It is argued that previous estimates of only 30% availability of methionine in meat meals are erroneous. Further problems in the use of values for fluorodinitrobenzene-available lysine and *Streptococcus zymogenes*-available trytophan are discussed.

Introduction

No single test can provide all the information required about the protein quality of a test material since this is a function of the quantities of each of the essential amino acids that it provides. It has long been realised that meat meals are low in some essential amino acids and this has been related to raw materials used in their manufacture.[1] Previous reports have shown only small differences among the digestibility values of meat meal samples, which were generally similar to those of fishmeals. All values are high, both by *in vitro* and *in vivo* methods.[2–6]

The presence of amino acids in an unavailable form in meat meals has been reported.[5,7–10] On the basis of microbiological determination,[8,9] meat meals, on average, appeared to have 1·8 g/16 g N total and 0·6g/16 g N 'available' methionine indicating a high degree of damage to methionine during meat-meal processing. However, lower total and higher available methionine figures by chemical and chick-assay methods respectively, have been found.[7,11] The biological performance of meat meals has been correlated with their available amino acid content.[5,12–15]

In the present study both commercial meals and laboratory preparations have been used to investigate the protein quality of meat meals.

Experimental

Materials

Most of these have already been described in Part I of this series.[16] Ossein samples were prepared by removing the connective tissues, etc., from bone which was then degreased at a low temperature. The mineral matter was removed by treatment with dilute hydrochloric acid at a temperature below 10° and the material was then washed until acid-free and dried at a temperature not exceeding 40°. This material was given the code X824. Half of it was heated in tallow at 110° for 1½ h and then refluxed with light petroleum ether for an hour and the solvent was removed; the product was given the code X825.

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Evaluation procedures

Amino acid analyses

Chemical procedures.—Acid hydrolysates, prepared as for the determination of total lysine,[17] were analysed for amino acids on an accelerated-column chromatographic system after the basic method.[18]

For further materials, samples were oxidised with performic acid and then hydrolysed by the procedure used in 'Laboratory B' as previously described.[19] Cystine and methionine were determined as cysteic acid and methionine sulphone, respectively. Total tryptophan was determined after alkaline hydrolysis of the protein.[20]

Fluorodinitrobenzene (FDNB)-available lysine was determined directly[21] and also indirectly from total and 'residual' lysine values.[17]

Microbiological procedures.—For the determination of total methionine with *Streptococcus zymogenes* two methods of acid hydrolysis were used: a mild procedure[22] of autoclaving a sample containing ~ 100 mg N with 40 ml 2N-HCl for 5 h at 115° or a stronger procedure[23] using 3N-HCl for 18 h at 121°. Available methionine and tryptophan were also determined with *S. zymogenes*[22] using slightly modified conditions of pre-digestion with papain.[24]

Biological assays.—Available methionine was determined using White Link 10-day-old cockerels (Sterling Chicks Limited, Welwyn, Hertfordshire) fed on a methionine-deficient diet 'groundnut basal diet 2'.[7] Modifications were made in that arachis oil replaced partly-hydrogenated vegetable fat; a modified version of the 'salts N' mineral mixture[26] was used in which the level of calcium hydrogen orthophosphate was halved and calcium carbonate was omitted, these being replaced by steamed bone flour at a level of 4%. L-Methionine (Mann Research Laboratories, Inc., New York) was added to the basal for the standard diets, up to a level of 0·06%. Test materials were added at two levels to contribute either 1·13 and 2·26 or 2·26 and 3·75% crude protein to the diet depending on their microbiologically available methionine content as determined in preliminary assays. The additions were made at the expense of steamed bone flour (to equilibrate all diets to constant ash content) and maize starch.

The response, measured as efficiency of food conversion over 10 days, was analysed in relation to the dose level (% of diet) by the slope-ratio procedure.[26] The design of the assay allowed for tests of statistical and biological validity and, if these proved satisfactory, the available amino acid content was calculated from a comparison of the regression coefficients of the test and standard lines.

Available tryptophan was also determined by a generally similar chick-assay procedure. The basal diet consisted of (% of diet): oxidised casein (N.B.C. Ltd., Cleveland, Ohio, U.S.A.), 5·5; gelatin (B.D.H., Poole, Dorset), 17·8; L-histidine HCl, 0·12; L-cystine, 0·35; DL-methionine, 0·65; L-tyrosine, 0·40; L-threonine, 0·10; L-isoleucine, 0·10 (all amino acids from Koch-Light Laboratories Ltd., Colnbrook, Buckinghamshire); vitamin mix,[7] 1; mineral mix (modified 'salts N'[25]), 3·6; steamed bone flour, 5·9; arachis oil, 5; choline chloride, 0·3; oxytetracycline carrier (Pfizer Ltd., "TM-5" Brand), 0·07; ground oat husks, 5; and maize starch, *ad* 100.

This basal diet is prepared so as to be deficient only in tryptophan. L-Tryptophan (Mann Research Laboratories Inc., New York) was added at levels up to 0·14% for determination of a standard curve. Test materials were added at 3 and 6 or 6 and 12% crude protein levels at the expense of gelatin and steamed bone flour to keep all diets isonitrogenous and equal in ash content.

There were 2 cages (of 3 birds/cage) per treatment for experiments 120 and 122, and 4 for experiment 126. The experimental period was 10 days for experiment 120, and seven days for 122 and 126.

The data for efficiency of food conversion were analysed as described for the chick-available methionine assays.

Net protein utilisation (NPU)

NPU_{10} (carc.) values (abbreviated to *NPU* for the remainder of the paper) were obtained with rats as described in the preceding paper.[16] The diets were equilibrated to constant ash level with steamed bone flour so as to give calculated levels of 1·3% calcium and 0·6% phosphorus in each diet, except as indicated in Table II.

Digestibility for chicks

This was carried out by a previously reported method using chicks operated on so as to allow separate collection of urine and faeces[27] with modifications in that the salt mix was 'salts N'[25] but calcium hydrogen orthophosphate and calcium carbonate were left out. Calcium and phosphorus were contributed to the basal protein-free diet by a mixture of equal parts of steamed bone flour and calcium hydrogen orthophosphate. The test materials were added at the expense of steamed bone flour, calcium hydrogen orthophosphate, arachis oil and corn starch, so that the ash and lipid contents of all diets were the same. 'Chromium bread'[28] was added at a level of 1% to contribute 0·3% Cr_2O_3 to the diet (and not to 'contain 0·3%' as previously reported incorrectly.[27]) The birds were operated on at 8 weeks of age, with an experimental period of 7 days and a collection period of 4 days.

Results

Amino acid analyses

In Table I are shown the total amino acid analyses of some of the test materials obtained by column chromatography, together with typical values that have been published for comparable materials. Figures for sulphur amino acids in unoxidised hydrolysates are in parenthesis because they are considered unreliable; so are tyrosine and phenylalanine values obtained after preliminary oxidation. Chemically determined tryptophan values have been added to the table for completeness. Further lysine values obtained by short-column chromatography are set out in Table II. With the exception of meat meal X804, these show a tendency to be lower than the values obtained with the long columns.

From Table II it is seen that the total methionine values obtained with *S. zymogenes* using mild hydrolysates are in some cases considerably higher than corresponding chemical

TABLE I

Amino acid analyses of laboratory preparations and meat meals, g/16gN

Amino acids	Meat			Gut			Tendon			Ossein	Meat meal				
	X706	X823		X752	X831		X599	X832		X825	X804			Literature values[d]	
	Control[a]	Autoclaved & oven-dried[b]	Literature values[d]	Control[a]	Autoclaved & oven-dried[a]	Literature values (rumen)[d]	Control[a]	Autoclaved & oven-dried[b]	Literature values (collagen)[d]	Heated[b]	a	b	c	I	II
Lysine	8·5	7·7	8·0	8·3	5·6	6·4	3·1	3·5	4·0	4·0	4·4	4·7	4·6	5·7	5·7
Histidine	3·9	2·9	3·0	2·7	1·9	2·1	1·1	0·9	0·7	0·8	1·7	1·4	1·5	1·8	2·5
Arginine	6·0	5·9	8·0	7·8	6·2	7·2	7·6	8·4	8·0	8·8	6·1	6·5	6·5	6·8	6·9
Aspartic acid	9·0	9·6	...	9·8	7·8	8·2	5·5	6·7	...	6·9	6·5	8·4	7·4	7·6	7·4
Threonine	4·4	4·6	4·5	4·4	3·5	3·8	1·9	2·3	2·0	2·4	2·6	3·3	3·4	3·3	3·1
Serine	4·0	3·6	...	4·7	3·4	4·3	3·2	3·9	...	3·8	3·8	4·6	4·7	4·0	3·7
Glutamic acid	14·4	15·7	...	17·8	15·4	14·4	9·5	11·5	...	12·1	10·5	12·3	12·3	11·3	12·1
Proline	3·6	4·4	...	...	...	...	10·1	11·5	...	12·9	5·3	7·8	7·5	9·4	7·9
Glycine	4·0	6·2	...	10·9	10·2	10·5	19·6	22·3	...	27·3	9·9	13·2	13·5	13·7	12·0
Alanine	5·5	6·5	...	7·4	7·0	6·5	7·9	8·9	...	10·5	6·1	7·4	7·2	7·2	8·6
Cystine	1·2	(0·4)	1·1	...	0·6	1·3	0·3	(0·0)	0·1	(0·2)	1·2	(1·2)	(0·2)	1·2	1·3
Methionine	2·7	(2·7)	3·3	2·2	1·5	2·4	0·8	(0·7)	0·8	(0·7)	1·1	(0·2)	(1·3)	1·5	1·4
Valine	4·9	4·8	5·0	5·0	5·3	4·6	2·6	2·9	2·8	2·8	4·3	4·2	4·2	4·2	4·1
Isoleucine	5·0	4·4	5·0	4·1	3·6	3·7	1·6	1·8	1·7	1·6	2·9	2·8	2·8	3·1	2·9
Leucine	7·9	8·1	7·7	8·2	6·8	6·9	3·4	3·9	3·3	3·7	5·6	5·9	6·0	6·1	5·9
Tyrosine	...	3·5	4·0	...	...	3·2	...	1·1	1·0	1·2	...	2·2	2·4	2·4	2·3
Phenylalanine	...	4·0	4·0	...	...	3·6	...	2·4	2·5	2·5	...	3·3	3·4	3·3	3·1
Tryptophan	[1·3]	[1·1]	1·3	[0·9]	[0·9]	1·5	[0·1]	[0·1]	0·0	[0·1]	...	[0·7]	...	0·8	0·6

[a] Hydrolysis on material pre-oxidised with performic acid
[b] Straight-acid hydrolysis
[c] Straight-acid hydrolysis under N_2 in presence of thioglycollic acid
[d] Abstracted from the literature: for meat and collagen[29,30–32] for rumen[29] and for meat meals I (mean of samples 1,2,3,5,6,7,8 from Table 4 of the paper quoted)[22] and II (composite of 23 American samples with 53–55% protein content)[66]
[e] Determined colorimetrically after alkaline hydrolysis

TABLE II

Chemical composition and nutritive value of commercial samples of meat meal and fishmeal and of model materials

		NPU (rats)	Digestibility for chicks,[d] %	Methionine, g/16 g N				Lysine, g/16 g N			Tryptophan, g/16 g N		
				Total		Available		Total	Available		Total	Available	
				S. zymogenes[e]	Chemical	*S. zymogenes*	Chick	Chemical[f]	By difference	Direct (+ HO-lysine)	Chemical	*S. zymogenes*	Chick
Model materials:													
Meat : control[a]	X724	74	93(3)	2·9	2·5	2·9	2·8	8·4	8·0	8·4	1·24	1·15	1·46, 1·47
Meat : oven-dried	X819	78	95(3)	3·0	...	2·2	2·3	8·2	7·5	7·2	1·22	0·69	1·32
Meat : autoclaved & oven-dried	X823	32	70(2)	2·8	2·2	0·8	1·2	7·4	4·9	4·3	1·08	0·30	0·82
Gut : control[a]	X752	...75[b]	88(1)	2·7	2·2	2·2	3·1	6·5	6·1	6·5	0·92	0·91	1·05
Gut : oven-dried	X830	37	...	2·4	1·6	1·5	1·6	5·8	5·6	4·3	0·90	0·40	0·71
Gut : autoclaved & oven-dried	X751	...24[b]	79(1)	2·7	...	1·5	...	5·8	5·3	4·5	1·00	0·48	...
Gut : autoclaved & oven-dried	X831	17	...	2·5	1·5	0·9	1·2	5·1	3·8	3·4	0·91	0·22	0·57
Tendon : control[a]	X599	24	...	1·0	0·8	1·0	...	3·0	2·9	4·7	0·13	0·02	...
Tendon : oven-dried	X600	...	...	0·9	...	0·8	...	3·0	2·9	3·7	0·12	0·06	...
Tendon : autoclaved & oven-dried	X832	11	...	0·7	...	0·8	...	3·1	2·7	3·2	0·13	0·03	0·11
Ossein : control[a]	X824	8	...	0·9	...	0·8	...	4·1	3·9	4·3	0·08	...	...
Ossein : heated	X825	12	...	0·8	...	0·8	...	4·0	3·7	4·1	0·09	...	...
Mixture of oven-dried gut and oven-dried tendon (2·5 : 1 : : N : N)[g]		(28–34)	...	(1·9)	...	(1·3)	(1·1–1·4)	(5·0)	(4·8)	(4·0)	(0·67)	(0·35)	(0·52–0·54)
Meat meals													
	X746	31[c]	87(1)	1·9	1·2	1·0	0·9	4·8	4·2	4·4	0·66	0·28	...
	X747	37[c]	...	2·0	...	1·0	1·1	5·5	4·8	4·7	0·75	0·24	...
	X748	36;25[b]	81(1)	1·9	...	0·9	1·1	4·6	4·0	3·7	0·60	0·17	0·58
	X749	...24[b]	86(1)	1·9	...	1·1	1·2	4·6	4·0	4·0	0·64	0·24	...
	X750	...29[b]	85(1)	2·1	...	1·2	1·1	5·3	4·6	4·7	0·69	0·24	...
Blend of X748, 749 & 750	X804	35;36[c]	87(3)	1·9	1·1	0·9	1·1	5·1	4·5	4·2	0·66	0·24	0·62, 0·65
White fishmeal	X759	58	94(2)	3·3	...	2·6	3·2	7·0	6·7	7·0	1·05	0·69	...

[a] Control materials were freeze-dried. Meat (fillet) and gut were then ether-extracted
[b] These *NPU* values were obtained with normally-reared Wistar rats, the remainder with 'specific pathogen-free' Sprague–Dawley rats
[c] Obtained with a dietary level of 2·0% calcium and 0·9% phosphorus
[d] Number of individual determinations in parentheses
[e] Obtained with 'mild' acid-hydrolysates
[f] Obtained by short-column chromatography[17]
[g] These values have been calculated by simple proportion between the values for X830 and X600; where a value for X600 is missing a range is given based on the extremes for the same materials processed in different ways

values. Further results, from a collaborative study of four samples, are set out in Table III. It is seen that there is good agreement where the same procedure has been carried out in different laboratories. However, with the two meat-meal samples the mild conditions of hydrolysis have given consistently higher values with *S. zymogenes* than those found after stronger acid hydrolysis, whether the analysis has then been carried out with the same organism or completed by column chromatography.

Table II also includes values for FDNB-available lysine content obtained by two procedures which in some cases have given different results. Materials containing collagen have significant proportions of hydroxy-lysine which is measured, along with lysine, when the direct procedure is used.[21] Determination of total hydroxy-lysine by column chromatography has given the following values (g/16 g N): control meat (X806), 0; control tendon (X599), 1·4; meat meal (X804), 0·6. The last value is similar to a previously determined figure for meat meals.[34] Of the 0·6 g/16 g N present in X704, 0·4 was still 'unbound' (i.e. reacted with FDNB).[35] The level in tendon largely explains the finding of direct FDNB-available 'lysine + hydroxy-lysine' figures being higher than the total lysine figures. Clearly, the direct values for meat meals should be reduced by a correction factor for the presence of HO-lysine. However for the meals, and for heat-damaged meat and gut the direct values are mostly lower than those obtained by difference and there is no immediate explanation for this.

The total tryptophan results set out in Table II for the different model materials show clear-cut differences between muscle (from meat or gut) and tendon or ossein.

Protein quality and digestibility

Three *NPU* experiments were carried out and the estimated standard error of each mean value obtained ranged from 1·4 to 3·6 in these experiments (Table II.) Meat meals X746 and X747, because of their high ash content, had to be assayed in diets containing altogether 2·0% calcium. Meat meal X.804 was also assayed at this level of calcium and gave a value of 36 which was not different from the value of 35 obtained with the level of 1·3% calcium used generally in this series of assays. Where the same material was assayed in two different experiments, the difference in result ranged from 1 to 11 units. None of these differences quite reached statistical significance and there was no obvious influence of the type of rat on the results obtained.

The meat meals all gave *NPU* values that were not significantly different from 30. Freeze-dried meat and gut gave very much higher values, but autoclaving and oven-drying reduced these to the level of meat meals. Oven-drying alone apparently damaged the gut severely, but had little effect on the meat. Even the carefully prepared tendon and ossein samples were of low quality.

The digestibility determinations with chicks were carried out without mishap and the results are summarised in Table II. Chicks receiving the basal, protein-free diet had a mean excretion of 0·24 g N/100 g diet eaten. This value was used as an estimate of metabolic N and was subtracted from the corresponding N excretion of chicks receiving the same basal diet supplemented with protein sources so as to provide the estimates of the 'true' digestibility of each protein source. From statistical analysis of the results with the three materials that were each determined with three chicks, it is estimated that the S.D. of a single value is $\pm$ 2·1. It is seen that the digestibility of the freeze-dried meat and gut is close to 90%. Autoclaving and oven-drying has reduced it to below 80%. The commercial meat-meal samples have given intermediate values of approximately 85%. Although these are still

reasonably high they are significantly less than the value of 94% obtained with the sample of fishmeal.

Chick growth assays

The assay used for available tryptophan has not been reported previously; a typical response to the standard and to two test materials is shown diagrammatically in Fig. 1. Almost without exception, statistical analysis indicated that the results were valid and the mean values are summarised in Table II. The standard error of the estimates ranged from $\pm$ 0·06 to $\pm$ 0·13 acccording to the level at which the proteins were included in the diet. With the freeze-dried samples (X724 and X752) the values are actually higher by approximately 15% than the corresponding 'total' values determined chemically, though the difference is not statistically significant. With the two commercial meals assayed, the values corresponded to the 'total' tryptophan values, and only for the deliberately damaged samples (i.e. autoclaved and then oven-dried) were they lower. The microbiological estimates of available tryptophan were lower than those obtained by chick assay throughout the series.

The chick assay for methionine gave satisfactory responses similar to those already reported.[7] The standard errors of the values set out in Table II ranged from $\sim$ 0·1 to $\pm$ 0·5 g/16 g N. The samples expected to be of highest value were added to the test diets at lower levels and the estimates for these, therefore, had the highest standard errors. There is generally good agreement between these values and the corresponding microbiological values for available methionine with all types of sample, whether freeze-dried or heat-processed.

Discussion

In the first paper of this series,[16] no evidence that the poor performance of animals receiving meat meals was due to growth-depressant factors was found.

Using a standard method of protein quality evaluation, the *NPU* values for all of the series of five commercial meat meals were around 30. These are in agreement with previously published results,[5,36–38] obtained by carcass analysis and are approximately one-half of the value found with a white fishmeal sample, which is also in agreement with literature values.[5,39] Where high *NPU* values for meat meals have been reported,[4] these were obtained using the balance technique which has been found in several laboratories to give higher values.[11,40] The present authors' values would also have been higher by 3–5 units[41] if the original calculation for the *NPU* (carcass) procedure had been used instead of the revised formula.[42]

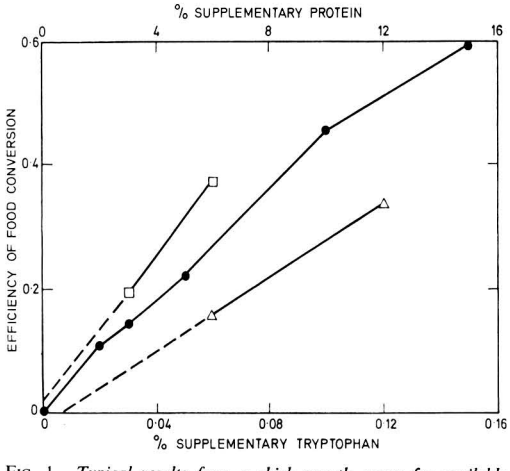

FIG. 1. *Typical results from a chick-growth assay for available tryptophan according to the supplements fed*

● Tryptophan; □ Control meat protein (X724); △ Meat-meal protein (X804)

Meat and gut tested in the same series of experiments have given high values, as has been previously reported.[43,44] These materials can, however, be damaged by heat to give values similar to those of meat meal. Tendon and ossein have given *NPU* values lower than those found for meat meals, even when dried so as to minimise heat damage. It is of interest, therefore, to consider how far the value of meat meals is due to higher quality raw material being heat damaged and how far to a large proportion of raw material being of initially low quality (e.g. tendon and ossein).

Indigestibility can only be a small contributory factor to the low protein quality of meat meals. The digestibility figures of 81–87% and 94% found here for meat and fish meals respectively (Table II) are in accord with previously published values,[2–5,11,45] except that one author[65] has obtained lower values of 69 (meat and bone meal) and 89 (white fishmeal) with hens. Freeze-dried meat and gut were found to have high digestibility for the chick but on autoclaving and oven-drying this was reduced to below the average value for meat meals.

An estimate of the overall value of a protein for rats can be made by comparing its essential amino acid composition with the ideal pattern for the rat.[46] The value for each amino acid is calculated as a percentage of the ideal and the lowest, i.e. that for the most limiting amino acid is taken and termed the 'chemical score'. Leaving problems of accurate amino acid analysis aside for the moment, the chemical analysis given in Table I for freeze-dried meat (sample X706) indicates that 'methionine + cystine' at 3·9 g/16 g N, would be expected to be the limiting factor. If 93% is taken as an estimate of digestibility of such material (i.e. the value obtained with chicks for X724), this gives an estimated supply of 3·6 g digestible sulphur amino acids/16 g N fed, which is approximately 77% of the estimated requirement, 4·7 g/16 g N.[46] This value of 77 is in good agreement with the actual *NPU* value of 74.

Repeating the calculation with meat meal X804, using the data set out in Table I, methionine appears to be limiting. With 1·1 g/16 g N corrected for an estimated digestibility of 87%, and compared with the standard of 2·7 g/16 g N, this gives a score of 35, which again agrees with the determined *NPU* value. The nutritional inferiority of the meat meals as a sole protein source for rats to that of freeze-dried meat (fillet) does seem therefore to be explained in terms of their large differences in amino acid composition, together with a small correction for the slightly lower overall digestibility of the nitrogen in the meat meal. However, the 'scoring' system does not explain why supplementary methionine fails to improve the *NPU* of meat meals.[16,36]

The mean methionine value for the two meat meals assayed with chicks (X746 & 804) was 1·0 g/16 g N (Table III), a value confirmed in further assays.[47] This is again in agreement with the mean of the chemically determined values (1·15 g/16 g N) multiplied by the percentage of digestibility, i.e. 87%.

The results of the chick-growth assays for tryptophan appear reasonable except that the estimates for freeze-dried

TABLE III

Methionine levels (g/16 g N) in test materials as analysed or assayed by different procedures

		Total				Available	
		Chemical		*Streptococcus zymogenes*		*Streptococcus zymogenes*	Chick
		Prior performic oxidation	Hydrolysis under nitrogen	Strong acid hydrolysis	Mild acid hydrolysis		
Meat: control	X724	2·5[a]	—	2·6, 2·7[e]	3·0, 2·7[d]	2·9, 2·7[d], 2·9[e]	2·9
Meat: autoclaved and oven-dried	X823	2·2[a]	—	2·8, 2·4[e]	2·8, 3·0[d]	0·8, 0·8[d], 1·1[e]	0·7
Commercial meat meal	X746	1·2[a]	—	1·3[e]	1·9	1·0	0·9
Commercial meat meal	X804	1·1[a], 1·1[b]	1·3[c]	1·2, 1·3[e]	1·8, 1·7[d]	0·9, 0·75[d], 0·9[e]	1·0, 0·95[d]

[a] Determined in Laboratory 'S'
[b] " " " 'R'
[c] " " " 'O'
[d] Determined in laboratory 'A'
[e] " " " 'N'

meat and gut are approximately 15% above the 'total' chemical values. A similar unsolved problem has been encountered in this laboratory with chick-growth assays for lysine.[48] For the two meat meals assayed (X748 & 804) the chick values are a little below the values obtained by chemical analysis. If they were reduced by 15% (as a correction factor, based on the apparent over-estimation of freeze-dried materials), the result would again fit with the 81 and 87% overall protein digestibility figures for these meals. In any case, there is no evidence of tryptophan being less available than would be expected from the 13–19% indigestibility of the total nitrogen. Other workers[49] using a rat-growth assay found that the tryptophan in raw beef-rib muscle was almost completely available and that the value was not significantly reduced by roasting.

In the case of lysine there are no chick or other biological assay results for guidance. However, the mean 'FDNB-available' value measured by the 'difference' procedure[17] for the six meals is 4·35 g/16 g N, which corresponds to 86% of the corresponding mean value for total lysine. The values obtained by the direct FDNB-available lysine procedure[21] have a mean of 4·3 g/16 g N which is in fair agreement with published[5,50,51] values; this lower value is, however, surprising since one would expect to have to subtract the contribution of biologically inactive hydroxy-lysine[21] (0·4 g/16 g N[35]) from these values, i.e. to give 3·9 g/16 g N before obtaining agreement with the results of the 'difference' procedure. It is hoped to consider this discrepancy, which has already been noticed to occur with damaged materials, in detail, in a later paper. In the meantime a biological assay of X804 (since re-coded MM 101) for lysines using rats, has given a value of 4·2 g/16 g N.[57] The indication is, therefore, that the availability of the lysine in the composite meat meal is 80–90% according to which 'total' value is taken, i.e. that obtained with long columns (Table I) or with short columns (Table II).

Evidence from 'model' materials

As already mentioned, it has been found that both meat fillet (mainly striated muscle) and gut (mainly smooth muscle) are of high nutritive value when carefully dried, i.e. they give *NPU* values of over 70.

'Oven-drying' the meat (X819) by keeping the mince in a forced-draught oven at 90° for 44 h, had no drastic effect on nutritive value, though the same treatment of gut (X830) almost halved its value both for rats and chicks. Possibly the greater sensitivity of the gut can be explained by the presence of some carbohydrates, (despite its having been washed out) which could be responsible for Maillard reactions.

With a more drastic heat-treatment (i.e. with autoclaving at 110° for 5 h as a preliminary to drying in an oven set at 160° for 5 h) both materials were severely damaged and gave *NPU* values under 35, i.e. no more than those obtained with commercial meat meals. Nevertheless there seem to be the following reasons for believing that this degree of damage is considerably greater than that occurring in commercial processing:

1. The 'total' values for methionine, lysine and tryptophan are all considerably higher in the heat-damaged meat or gut samples (X823, 751 and 831) than in commercial meals, although the nutritive value is similar.
2. The overall digestibility of N in the deliberately heat-damaged samples is lower.
3. The ratio of 'chick available' to 'chemical total' figures for methionine or tryptophan range from 55–76%, whereas the corresponding figures for meat meals (as has been discussed already) are all 85% or more.

The non-muscle models, i.e. tendon and ossein are closely similar to each other but show quite different amino acid patterns from those of meat or gut. This is in line with other values already published and summarised in Table I. The low tryptophan values limit the chemical score of tendon and ossein to 13 and 8 respectively. Since the rat can apparently re-cycle some endogenous tryptophan for maintenance,[36] the low levels of 'methionine and cystine' may be equally limiting. In any case the low, determined *NPU* values are fully explained.

The total methionine and tryptophan levels in these two materials are, of course, markedly, lower than in meat meals.

None of the present authors' calculations of the composition of hypothetical mixtures of model materials has given a complete fit with that of a commercial meal. The hypothetical composition of one combination, a 2·5 : 1 (N : N) mix of oven-dried gut (X830) and oven-dried tendon (X600) which shows points of resemblance to commercial meals, is set out in Table II for ease of comparison. No allowance has been made for any interactions which might have occurred if such a mixture had actually been dried, nor for the small mutual supplementation in *NPU* that might occur between gut (calculated to be first limiting in methionine) and tendon (first limited in tryptophan).

The proportions of different tissues taken for meat-meal production fluctuate from one charge to another. Further, the division of the total by-products of the meat industry into meat-and-bone meal, blood meal etc. as well as into meat meal, invalidates any theoretical calculation based on the division of a whole carcass between food and animal feedingstuffs. However, if on the one hand muscle (including gut), and on the other non-muscle (i.e. tendon and bone proteins) are taken, a nitrogen ratio of between 2 : 1 and 3 : 1 does seem consistent with observations at the factories where the present samples were obtained.[16,41] It is also resonably consistent with the observed ash and hydroxy-lysine contents of our commercial meals.

If the ratio of connective tissue to muscle protein were a major factor in determining the nutritive value of meat meals one might expect the level of hydroxy-proline (only found in connective tissue), or the ratio of hydroxy-proline to tryptophan[52] to be indicators of quality, but they have proved disappointing[41] in relation to the results of feeding tests obtained with collaborative samples.[5]

Application of microbiological assays

The highest total lysine value obtained for any of the present meat meals (5·5 g/16 g N for X747, using a short column) is considerably below values listed in some of the older compilations of amino acid values. For example, U.S. National Research Council publications[54] list for 'meat meal' (code ref. 5–00–385) a mean crude protein (N × 6·25) content of 53·4%, and a mean lysine content of 3·80% which corresponds to 7·1 g lysine/16 g N. The mean lysine value of 3·8% is seen, from an earlier publication,[55] to include individual values ranging from 2·5 to 8·3%. The higher values almost certainly arise from early microbiological assays,[12] and it is now realised that the hydroxy-lysine present is, at any rate, one component which can cause considerable stimulation of the micro-organisms used for assay.[34] A value of 9·9 g/16 g N in the British literature[56] was also subsequently reported[57] to be the result of a miscalculation. There is general agreement amongst workers who have used column chromatographic procedures, as is illustrated in Table II, that lower values of the kind reported here are applicable to a wide range of meat meals[33,58] and it seems reasonable to regard the earlier values as being in error, rather than as truly representing the composition of a superior type of product.

In the present experiments all the microbiological assay work has been carried out with the proteolytic organism *Streptococcus zymogenes*. The 'available' methionine assays, carried out with papain digests of the test materials, have given results that agree well with those from the chick assays, which is in agreement with earlier findings in this laboratory.[7] The procedure is, therefore, extremely useful for relatively rapid evaluation of materials of this type. It is, however, important to standardise the conditions of pre-digestion with papain.[7]

In contrast, it appears that the microbiological assay for total methionine, as carried out by the original procedure[22] may have given the present authors, and others, misleadingly high results in some cases. Thus, the value for X804 was 1·8 g/16 g N as compared with chemical values (obtained in three different laboratories) of 1·1–1·3 g/16 g N. When the microbiological assays were carried out with hydrolysates prepared under stronger conditions,[23] so as to cause complete hydrolysis, they also gave values of 1·2–1·3 g/16 g N.

Similar comparisons can be drawn from the literature where several laboratories have worked on the same series of samples of protein concentrates.[59] Table IV summarises these for two meat meals on which most work has been done and on the two 'extreme' samples from a series of whale meals. Thus, some, but not all,[60] workers have obtained

TABLE IV

Literature values for 'available' and 'total' methionine (g/16 g/N) in four protein concentrates

	Meat meals				Whale meat meals			
	MM 10		MM 16		WM 7		WM 13	
	$\frac{\text{available}}{\text{total}}$	= availability	$\frac{\text{available}}{\text{total}}$	= availability	$\frac{\text{available}}{\text{total}}$	= availability	$\frac{\text{available}}{\text{total}}$	= availability
S. zymogenes (Papain digest/ mild hydrolysate):								
Ford[22]	1·0/1·1	= 91%	0·6/1·0	= 60%	0·8/2·2	= 36%	2·5/3·0	= 83%
Waterworth[8]	0·48/1·7[a]	= 28%	0·32/1·5[a]	= 21%	0·4/2·5[a]	= 16%	2·3/3·1[a]	= 74%
Miller *et al.*[7]	0·9/2·0	= 45%	0·5/1·6	= 31%	1·0/2·8	= 36%	2·8/3·5	= 80%
Boyne *et al.*[9], giving a range of values from five laboratories[b]	0·6 (0·5–0·8)/ 1·8 (1·5–2·0)	= 33%	0·4 (0·3–0·5)/ 1·5 (1·2–1·6)	= 27%	0·5 (0·4–0·6)/ 2·6 (2·4–2·8)	= 19%	2·2 (1·7–2·5)/ 3·1 (2·8–3·5)	= 71%
'Chick' (available/'*chemical*' total[c])								
Miller *et al.*[7]	0·9/1·3[d]	= 69%	0·8/1·05	= 76%	1·2/2·2	= 55%	2·4/2·55	= 94%
Guttridge & Lewis[10]	—		0·6/1·0	= 60%	—		—	

[a] Intermediate hydrolysis conditions, using 3N-HCl at 121°C for 9 h

[b] The 'available' figures shown are from Table 2 of the paper;[9] further results using a stronger papain (Table 5) have given generally similar results. The hydrolysis conditions for estimating 'total' methionine may have varied between laboratories

[c] Mean results from the two chemical methods used (chromatography of oxidised hydrolysate and iodometric titration)

[d] Another laboratory also obtained the same value by a chemical procedure[36]

values by microbiological assay which correspond to a very low 'percentage availability' of methionine in the meat meals, whereas the corresponding estimate from comparison of chick assays with chemical analyses is much higher. Although low 'percentage availabilities', approximately 30%, were still found in the most recent work involving five laboratories[9] and using nine meat meals, it is doubtful whether such results represent a true indication of the degree of damage that occurs during the processing of meat meals. In some instances the available figures may be low because of inadequate pre-digestion with papain but the present authors believe, in particular, that with meat meals the partial hydrolysates (produced with 2 N-HCl at 115° for 5 h) used to give 'total' methionine values, may contain material with an unexplained stimulatory effect on the growth of *Streptococcus zymogenes*, as has already been discussed in connexion with the analysis of casein;[24] the original author is in agreement[61] and now uses stronger hydrolysis conditions.[23] The present authors feel driven to this view although the hypothetical stimulant can hardly be an ordinary peptide since the organism has a strong proteolytic power and, in any case, is not apparently stimulated unduly either by mild acid hydrolysates of pure meat or by papain digests of meat meals.

Turning to the results for microbiological assay for available tryptophan the present authors' mean value of 0·23 is the same as the mean of four published values in one series[22] and slightly higher than the mean, 0·17 g/16 g N from a further laboratory[8] for nine samples (with omission of two samples, MM3 and 5 made from 'stick liquor'[62] that contained over 85% protein[59]). Nevertheless, these values are very much lower than those of approximately 0·6 g/16 g N given in the chick growth assays of the present authors. One further assay carried out with a different basal diet[63] has given a value of 0·5 g/16 g N.[47] The present authors have seen no value for meat meal in the literature except one of 0·5 g/16 g N for a sample of meat- and bone-meal,[64] whose protein quality tends to be lower than that of meat meal.

It does seem therefore that, although *S. zymogenes* values give a useful indication of the relative potencies of samples of the type investigated here, the absolute values for commercial meat meals are probably much too low. The results of a recent collaborative investigation confirm that, while the ranking of samples is consistent in different laboratories, absolute values differ.[61] It would be premature to use such values for available tryptophan for the formulation of balanced animal diets.

Acknowledgments

The authors thank Dr. R. C. Campbell for assistance with the slope-ratio analyses, Dr. D. W. T. Crompton for assistance with the chick operations and Mr. A. W. Hartley, Central Laboratories, Spillers Ltd., Cambridge; Dr. G. M. Ellinger, Rowett Research Institute, Aberdeen; Dr. J. E. Ford, National Institute for Research in Dairying, Reading; Dr. V. H. Booth and Mr. G. Koreleski for collaboration with various aspects of the amino acid analysis. The authors are grateful to Dr. D. R. Ashworth, B.G.C. (Marketing) Limited, London, W.C.1 for the gift of the ossein samples and to Mr. J. G. Corrie, Robert Wilson & Sons (Ulster) Ltd., Belfast for information about the meat meal industry. One of the authors (J.A.) is indebted to the Pig Industry Development Authority, London, for a grant to carry out the work.

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Received 11 February, 1970

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NUTRITIVE VALUE OF MEAT MEALS

III.*—Value of meat meals as supplements to a cereal basal diet: limiting amino acids in these diets

By J. ATKINSON† and K. J. CARPENTER

When fed to rats and pigs as supplements to a cereal-based diet, meat meals were of significantly lower quality than fishmeal. The order of amino acid limitation in the meat meal-plus-cereal diet was first lysine and equal second methionine and threonine. Supplementation of meat meal with these three amino acids gave performance approximately equal to that obtained with fishmeal. Results obtained with the two species were very similar.

Introduction

Several authors have reported the results of experiments on the feeding of meat meals in commercial-type rations to pigs (c.f. references in Table IV below); it has generally proved to be a poorer supplement to the basal diet than the other protein concentrates tested. In the Parts I and II[1,2] it has been shown that the low value of meat meals may be due to their low available amino acid content. In this paper, experiments with rats and pigs, given diets of a form used commercially in the U.K., i.e. based on barley and wheat middlings, are described. These experiments were designed to investigate the relative nutritive value of meat meal and fish meal in a cereal-based diet, and how the value of a meat meal-plus-cereal diet could be improved by amino acid supplementation.

The amino acid composition of meat meal X804, as reported previously,[2] was taken and it was assumed that 50% of the essential amino acids were present in an available form except for lysine, methionine and tryptophan, for which actually determined chemical and microbiological 'available' figures were taken. The previously determined amino acid composition of white fishmeal, X759, was taken[2] and 80% amino acid availability was assumed. The composition of the cereals used was estimated from consideration of published analyses[3–6] and the amino acids were all assumed to be 80% available, which is the average availability figure reported for wheat.[7]

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Experimental

Experiment with rats

Male, Sprague–Dawley, 'barrier-maintained', 'specific pathogen-free' weanling rats[8] of 40–45 g body weight were kept on a cereal-based preliminary diet for 4 days. After this the rats were weighed and those of middle weight selected, stratified and randomised onto experimental diets, 4 individually caged rats per treatment. The rats were kept for 14 days on the test diets, the basal form of which consisted of barley meal, 67; wheat middlings, 12; arachis oil, 5; steamed bone flour, 3·5; vitamin mix 1B,[1] 1; mineral mix 1D[1], 2·1; and maize starch *ad* 100.

The meat meal (X804) or white fishmeal (X759) were each added at the expense of steamed bone flour, arachis oil and maize starch, so as to contribute 6% crude protein without changing the ash and fat levels.

The further amino acid additions are shown in Table I. All the essential amino acids used were of a 'chromatographically homogeneous' grade (Koch-Light Laboratories Limited, Buckinghamshire) and amino acid additions were equilibrated to an equimolecular level with L-glutamic acid. It was the intention to choose a level of each that would bring the available amino acid content of the diet up to the full requirement of the rat.[9]

Experiments with pigs

Experiment 1

10 sets of litter-mates each of three males and three females of Welsh × Wessex pigs, weighing 35–40 lb each on arrival,

were fed *ad libitum* for six days on a cereal-based preliminary diet. Each sex was divided into three strata according to weight and performance during the preliminary period, and randomised within strata into 10 groups, so that each contained three males and three females. One group was eliminated and two of the remaining nine groups (each group in a single pen) were randomly allotted to each of the supplemented diets and the remaining group to the basal diet.

The basal diet consisted of steamed bone flour, 3·0; maize starch, 7·2; barley meal, 77; wheat middlings, 12·0; and 'vitamin–mineral–antibiotic' mix, 0·37. Meat- or white fishmeal were added to this at the expense of maize starch and steamed bone flour so as to add 6% crude protein to the diet, without changing the ash content. The cereal contribution of protein to the diet, as in the previous rat experiment described, was 9%. The origin of the three meat meals has been described in a previous paper.

The 'vitamin–mineral–antibiotic' mix contained (% of mix): Rovimix A-50 (Roche Products Ltd., London: 50,000 I.U. vitamin A/g), 1·72; Rovimix D_3-100 (100,000 I.U. vitamin D_3/g), 0·05; Rovimix E-10% (10% α-tocopheryl acetate), 3·97; riboflavin, 0·04; pantothenic acid, 0·19; basic $ZnCO_3$, 4·44; ferric nitrate $\cdot 5H_2O$, 7·12; $MnSO_4.H_2O$, 2·52; NaCl (feeding grade), 59·93; antibiotic supplement 'TM-5' (Pfizer Ltd., Sandwich, Kent; 5 g oxytetracycline HCl/lb), 26·47.

The diets were acceptable to the pigs and spillage was low, although the pigs were fed *ad libitum*; the experiment was run for 37 days, until the pigs reached approximately 80 lb liveweight.

Experiment 2

Five sets of litter-mates each containing 3 male and 3 female Large White × Essex weanling pigs of 30–40 lb liveweight on arrival, were fed on a cereal-plus 'mixed animal protein' diet for 5 days. They were then stratified and randomised onto test diets; The pigs were kept in the same litter group as in the preliminary period but were fed individually on a modified *ad libitum* system[10] with each group member receiving a different test diet. The randomisation was also restricted so that there were either 2 or 3 of each sex receiving each dietary treatment. In the statistical analysis of the results, means were adjusted for the differences in proportion of the two sexes.

The basal diet consisted of barley meal, 83; steamed bone flour, 2·8; vitamin–mineral–antibiotic mix (as for Expt. 1), 0·37; and the maize starch, *ad* 100. Meat meal (X804) or white fishmeal (Provimi Brand, X807) were added to contribute 6% crude protein at the expense of maize starch and steamed bone flour, to keep the diets equal in ash content. Meat meal (X804) consisted of a mixture of equal parts of the three meals used in Expt. 1. The barley meal contributed 9% crude protein to the diet. 'Feeding grade' amino acids (97% purity, A.E.C. Ltd., Commentry, France) were added in combination with meat meal in three further treatments. The levels added (% of diet) were DL-methionine, 0·14; L-lysine HCl, 0·25; and DL-threonine, 0·24.

The basis of the calculation of amino acid additions was similar to that used in the rat experiment except that each supplement was the amount of the particular amino acid required to bring the level to that estimated to be present in available form in the white fishmeal-plus-cereal diet. It was assumed that the D stereoisomer was fully active in methionine, but inactive in threonine.

The experiment was run for 35 days, from 40 lb liveweight to a mean finishing weight of 80 lb approximately.

Results

The results of the rat experiment are summarised in Table I. The meat meal (X804) has proved inferior to fishmeal as a supplementary protein source for rats under the present conditions. Calculating the response to each protein supplement as food conversion efficiency (*FCE*) on test diet minus *FCE* on basal diet', it can be seen that the response to meat meal is approximately 50% of that to fishmeal. This is a similar proportion to that found for the *NPU* (net protein utilisation) of meatmeal and fishmeal as sole proteins.[2]

Addition of the three amino acids lysine, methionine and threonine, to the meat-meal diet has brought its value up to that of the white fishmeal diet. Further addition of valine, phenylalanine, tryptophan and leucine gave a small additional response which took the value above that of the white fish-

TABLE I

Food conversion efficiency of rats on cereal diets supplemented with either fishmeal or meat meal (+ amino acids)

Essential amino acids[a]	None	White fishmeal (X759)		Meat meal (X804)								
Threonine	—	—	+	—	+	+	—	+	+	+	+	+
Methionine	—	—	+	—	+	—	+	+	+	+	+	+
Lysine	—	—	+	—	—	+	+	+	+	+	+	+
Valine	—	—	+	—	—	—	—	—	+	+	+	+
Phenylalanine	—	—	+	—	—	—	—	—	—	+	+	+
Tryptophan	—	—	+	—	—	—	—	—	—	—	+	+
Isoleucine	—	—	—	—	—	—	—	—	—	+	—	+
Leucine	—	—	—	—	—	—	—	—	—	—	+	+
Histidine	—	—	—	—	—	—	—	—	—	—	—	+
Glutamic acid, %	—	2·06	1·18	2·06	1·57	1·32	1·30	1·10	0·79	0·34	0·51	—
Food conversion efficiency[b]	0·217	0·445	0·475	0·343	0·340	0·382	0·372	0·446	0·444	0·429	0·469	0·469

[a] Essential amino acids were added as supplements to the meat meal diet at the following levels (with the corresponding additions to the fishmeal diet in parenthesis): L-threonine, 0·21 (0·11); L-methionine, 0·22 (0·12); L-lysine HCl 0·58 (0·40); L-valine, 0·25 (0·08); DL-isoleucine, 0·33; L-phenylalanine, 0·30 (0·20); L-tryptophan, 0·06 (0·02); L-leucine, 0·21 and L-histidine HCl, 0·08

[b] Estimated standard error of each value is ±0·009

meal diet. However, addition of amino acids to the white fishmeal diet also increased its value. The first limiting amino acid for rats in the meat meal-plus-cereal diet seems to be lysine, followed by methionine and threonine (equally second).

The results of pig experiments 1 and 2 are shown in Table II. In pig experiment 1, the difference between the meat meals were small and not significant. The proportional responses to meat meal or fishmeal as supplements to cereals were similar to those found in the rat experiment. This was confirmed in pig experiment 2, and the order of amino acid limitation in the meat meal-plus-cereal diet was the same for pigs as for rats, although the response to threonine was not statistically significant.

The relative responses on similar diets for pig experiments 1 and 2 and for the rat experiment are set out in Table III and the correlation coefficient between the results of the rat experiment and pig experiment 2 was calculated to be $r = +0{\cdot}86$ ($P < 0{\cdot}001$).

Discussion

The results reported here are compared with other published experiments in Table IV. For pigs reared to a greater weight than in experiments described here only small differences between meat meal and fishmeal as supplements to cereal diets have been reported.[15,16] It seems that where experiments have been conducted over the weight range of maximum requirements and the test-protein level has been critical, then a difference has always been found between the value of meat meal and that of fishmeal as sources of supplementary protein.

In experiments described here and previously,[2] similarity between meat meals of widely varying protein contents has been seen. This is in agreement with previous reports.[17]

Limiting amino acids in cereal diets have been investigated by many workers. The order of limitation of amino acids in pearl barley for the rat was found to be lysine, threonine and methionine.[18] Extensive work in Czechoslovakia showed lysine to be first limiting and threonine second in a barley-only

TABLE II

Mean performance of pigs fed cereal-based diets, supplemented with fishmeal or meat meal (+ amino acids)

Dietary supplement	Expt. 1		Expt. 2	
	Wt. gain, g/pig/day	*FCE*	Wt. gain, g/pig/day	*FCE*
None	230	0·22*	218	0·21
White fish meal	518	0·34	538	0·36
Meat meal (X748)	419	0·30	...	...
Meat meal (X749)	409	0·27	...	...
Meat meal (X750)	404	0·27	...	...
Meat meal (X804)	...	...	352	0·29
Meat meal (X804) + L, T†	...	...	390	0·33
Meat meal (X804) + L, M	...	...	500	0·33
Meat meal (X804) + L, T, M	...	...	488	0·35

* Estimated standard error of each *FCE* value is ±0·01, with the exception of that for the unsupplemented treatment in Expt. 1 which was not replicated

† L, lysine; T, threonine; M, methionine

TABLE III

Comparison of mean performance of rats and pigs fed similar diets (food conversion efficiency)

Supplement		*FCE* of test diets as % of *FCE* of fishmeal diet			Response to test supplement as % of response to fishmeal*		
		Rat	Pig expt. 1	Pig expt. 2	Rat	Pig expt. 1	Pig expt. 2
None (Cereal only)		49	65	58	0	0	0
Meat meals	X748	—	87	—	—	67	—
	X749	—	80	—	—	42	—
	X750	—	80	—	—	42	—
	X804	76	—	81	52	—	53
Meat meals (X804) plus amino acid	L,T†	84	—	92	70	—	73
	L,M	84	—	92	70	—	73
	L,M,T	100	—	97	100	—	87

* Calculated as $\frac{FCE \text{ (test diet)} - FCE \text{ (basal)}}{FCE \text{ (fishmeal diet)} - FCE \text{ (basal)}} \times 100$

† L, lysine; T, threonine; M, methionine

TABLE IV

Value of meat meals for pigs in present experiments and in published papers

Reference	Weight range of pigs, lb	% protein from basal diet (and type)	% protein from meat meal in test diet	% protein from positive control (and type)	*FCE* on meat meal diet as % of *FCE* in control diet
11.	33–75	7 (maize)	10	11 (soya)	72
12.	36–100	8 (maize)	10	10 (soya)	71
13.	48–100	11 (sorghum)	7·8	6·4 (fishmeal)	79
14.	46–100	9 (maize)	8·0	6·7 (fishmeal)	71
Present work	45–80	9 (barley and wheat)	6·0	6·0 (fishmeal)	82
15.	53–130	9 (maize and barley)	7·0	7·5 (fishmeal)	87
16.	60–230	11 (soya and barley)	3·0	3·0 (fishmeal)	94

diet for fattening pigs.[19] Lysine and methionine were found to be limiting in other barley-based diets for pigs.[20] On feeding meat meals as supplements to maize-based diets for pigs, American workers found tryptophan to be the first limiting amino acid.[11,12,21–23] Lysine was found to be the first limiting amino acid in a wheat-plus-meat meal diet for poultry.[24] When meat meals were tested by a method in which the cereals contributed 8% protein and the test supplement 3% and also by a second method in which each contributed 6·5% protein, it was found that in the first type of chick experiment, there was a good response to lysine only, whereas lysine and methionine were the joint limiting factors in the second experiment.[25]

The effect of the type of basal protein on the order of amino acid limitation in a cereal-plus-meat meal diet is well seen here, in that the order is essentially the same as in a barley-only-diet as reported above and found in this laboratory.[26] In meat meal-plus-maize diets the order of amino acid limitation is different to the one found here but is the same as that found in maize-only diets.

On supplementation of a meat meal-plus cereal-diet with amino acids, growth equal to that on a white fish meal-plus-cereal diet has been obtained. Thus there is no evidence of growth depressant factors when meat meals are fed to rats and pigs in cereal-based diets.

Acknowledgments

The authors thank Mr. O. Fabb for his care of the experimental pigs. One of the authors (J. A.) is indebted to the Pig Industry Development Authority, London for a grant to carry out the work.

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Received 6 March, 1970

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STUDIES ON THE LIPIDS OF FLOUR
V.*—Effect of air on lipid binding

By N. W. R. DANIELS, P. S. WOOD, P. W. RUSSELL EGGITT and J. B. M. COPPOCK

Lipid distribution in mechanically developed doughs was found to be sensitive to relatively small amounts of air present in the dough mixer atmosphere. In doughs mixed to high work levels, less than 5% air present in the nitrogen–air mixture fed into the mixing bowl was sufficient to cause a significant decrease in bound lipid. Although flour pigments were readily bleached by small amounts of air, lipid peroxides did not increase significantly until at least 50% air was present. Polyunsaturated free fatty acids were most susceptible to peroxidation although, at high work levels in air, peroxides were found in all lipid classes. While lipids bound during nitrogen mixing were readily released by subsequent mixing in air, the effect of air on lipid binding was not reversed by further mixing in nitrogen. Addition of peroxidised lipid to the dough did not prevent lipid binding in nitrogen-mixed doughs and it was concluded that a mechanism of lipoxidase-coupled site oxidation was responsible for the effect of air on lipid binding rather than the direct action of the lipid peroxides themselves.

Introduction

During dough mixing and baking, lipids are known to be involved with other dough constituents in interactions that are believed to be important in breadmaking.[1] The mere addition of water to flour results in a decrease in readily extractable lipid[2] even when mechanical work is rigorously excluded.[3] Lipid binding is further increased during dough mixing to an extent that depends on the rate of dough development used.[4]

Recently, lipid binding was found to be influenced by the atmosphere in the dough mixing chamber,[5] an effect now confirmed in doughs mixed to near optimum development.[6,7] In such doughs more lipid was bound if the mixer was flushed continuously with nitrogen in place of air. As the level of mechanical work was raised, nitrogen-mixed doughs showed an increase in bound lipid whereas in air a fall was observed.[5] Vacuum applied to the dough mixer also produced a rise in lipid binding similar to that found in a nitrogen atmosphere.[8] These observations raise again the question of the part played by atmospheric oxygen in the breadmaking process. Oxidative reactions have long been recognised as important in dough development and baking.[9] For example, oxygen is known to act either alone or with other oxidising agents in the maturing and so called 'improvement' of flour protein[10] and is also involved both as a pigment bleach and as an improver during dough mixing.[11] Flour lipids are also appreciably involved in the uptake of oxygen during dough mixing[12,13] and are said to compete with the sulphydryl groups of the dough protein for available oxygen in the dough.[14]

The findings of the previous paper in this series[7] implied that the polyunsaturated lipids of the flour were involved in the effect of air on lipid binding and it was concluded that oxidative reactions could influence the behaviour of the dough lipids (including added shortening) during the high-energy development of bread doughs. The object of the present work was to investigate further the oxidative changes taking place in doughs and to relate these where possible to the effect of air on lipid binding.

Experimental

Dough mixing and extraction

Because it was intended to investigate the effect of air on lipid oxidation and binding, all other oxidants were excluded from the basic dough formula. An untreated, unbleached, bakers' grade flour was used, free from additives. The flour (14% moisture) on a dry weight basis contained 12·6% protein (N × 5·7) and 0·50% ash. The dough formula used earlier was further simplified (Table I) by the omission of all ingredients not significantly contributing to the lipid content of the dough.

Doughs were mixed at a constant work rate of 0·3 h.p. min/lb/min. (0·5 kW/kg) in a controlled atmosphere as described earlier.[7] The proportion of air to nitrogen was varied by filling a previously evacuated gas cylinder with air and oxygen-free nitrogen at appropriate partial pressures. It was possible to change the dough atmosphere during mixing simply by switching from one cylinder to another. The change was instantaneous and did not interrupt either the gas flow or dough development.

The atmosphere in the dough mixer was varied over the range: nitrogen alone, 1% air in nitrogen by vol., 3%, 5%, 10%, 20%, 50%, and air alone (corresponding to 0·0%, 0·2%, 0·6%, 1·1%, 2·1%, 4·2%, 10·5% and 21·0% oxygen in the dough atmospheres respectively). Doughs were mixed to work levels of 0·4, 1·0, 2·0, and 4·0 h.p. min/lb (11·0, 27·4, 54·8 and 109·7 W h/kg) using a modified Brabender Do-Corder fitted with a Farinograph mixing bowl (stainless steel, 300 g flour capacity) held at 30°. Mean results for

TABLE I
Experimental dough formula

Flour	305 g
Salt	5·45 g
Soya flour*	2·20 g
Shortening fat	2·195 g
Water	180 g

* Full fat, enzyme active

* Part IV: *J. Sci. Fd Agric.*, 1969, **20**, 129

lipid analyses were taken from at least two separate dough mixings and up to four mixings in critical atmospheres (e.g. 5% air or less).

After mixing, doughs were freeze-dried and ground and the free and bound lipids extracted as described previously.[4] Free fatty acids present in the free lipid were isolated as sodium soaps by the method of Mattick & Lee.[15] After acidification, the free fatty acids were converted to methyl esters by reaction with diazomethane[16] before analysis by gas chromatography.[4]

Lipid analysis

Peroxide determinations were made on freshly prepared dough powders using the ferric thiocyanate method.[17] Lipid was extracted by shaking the dough powder with redistilled toluene rather than benzene. The change to a less toxic solvent was found not to affect the subsequent determination of lipid peroxide.

Bleaching of the dough pigments was studied during dough mixing in the various atmospheres. The concentration of fat-soluble pigments in the free lipid was determined by measuring the optical density of a toluene solution at 454 nm in a spectrophotometer (Unicam SP500). Extinction was calculated on a dough weight basis and the percentage colour retention after dough mixing obtained by reference to the amount of pigment extracted from the unmixed ingredients.

Thin-layer chromatography

The appearance of lipid peroxides in the free lipid of the dough was followed by thin-layer chromatography (t.l.c.). Lipid peroxides in the free lipid were effectively separated from non-peroxidised lipids by the method of short-column chromatography described by Hunt & Rigby.[18] Since the greater part of the extracted lipid was found to be non-peroxidised triglyceride, this greatly increased the sensitivity of the subsequent t.l.c. analysis.

Freshly prepared dough powder (2·0 g) was extracted with toluene (3 ml) by percolation and the solvent was removed without heating, by evaporation under a stream of oxygen-free nitrogen. The free lipid (~ 30 mg) was taken up in 0·5 ml of eluting solvent (45% ethyl ether in light petroleum ether, b.p. 40–60°) and placed at the top of the chromatography column (10 × 1·0 cm, prepared using 4·0 g Silica gel G (E. Merck, Darmstadt) slurried in 17·0 ml eluting solvent). A marker dye (2,6-dichlorophenol-indophenol) was added and the chromatogram was developed under a nitrogen pressure of 10 cm mercury.

Preliminary investigation showed that 80% of the total lipid was eluted during the movement of the marker dye over the first 20% of the column length. This fraction consisted entirely of triglycerides and sterol esters and was peroxide-free. The second fraction (dye movement between 20% and 70% of the column length) was rich in lipid peroxides together with free fatty acids, diglycerides and free sterol. Monoglycerides and polar lipids remained on the column and were usually discarded. After removal of solvent by evaporation in a stream of nitrogen, the concentrated lipid peroxides were dissolved in 0·1 ml of chloroform for t.l.c. analysis.

Chromatoplates (20 × 20 cm) were coated with Silica gel G (0·25 mm thickness) and developed using a solvent comprising light petroleum ether (b.p. 40–60°)–ethyl ether–glacial acetic acid (60 : 40 : 1 by vol.) as described earlier.[4] Duplicate t.l.c. plates were prepared and the total peroxidic lipid extracted from each dough sample loaded equally between the two plates (~ 50 µl on each). After identical development, one plate was sprayed with 50% sulphuric acid to visualise all lipid material and the other was sprayed firstly with a 5% (wt./vol.) potassium iodide solution and 1 min later with 5% (wt./vol.) starch in 1% acetic acid. Peroxides appeared as blue brown spots within a few minutes and were tentatively identified by comparison with the duplicate sulphuric acid charred plate and with the retention data of Oette.[19]

Gas–liquid chromatography

Extracted lipids were converted to their component fatty acid methyl esters by the rapid, sealed tube method of direct transesterification.[20] Fatty acid ester mixtures were analysed by gas–liquid chromatography (g.l.c.) on polyethylene glycol adipate (10% on Chromosorb W, 80–100 mesh) as described earlier.[4] High temperature g.l.c. was used to analyse freshly extracted free lipid directly for component triglycerides,[21] diglycerides, monoglycerides, free fatty acids and sterols after non-selective hydrogenation and silylation with bis(trimethylsilyl) acetamide (BSA).[22] Hydrogenation of the lipids (~ 100 mg) in n-hexane (~ 20 ml) was catalysed by platinum oxide (Adam's catalyst). After removal of the spent catalyst by filtration, the bulk of the solvent was evaporated and BSA (0·5 ml) was added. After 5 min the hydrogenated, silylated lipids were analysed directly by g.l.c. (Pye 104, dual flame-ionisation detectors) using 18 in. columns of 2% S.E. 30 on 100–120 mesh Celite and a temperature programme from 150 to 360° at 4°/min. (For details of this technique see Wood[23]). Peak areas were calculated by triangulation and the results were expressed as percentages of the total peak area.

Results

Composition of the mixer atmosphere

Lipid binding

Fig. 1 shows the combined effect of atmosphere and work level on lipid binding. At all work levels, increasing the amount of air available to the dough led to a decrease in lipid binding during mixing. However, as the work level was increased, the effect of air became more pronounced so that

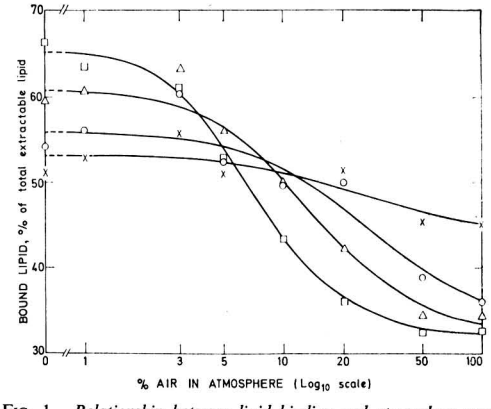

FIG. 1. *Relationship between lipid binding and atmosphere composition in doughs mixed to different work levels*
× 0·4; ○ 1·0; △ 2·0; □ 4·0 h.p. min/lb

at 4·0 h.p. min/lb an overall drop of 32% in bound lipid was observed compared with only 7% at 0·4 h.p. min/lb. At the highest work level the dough was surprisingly sensitive to the presence of air and as little as 5% air (~ 1·1% oxygen in the mixer atmosphere) resulted in a significant fall in bound lipid. The greater part of the effect of air occurred between 3% and 20% air. Above 50%, air was no longer a limiting factor in relation to lipid binding in the dough. This finding was in agreement with previous work[8] which showed that only when the absolute air pressure in the mixer was reduced below 10 in. mercury (vacuum of 20 in. mercury, equivalent to 33% of air available at atmospheric pressure) could an increase in lipid binding be observed.

The results of Fig. 1 support the suggestion made earlier[7] that two separate systems control lipid binding during dough mixing. Below 5% air, lipid binding increased with work in accordance with the first, essentially anaerobic, system of binding. Above 10% air, bound lipid was released as work level was raised indicating that the second, oxidative, system was becoming increasingly active at these higher oxygen tensions in the dough atmosphere. Between 5% and 10% air the convergency of the four plots suggests that the two systems were in balance, lipid binding remaining constant irrespective of work input.

Lipid peroxidation

Lipid peroxides are known to be formed during the aerobic mixing of dough[10] although their influence on dough properties is less certain.[9] The possibility that lipid peroxidation could be related to the changes in lipid binding observed in Fig. 1 was investigated. Preliminary work showed that while peroxide formation was readily detected in the free lipid of the dough, bound lipid remained low in peroxides even in dough mixed to high work levels in air. Peroxide determinations were therefore confined to the free lipids extracted from doughs mixed as described above.

The results obtained are given in Table II and show that high levels of lipid peroxides were found only in doughs mixed to the highest work levels (2·0 and 4·0 h.p. min/lb) and in the presence of an abundant supply of air (50 or 100% air). In fact, no consistent increase in peroxide value was found until either 20% air was present at the higher work levels or until at least 50% air was present in doughs mixed to near optimum development (0·4 h.p. min/lb).

Comparison of these results with Fig. 1 shows that, particularly at the higher work levels, the introduction of air influenced lipid binding at a much earlier stage than the appearance of a measurable increase in the level of lipid peroxide. At 4·0 h.p. min/lb, increasing the air content from 0 to 10% resulted in a fall in bound lipid from 65 to 45% of the total lipid without any marked appearance of peroxide in the free lipid. At the lower work levels, lipid binding was already at a minimum before peroxides were detected. It was concluded that the effect of air on lipid binding was not dependent on the presence of high levels of lipid peroxides in the dough.

TABLE II

Peroxide formation in free lipid during dough mixing

Work level, h.p. min/lb	Air in mixer atmosphere, %							
	0	1	3	5	10	20	50	100
0·4	—	—	—	—	—	—	+	+
1·0	—	—	—	—	—	—	+	+
2·0	—	—	—	—	—	+	++	++
4·0	—	—	—	—	±	+	++	++

Peroxide values (μmole ½ O_2/g lipid) expressed as < 10 = —
10–100 = +
>100 = ++

Pigment bleaching

Unlike the appearance of lipid peroxides, pigment bleaching occurred over the whole range of atmospheres studied. Fig. 2 shows that even in doughs mixed in nitrogen there was some loss of pigment as the work level was raised. Pigment bleaching increased at all work levels as air was admitted to the dough atmosphere. The trend followed was similar to that observed for lipid binding (Fig. 1). Thus, at 2·0 and 4·0 h.p. min/lb, dough pigments were destroyed rapidly as the proportion of air increased, reaching a minimum between 20 to 50% air, a level similar to that associated with minimum lipid binding at these work levels. Pigment bleaching at the lower work levels was slower and was never complete, again corresponding to the pattern of lipid binding at these work levels shown in Fig. 1.

The bleaching of plant pigments is known to take place through an oxidation reaction coupled to the oxidation of polyunsaturated lipid.[24] The similarities observed between the bleaching of dough pigments and the release of bound lipid suggested that both effects may be linked through a similar lipoxidase-coupled oxidation mechanism involving polyunsaturated lipids present in the dough. The relationship between lipid oxidation and lipid binding was further investigated to test this hypothesis.

Oxidation and lipid binding

Peroxidised fat

The effect of lipid peroxides on lipid binding was investigated directly by the addition of pre-oxidised lipid to dough mixed under nitrogen. Free lipid containing a high level of peroxide was extracted from dough mixed to 4·0 h.p. min/lb in air. This was added in place of shortening to a second series of doughs mixed to different work levels under nitrogen. The results (Fig. 3) show that while lipid binding was depressed overall by about 15% compared with the control, binding

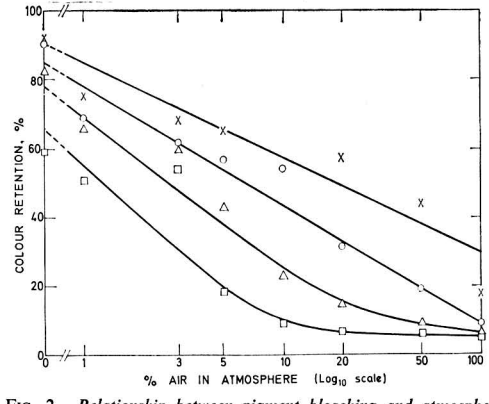

FIG. 2. *Relationship between pigment bleaching and atmosphere composition in doughs mixed to different work levels*
Symbols as in Fig. 1

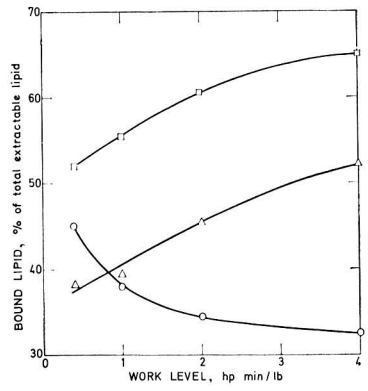

FIG. 3. *Effect of peroxidised lipid on lipid binding in nitrogen-mixed doughs*

□ Control doughs mixed in nitrogen; ○ control doughs mixed in air; △ nitrogen-mixed doughs with added peroxidised lipid in place of shortening fat

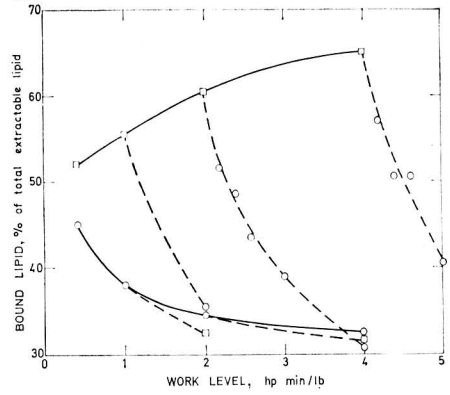

FIG. 4. *Lipid binding in doughs mixed either in air or nitrogen or a combination of the two atmospheres in succession*

□——□ Nitrogen alone; □- - - -□ nitrogen preceded by air; ○——○ air alone; ○- - - -○ air preceded by nitrogen

increased with increasing work in spite of the presence of peroxidised lipid in the dough.

This result supports the hypothesis that, as with pigment bleaching,[15] the effect of air on lipid binding is related more to the active peroxidation of the unsaturated lipids than to any direct effect of the lipid peroxides themselves. The finding that substantial quantities of lipid peroxides appeared in the dough only after both pigment oxidation and the release of bound lipid had proceeded nearly to completion (Table II, Figs 1 and 2), agrees with the known sparing of lipid peroxidation that occurs during the lipoxidase-coupled oxidation of a secondary substrate (e.g. carotene).[25] It is suggested that during aerobic mixing the sites of lipid binding act as secondary substrates for coupled lipoxidase oxidation and, as a result of this site oxidation, bound lipid is released without the intervention of lipid peroxides, which are only formed in quantity in the dough after such coupled oxidations have neared completion. It has been reported that lipid peroxides themselves are not involved in the oxidation of sulphydryl groups in the dough protein to any significant extent[26] and it would appear that, once formed, they are equally unable to affect lipid binding in mechanically developed doughs.

Atmosphere control

To investigate the way in which site oxidation was controlled by the dough atmosphere, doughs were mixed either in air or in nitrogen and, after specific work levels had been reached, the atmosphere composition was reversed and mixing was continued in the new atmosphere. The results (Fig. 4) show that, following the normal rise in bound lipid in nitrogen, the introduction of air into the mixing dough led to an immediate and rapid release of bound lipid as the work level was raised in the dough. In fact, the release of bound lipid was more rapid than in doughs mixed continuously in air and it would appear that during dough development in nitrogen, new sites for lipid binding are formed which, by their susceptibility to oxidation, lead to a more rapid release of bound lipid when air is admitted.

Conversely, when nitrogen was admitted to doughs already mixed in air, there was no reversal of the effect of site oxidation already established and lipid was not bound during further mixing in nitrogen. Thus, it was clear that site oxidation was irreversible, further mixing leading to a continued release of bound lipid. Having established the lability of lipid binding sites to irreversible oxidation, probably coupled to the lipoxidation of polyunsaturated lipid, the mechanism of lipid oxidation in the dough was studied to ascertain the initial reactions occurring during aerobic mixing.

Lipid oxidation

Formation of specific lipid peroxides

Thin-layer chromatography was used qualitatively to follow the appearance of specific lipid peroxides in doughs mixed in air to increasing work levels. Free and bound lipids were extracted from the freeze-dried dough powder and the peroxidic lipids were separated from the unoxidised triglycerides and sterol esters by short-column chromatography.[18] Removal of the major triglyceride fraction in this way greatly improved the sensitivity of the method, allowing a large increase in the loading of lipid peroxides without over-loading the t.l.c. plate.

Fig. 5 shows the distribution of peroxides in the free and bound dough lipids as the work level was increased from 0·4 to 4·0 h.p. min/lb. As predicted by peroxide determinations on the free lipid of air-mixed doughs (Table II, 100% air), high levels of free lipid peroxides were found only in doughs worked to 2·0 and 4·0 h.p. min/lb. Much less peroxide was present at the lower work levels and it was possible to follow the sequence of formation of peroxidic lipid as the work level was raised.

At the lowest work level (0·4 h.p. min/lb) peroxide formation in the free lipid occurred initially in the free fatty acids with only trace amounts present in the 1 : 2- and 1 : 3-diglycerides. The level of peroxide in these lipids increased as the work level was raised to 1·0 h.p. min/lb and was accompanied by the appearance for the first time of peroxidic triglyceride. Above this work level, peroxides were abundant in all lipid classes and at the highest work level streaking of the chromatogram suggested that some breakdown and polymerisation of the oxidised lipids was taking place.

By comparison, there was little change in the low content of peroxidic bound lipid as work level was increased. Even at

4·0 h.p. min/lb little more than a trace of fatty acid and diglyceride peroxides were found and triglyceride peroxides were never more than barely detectable. The low and unchanging level of bound lipid peroxide was in agreement with the negative results for peroxide in the bound lipid obtained by the thiocyanate method. It would appear that bound lipids are either protected against peroxidation or, if oxidised, are no longer held in the bound form. The finding that the first peroxides to appear in the dough are formed by the oxidation of the free fatty acids is in agreement with the work of Morrison who reported the rapid oxidation of free fatty acids in aerobic flour–water mixtures.[13,27]

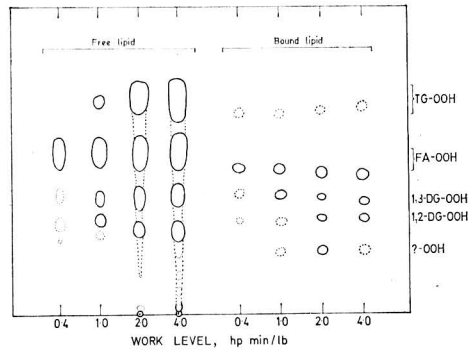

FIG. 5. *Thin-layer chromatogram of lipid peroxides selectively isolated from the free and bound lipids of air mixed doughs*
Solvent: light petroleum ether–ethyl ether–acetic acid (60 : 40 : 1 by vol.)
Spray: starch–potassium iodide solution

Fatty acid analysis

In order to assess the extent of lipid oxidation taking place during aerobic mixing, the component fatty acids of the total free lipid were analysed by g.l.c. and compared with the results obtained from similar doughs mixed in nitrogen. The results are given in Table III and show that while there was no change in the fatty acid composition during nitrogen mixing there was some evidence of loss of polyunsaturated acids as the work level was raised in air. However, the changes were small compared to the large increase in free lipid resulting from aerobic dough mixing (55–68% of total lipid), an observation which supports the suggestion that the formation of lipid peroxides was not directly responsible for the release of bound lipid.

A similar investigation of the free fatty acids present in the free lipid again showed that there was little change during nitrogen mixing (Table IV). However, in air, the g.l.c. analysis showed extensive losses of the polyunsaturated free fatty acids increasing through all the work levels examined. These results clearly demonstrate the susceptibility of the polyunsaturated free fatty acids to oxidative attack during the aerobic mixing of doughs[27] and confirm the initial oxidation of the free fatty acids observed by t.l.c. (Fig. 5).

Whole lipid analysis

The effect of aerobic mixing on the whole lipids of the dough was measured directly and semi-quantitatively by high-temperature g.l.c. of the intact glycerides.[23] The extracted free lipid was hydrogenated and the partial glycerides and fatty acids converted to their silyl derivatives before analysis. Fig. 6 shows a typical chromatogram in which the fatty acids, mono-, di and tri-glycerides are separated on the basis of their total fatty acid carbon numbers.

TABLE III
Fatty acid composition of total free lipid, % as methyl esters

Fatty acid	Nitrogen				Air			
	0·4*	1·0	2·0	4·0	0·4	1·0	2·0	4·0
Myristic acid	2·0	1·8	1·8	1·8	2·0	2·3	2·4	2·3
Palmitic acid	19·0	23·5	23·8	23·3	20·8	22·5	23·2	25·8
Stearic acid	7·9	8·8	9·0	9·2	6·9	7·0	9·2	9·4
Oleic acid	30·4	25·8	26·2	27·2	34·0	31·6	33·2	32·3
Linoleic acid	37·7	36·3	35·5	34·9	36·3	36·5	31·9	30·2
Linolenic acid	3·1	3·9	3·7	3·6	tr.	—	tr.	tr.
% Free lipid	48·0	44·5	39·5	35·0	55·0	62·0	65·6	67·6

* Work level, h.p. min/lb

TABLE IV
Fatty acid composition of free fatty acids, % as methyl esters

Fatty acid	Nitrogen				Air			
	0·4*	1·0	2·0	4·0	0·4	1·0	2·0	4·0
Palmitic acid	15·3	16·9	17·0	18·9	17·0**	17·0	17·0	17·0
Stearic acid	1·1	tr.	1·2	tr.	tr.	0·8	tr.	0·8
Oleic acid	12·6	12·0	12·5	12·7	14·7	13·2	13·5	12·3
Linoleic acid	67·0	66·5	63·5	64·0	52·0	41·4	28·9	17·4
Linolenic acid	4·1	4·4	4·7	4·5	tr.	tr.	—	—

* Work level, h.p. min/lb
** Mean value for palmitic acid in nitrogen-mixed doughs taken as internal standard in air-mixed doughs

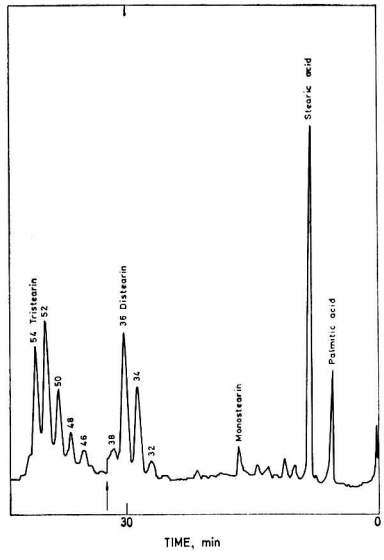

FIG. 6. *High-temperature gas–liquid chromatogram of free lipid from air-mixed dough*

Temperature programmed from 150 to 360°C at 4°C/min using a column (46·0 × 0·5 cm) packed with S.E.30 (2%) on acid-washed, silylated Celite (100–120 mesh) Recorder response attenuated at arrow

Table V shows the results obtained from the analysis of the free lipids of doughs mixed either in air or nitrogen to increasing work levels. Triglycerides accounted for over 80% of the total free lipid in all doughs, the remainder being mainly diglycerides (~ 10%) and fatty acids (3–7%). In spite of the large difference in the amount of free lipid present in the doughs (free lipid varied from 35% in nitrogen to 68% in air) there was little change in the proportions of the various glycerides found in the free lipid. There was no evidence of either preferential binding or release of any major lipid class in the doughs examined. While aerobic mixing gave rise to a number of minor peaks in the monoglyceride region which were not seen in nitrogen doughs, the proportions of di- and tri-glycerides remained unchanged even at the highest work levels.

The only significant change in the free lipid that occurred as a result of mixing in air was in the free fatty acid fraction. While there was little change in the proportion of palmitic acid during dough mixing, the unsaturated free fatty acids (analysing as stearic acid after hydrogenation) were reduced from 6% in nitrogen to 3% in air at the highest work level. This result confirms again the readiness with which the free fatty acids may be oxidised during dough mixing in air (cf. Table IV and Fig. 5).

It would appear from these results that the large amount of lipid released from lipid-binding sites during aerobic mixing of the dough is released without appreciable oxidative change taking place in the lipid itself. While triglycerides comprise over 80% of this lipid, the only evidence of triglyceride peroxidation was found by selective thin-layer chromato-

TABLE V

High temperature g.l.c. of free lipids

Percentages based on peak areas without calibration

Lipid class and carbon number*	Nitrogen				Air			
	0·4**	1·0	2·0	4·0	0·4	1·0	2·0	4·0
Fatty acids:								
Palmitic acid	1·1	1·0	1·2	1·3	1·4	1·3	0·9	1·4
Stearic acid	6·0	5·9	6·2	5·7	5·4	4·4	2·4	3·0
Class total, %	7·1	6·9	7·4	7·0	6·8	5·7	3·3	4·4
Monoglycerides:								
C 16	—	—	—	—	0·6	0·4	0·6	1·0
C 18	—	—	—	—	tr.	0·4	0·8	tr.
Class total, %	—	—	—	—	0·6	0·8	1·4	1·0
Free sterols	0·3	0·4	0·3	0·3	0·3	0·3	0·3	0·4
Diglycerides:								
C 32	0·5	0·5	0·6	0·4	0·5	0·7	0·6	0·5
C 34	3·6	3·5	3·9	2·8	3·2	3·5	3·4	2·9
C 36	6·7	6·6	7·3	4·8	5·5	6·1	5·2	4·6
C 38	tr.	tr.	tr.	tr.	1·2	1·4	1·3	1·4
Class total, %	10·8	10·6	11·8	8·0	10·4	11·7	10·5	9·4
Triglycerides:								
C 46	4·8	5·9	4·7	6·5	5·9	5·0	5·5	5·8
C 48	6·0	8·2	8·6	7·9	7·1	7·5	7·8	7·9
C 50	15·6	14·8	14·3	15·0	15·1	14·3	14·8	14·9
C 52	31·0	29·8	29·4	30·3	28·6	29·3	30·0	30·6
C 54	24·4	23·3	23·5	25·1	25·1	25·1	24·3	25·3
Class total, %	81·8	82·0	80·5	84·8	81·8	81·2	82·4	84·5
Free lipid, %	48·0	44·5	39·5	35·0	55·0	62·0	65·5	67·6

* Total number of fatty acid carbons in the molecule
** Work level, h.p. min/lb

graphy of lipid from doughs mixed to work levels above those at which the greater part of the bound lipid had been released (Figs 1 and 5). The constant composition of the free lipid revealed by high temperature g.l.c. adds support to the hypothesis that bound lipid is released by a lipoxidase-coupled site oxidation without the peroxidation of the released lipids themselves.

Discussion

The results of this investigation have confirmed that in doughs based on commercial formulations such as have been examined here and in earlier papers of this series, lipid bound either in the early stages of hydration of the dough[3] or after mechanical development[4,5] is readily released by mixing the dough aerobically. Lipid release was found to be suprisingly sensitive to small quantities of air admitted to the dough and preceded the formation of appreciable amounts of lipid peroxides in the dough. The presence of peroxidic lipid was unable to prevent lipid binding in doughs mixed under nitrogen and it was concluded that peroxides were not directly involved in lipid release.

Similarities noted between the release of bound lipid and the bleaching of the flour pigments suggested that the mechanisms involved may be related. During the lipoxidase-catalysed oxidation of polyunsaturated fats, carotenoid pigments are simultaneously bleached in a coupled oxidation-reaction[24] although pigments are not bleached by lipoxidase or peroxides acting alone.[25] It is suggested that sites of lipid binding in the dough are vulnerable to a similar coupled oxidation mechanism in which bound lipid is released as a result of site oxidation rather than oxidation of the lipid itself.

Although much research has been directed towards the study of both lipoxidation and autoxidation of unsaturated oils and fats, the reactions involved in the initial stages of lipid oxidation are still not fully understood. Some evidence has been presented suggesting that unstable reactive intermediate oxides may be responsible for the coupled oxidation of secondary substrates during lipoxidase action[25] and the formation of compounds with strong pro-oxidant properties has been detected before the appearance of stable hydroperoxides during the induction period of methyl linoleate autoxidation.[28] The possibility that transient intermediates of lipid oxidation could be involved in the oxidative changes taking place during dough mixing was noted earlier[29,30] and it is now suggested that such pro-oxidant lipid intermediates, because of their lipophilic nature, could be ideally suited to reach sites of lipid binding in the dough.

Triglycerides accounted for about 80% of the bound lipid and may be bound by hydrogen bonding to hydrophobic sites in the dough protein.[31] While such sites would be inaccessible to aqueous oxidation systems such as the lipoxidases or commercial oxidative improvers (e.g. potassium bromate and ascorbic acid), they may be more readily reached by the pro-oxidant lipid intermediates produced during the oxidation of poly-unsaturated lipids. The possibility that oxidised lipid may react with sulphydryl groups in the dough protein has been put forward to explain the initial loss of —SH observed when doughs are mixed in a nitrogen atmosphere.[32] These results with air-mixed doughs may indicate a further oxidation of protein sulphur by oxidised lipids in areas not readily accessible to water-soluble oxidants. If oxidation within the hydrophobic sites of lipid binding proceeds by the second oxidation pathway of Hird & Yates[33] leading to the formation of cysteic acid as found by Zentner in mechanically developed doughs,[34] the formation of ionisable sulphonic acid at the expense of disulphide bonds would lead to a marked change in the charge distribution on the protein surface, reversing the previously hydrophobic nature of the lipid-binding sites. The resulting collapse of the now unstable three-dimensional protein network surrounding the bound lipid could result in an inversion of the lipoprotein micelles, followed by the displacement of lipid held by either hydrogen bonding or hydrophobic bonding [35] as water molecules enter the protein structure. Loss of titratable sulphur (total –SH + –SS–) has been reported in doughs developed in both air and nitrogen atmospheres[36] and may indicate the occurrence of such extreme oxidation as suggested here owing to the presence of oxidised lipid. Further support for the oxidative breakdown of the three-dimensional lipid-binding protein network during air mixing is given by the observation that doughs mixed in air became noticeably softer as the work level was increased compared with similar doughs mixed in nitrogen.[5]

A tentative scheme for the mechanism involved in the effect of air on lipid binding is given in Fig. 7. Such a mechanism of lipoxidase-coupled site oxidation, as opposed to the direct oxidation of bound lipid before liberation, is consistent with the observed absence of gross change both in the fatty acid composition of the total free lipid (Table III) and in the relative proportions of the major lipid classes (Table V), despite the release of substantial amounts of bound lipid during air mixing. Support for this hypothesis is also provided by the results of earlier work[7] in which removal of the free lipid of the flour, the main source of the linoleate substrate for lipoxidase action, resulted in a marked increase in lipid binding even in the presence of air. The finding that the poly-unsaturated free fatty acids, alone among the dough lipids, are oxidised extensively from the onset of aerobic dough mixing, implicates this class of lipid as the primary substrate for lipoxidase attack leading to the coupled oxidation of lipid binding sites. However, the possibility that other substrates may be involved cannot be excluded and more work is planned to resolve this question and to test the proposed hypothesis.

In commercial doughs, such as have been used as a basis for this work,[5,7] the most abundant source of lipoxidase enzymes is the full-fat, enzyme-active soya flour added to promote a greater bleaching of the flour pigments during dough mixing. It has recently been shown that, while both soya and wheat lipoxidase readily oxidase poly-unsaturated free fatty acids, the soya enzymes are far more reactive towards esterified

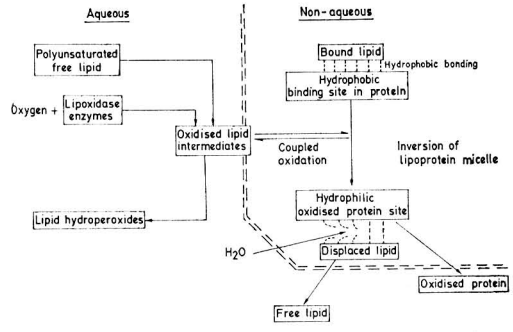

FIG. 7. *Proposed mechanism for the release of bound lipid during dough mixing in the presence of air*

fatty acids including the triglycerides.[37] The possibility that this difference in enzyme specificity may help in understanding the part played by the various lipoxidase substrates in the dough during mixing and bread baking is at present under investigation.

Acknowledgment

The authors would like to thank Mr. J. V. Ireland for his valuable technical assistance.

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Received 14 January, 1970;
amended manuscript 5 March, 1970

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ELECTROPHORETIC STUDIES OF α-AMYLASE IN WHEAT. II*

By R. OLERED and G. JÖNSSON

The lack of correlation between the degree of sprouting and the α-amylase activity in wheat and rye, as well as the apparent variation in the falling number during ripening, can be explained as the result of two amylase systems acting during different stages of the development of the grain. During the early stages of the development of the grain, α-amylase is continuously inactivated. This process is reversible, however, and when the evaporation of moisture is retarded the α-amylase activity increases as a consequence of the higher amount of dissolved enzyme. This results in an occasional decrease in the falling number, which might amount to more than 50 sec. During germination, a new kind of α-amylase develops. The synthesis of the new form of α-amylase is irreversible and causes a much greater and permanent reduction in the falling number. During the initial stages of germination, the amylase activity thus increases owing to the combined action of both the original amylase and the new form. The two kinds of amylases show different electrophoretic patterns. Drying the grain after harvesting reduces the activity of 'green' amylase (amylase in unripened grain), which might explain the frequent observations of increasing falling number during storage.

Introduction

The adoption in 1964 of the Falling Number Method as the exclusive official Swedish procedure for the determination of sprouting damage in wheat and rye encouraged farmers to harvest very early. By doing so, they hoped to avoid sprouting damage and to secure an optimum value in the falling number. The method was combined with a formula for price regulation. However, experiences during the past 5 years have shown that it is doubtful whether the farmer can fix a day for maximum falling number. Instead, an irregular variation with an amplitude of about 50 sec is often observed even in sound wheat throughout the usual harvest period[1] – a variation which seemed inexplicable from the farmer's point of view. Another factor that might cause doubts among farmers concerning the reliability of the new method is the observation that the falling numbers often increase during storage after the wheat has been harvested at a comparatively low falling number. These changes generally proceed only for a few weeks after harvest. Of interest is that the changes might improve the baking quality considerably when the wheat has been harvested in an early stage of ripeness. If the farmer is not able to dry and store the crop on the farm, taking advantage of these improvements himself, the extra profits will be gained on the grain market. These problems associated with the Falling Number Method necessitated a study of the biochemical background of the Falling Number Method, which on the whole has been successful both in agriculture and in the grain trade.

Figs 1 and 2 provide an idea of the variation that might be observed under different growing conditions, and even when no sprouting has occurred. The Figures show the variation in the falling number and α-amylase activity in two adjacent areas in a field of winter wheat in 1968. In one area there was 100% lodging, whereas in the other one, there was no lodging. Only during the last days of sampling were a few kernels with visible sprouts observed in the area with 100% lodging. In the area without lodging, and with apparent homogeneous development, the range of variation in the falling number amounted to about 50 sec. In the other area, the variation was more than 100 sec during a time interval of 3 or 4 days. The variations are strongly and negatively correlated with a periodic variation in α-amylase activity, although an obvious influence from changes in starch quality can also be observed in samples with high falling numbers; this is quite natural. When the enzymic activity has reached a certain level, the α-amylase activity is by far the predominating factor with respect to the viscosity; at this time, a strong correlation is to be expected between the amylase activity and the falling number. When the amylase activity is very low, its influence will be overlapped by variations in starch properties.

From the results presented in Figs 1 and 2, it can be concluded that an increase in α-amylase activity does not necessarily presuppose a heavy rainfall. It can be induced by changes in environmental conditions that retard moisture evaporation from the grain, and disappears again as the drying process continues.

The occurrence of α-amylase activity in unripe grain has been reported earlier.[1-3] However, little has been published concerning the relationship between the 'green' amylase and the 'malt' α-amylase with regard to their influence on the general concept of 'sprouting damage'. In a previous paper,[4] preliminary results have been presented on the electrophoretic pattern of the α-amylase system in unripe wheat. It was shown that the physico-chemical properties of these enzymes differed from those of 'malt' α-amylase but that their effect on the falling number was the same.

This paper reports some additional experiments aimed at elucidating the nature of those amylases that are found in the grain before 'full ripeness.' The main objective was to distinguish between the α-amylase in unripe grain and those that appear during germination with regard to their influence on the falling numbers and 'sprouting damage' with the hope of finding a method that could be used to differentiate between reactivation of α-amylases and germination.

The complex nature of the amylases is now well evidenced. Cooper & Pollock[5] isolated several fractions of α-amylase from barley by column electrophoresis. All the fractions were characterised by the same activity, although the molecular weight varied between 20,000 and 80,000. Greenwood & Milne[3] reported that the α-amylases found in ungerminated and

* Part I: *Getreide Mehl*, 1968, **18**, 95

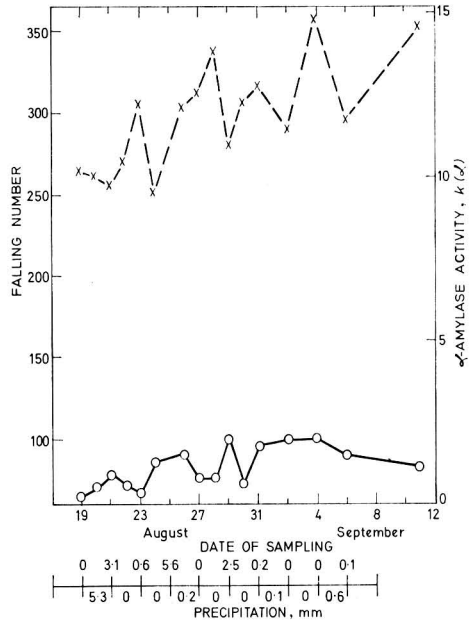

FIG. 1. *Variation in α-amylase activity (○) and falling number (×) in winter wheat during ripening*
No lodging

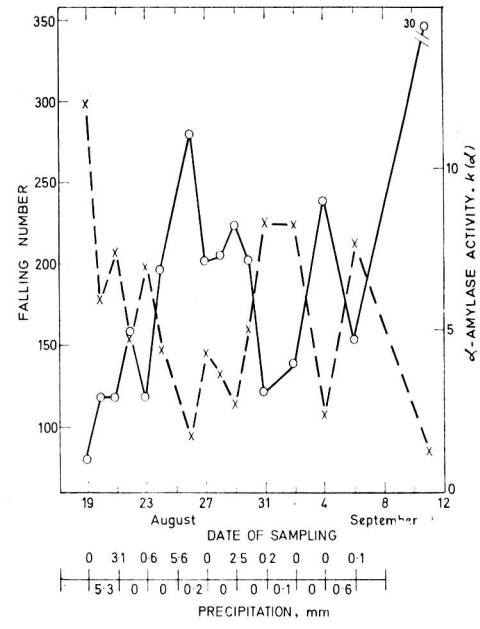

FIG. 2. *Variation in α-amylase activity (○) and falling number (×) in winter wheat during ripening*
100% lodging

germinated cereals had similar modes of action and molecular sizes. Grabar & Daussant[6] found that fractions of β-amylase from barley and from barley malt showed identical immunochemical properties, whereas the enzymes differed with regard to solubility and migration rate. Waldschmidt-Leitz *et al.*[7] discovered that the molecular weight of β-amylase from barley was 63,000, while the β-amylase of barley malt had a molecular weight of 93,000. These observations indicate that the activation of β-amylase might be dependent on synthesis and not solely on hydrolytic processes. Frydenberg & Nielsen[8] isolated as many as nine different iso-enzymes of barley malt amylases by agar electrophoresis. They localised three different groups of amylases by means of their specific reactions with copper ions, heat treatment and calcium precipitating agents, such as hexametaphosphate. Besides the α- and β-amylases, they also found a third group of related amylases – the 'unknown' amylases, which displayed many properties in common with α-amylase.

Experimental

Materials

All samples of wheat were collected from an open field and analysed without delay unless otherwise stated. When it was necessary, the grains were dried with ventilation at 30°. Steeping of dry grains, described in some experiments, was performed in Petri dishes on moistened filter papers. The youngest stages investigated contained about 50% water, and the later stages about 25% moisture. Green undried kernels were crushed in a mortar with a small volume of water immediately after harvest, whereas the ripe and dried kernels were milled before extraction with water. After 1 h, the extracts were centrifuged and pipetted immediately into the basins in the agarose gels as described below.

Electrophoresis

The electrophoretic technique used in these experiments was similar to that described by Frydenburg & Nielsen.[8] The electrophoresis was performed on 25 × 75 mm microslides using a gel of 1·0% agarose in citrate–phosphate buffer with an ionic strength of 0·02 and a pH of 6·0. By pipetting 3 ml of the boiling agarose solution onto the microslides, these were completely covered with a uniform layer. The gel solution stiffened quickly when poured on to the glass. When the gel had cooled, a small basin (2 × 10 mm) was cut in the gel about 20 mm from the cathode end of the slide with a sharp-edged brass punch.

The amylase solution, prepared by extracting the crushed or milled grains with water at room temperature, was mixed with an equal volume of boiling agarose solution. It was necessary to boil the agarose solution before mixing to permit pipetting. The temperature of the mixture did not, however, exceed 50°, and by working rapidly, the thermal inactivation of the enzymes could be held at a minimum.

About 40 μl of the enzyme–agarose mixture were pipetted into the basin, which should be filled exactly. The best concentration of the enzyme solution can be found by trial and error. The connexion between the slide and the buffer solution in the electrophoretic cells consisted of three layers of filter paper, of the same width as the slide, dipping into the buffer solution in the anodic and cathodic cells. The electric

current was stabilised to 4 mA per plate and a voltage gradient of about 17 V/cm was established. During electrophoresis, the slides must be cooled by filling the space between the electrode cells with crushed ice. Blocks of ice were also placed on the cover of the vessel.

The electrophoresis was run for 1 h and 25 min, and after being dried a few minutes at room temperature, the slides were sprayed with a solution of 0·15% agarose and 1·0% Smithies' modified starch. After incubation for 25 min at 45°, the slides were immersed for a few minutes in a 0·25% iodine solution containing 0·5% potassium iodide. Finally, the slides were rinsed in distilled water. Violet to colourless bands appeared where the starch solution had been partly digested by enzymes.

All experiments were run in duplicate or eventually in triplicate when the α-amylase activity was determined for the different fractions of enzymes. The normal procedure included the following steps: (a) electrophoresis of the extract as described above; (b) heating the microslides to 70° for 15 min before the treatment with agarose–starch solution; and (c) the same procedure as under (a) and (b) after heating the enzyme extract to 60 or 70° for 15 min. The last treatment (c) was included to differentiate between the α-amylase groups and the β-amylase group.

The treatment with the starch solution is a critical procedure. It is necessary that the substrate be spread out in a thin and uniform layer – with the best volume of the starch solution being found by trial and error. If the layer is too thin, eventual limits between the different amylase sections will disappear, whereas too thick layers will make it impossible for all of the amylase bands to break through the covering starch.

Determination of the α-amylase activity

For determining α-amylase activity, the agarose slide was cut into three or four sections, which were disintegrated with a 0·2% calcium chloride solution in a glass homogeniser for 5 min. The suspension was stored for 1 h and then centrifuged. The activity of the enzyme in the supernatant was determined by a kinetic method based on a colorimetric estimation of the decrease of the colour produced when aliquots of the mixture of starch and enzyme were pipetted into a dilute iodine solution at 40° (± 0·2°). When grain or flour was analysed, the normal procedure was to extract 1–5 g of flour with 100 ml of calcium chloride solution with a mixer for 3 min. The extract was filtered through a fluted filter. 10 ml of the enzyme solution were mixed with an equal volume of substrate and the determinations were then performed as described below.

Reagents

A stock solution of iodine was prepared by dissolving 5·5 g iodine crystals and 11g potassium iodide and diluting to 250ml. The solution was stored in the dark in a brown bottle. (A fresh solution should be prepared once a month.)

A dilute iodine solution was prepared by dissolving 40 g potassium iodide in water, adding 4 ml of the stock solution of iodine and diluting to 1000 ml. (This solution should be freshly prepared every day.)

The buffer solution was prepared by dissolving 120 ml conc. acetic acid and 164 g anhydrous sodium acetate (or 272 g hydrated sodium acetate), and diluting to 1 l.

A 0·2% calcium chloride solution was prepared.

To prepare Zulkowsky's starch substrate, 10 g of dry starch were dissolved in about 400 ml of distilled water at room temperature. 25 ml of the buffer solution and 50 mg of β-amylase (free from α-amylase activity) were added. 10 drops of toluene were added to this and the volume was made up to 500 ml. The solution should not be used within 24 h. The extinction of the substrate when 1 ml of a mixture of equal volumes of substrate and water were mixed with 5 ml iodine solution should not be higher than 0·6 on the logarithmic scale.

Colorimeter

The wavelength region of the colorimeter filter used in the routine work was 550–600 nm. It should be noted that not only the intensity but also the wavelength of the iodine colour produced by the substrate changed with decreasing chain length of amylose. An instrument with a filter giving a fairly wide region of wavelengths was therefore necessary.

Control of the colorimeter

The blank was prepared from a mixture of 1 ml of water in 5 ml iodine solution. When this method is applied to cereal grains in which sufficient extract is available, the blank should preferably be prepared from 1 ml of the extract and 5 ml of the iodine solution to compensate for the soluble starch in the enzyme extract.

Determination of the amylase activity

2 ml of the extract were added with a rapid-running pipette to 2 ml of the starch substrate, with the temperature of both solutions being brought to 40° immediately before mixing. This temperature was chosen in order to increase the rate of starch hydrolysis. A stop-watch was started the moment the solutions were mixed. After 5 and 125 min, 1 ml of the mixture was withdrawn and pipetted into 5 ml of the iodine solution and the extinction was read on the colorimeter. In determinations of α-amylase activity in samples of grain, 10 ml of enzyme extract and 10 ml of substrate were used; three readings were made at intervals of 5 min to give three points on a straight line when the logarithm of the extinction was plotted against the time of reaction. In all other details, the same procedure was used.

Calculation of the enzyme activity

The α-amylase activity was calculated from the equation:

$$k(\alpha) = \frac{1000}{a.\ t} (\log E_o - \log E)$$

where $k(\alpha)$ is the activity of the extract, E_o is the extinction on the colorimeter at the time chosen as the start of the reaction, E is the extinction of the starch–iodine complex t minutes after the start. When the method was used with flour or grain, $k(\alpha)$ was calculated for 1 g of flour – extracted with 100 ml 0·2% calcium chloride solution – by dividing with the weight a, which was the quantity of flour extracted with the solution.

Results and Discussion

In Figs 3 and 4, the main characters of the zymograms are presented from extracts of both unripe wheat and malted wheat. In the Figures, areas of more or less complete digestion of the starch are shown by cross-hatching, whilst violet-fringed or ill-defined areas of digestion are indicated by diagonal-line shading. Areas where little digestion has occurred are shown by diagonal lines unbounded by distinct limits.

The extract from malted wheat was characterised by a broad clear band on the negative side, while the extract from green kernels contained no enzymes of this type. The strong fractions farthest from the starting point in the positive direction in Fig. 3 consisted of β-amylase. The fact that these bands were absent in zymograms from malted grain or from reactivated wheat does not necessarily mean that these extracts did not contain any β-amylase. Their absence can be explained as an effect of the dilution, which is necessary in samples with a very high α-amylase activity.

Heating the slides after electrophoresis but before incubation with starch nearly always increased the amylase fractions. This might be interpreted as an activation of amylase by gentle heating; but a more probable explanation perhaps is that the agarose shrinks, causing a local increase in enzyme concentration.

The general appearance of the zymogram in extracts from malted wheat was different from those of malted barley reported by Frydenberg & Nielsen.[8] In order to simplify the discussion on the nature of α-amylases in malt and in green or immature kernels, the zymograms have been divided into three or occasionally into two sections as indicated on the drawings in Figs 3–5. The limits between these groups used in this work are based on previous observations concerning the general nature of the electrophoretic pattern. In most cases each section is composed of several fractions of amylase that, however, very often overlap in the routine work when the concentration is not sufficiently controlled.

Section 1 includes the enzyme fractions remaining in the vicinity of the basin or moving in the negative direction during electrophoresis under the experimental conditions described. These fractions were characteristic for malted wheat and thus consisted mainly of α-amylase in the conventional meaning of this term. By very long malting (8–10 days) several separate bands were always distinguished in this group.

In section 2, which constituted the middle part of the slide, several weak bands of amylase were observed in extracts from unripe wheat. When the grains are approaching the stage of full ripeness or when they are dried slowly, the fractions in this section become very weak and ill-defined and finally disappear completely in the ripe grain. From the reactions to heat treatment, it was concluded that these fractions consisted of α-amylase, though they seemed to differ in some respects from the α-amylases in malted grain, which are shown in section 1. They are probably somewhat more sensitive to heat treatment than the malt amylases, and seem to occupy a position between α- and β-amylase in this respect. Enzymes in section 2 were extracted from the agarose slide and analysed for α-amylase activity. From their appearance, it was concluded that they were associated with the variation in enzymic activity and falling number during ripening reported previously (cf. Figs 1 and 2). They were thus the principal objects of investigation in this work. The different reactions to heat treatment of the individual fractions of amylases in section 2, which are observed in slides marked A3 and B3 in Fig. 3, indicate that the properties of the amylases belonging to this group are not constant during ripening but that the α-amylases in this group become more temperature-sensitive as the kernels approach the stage of full ripeness.

Finally, section 3, which is made up of the amylase fractions at the farthest end in the positive direction, is composed of one or two separate fractions of enzymes with distinct β-amylase properties. No α-amylase activity can be detected by use of the method described above. Whenever a small amount of α-amylase action can be observed, this probably should be assigned to traces of α-amylase belonging to section 2. The enzymes in this section lose their activity when the extract is heated to 60° before electrophoresis. The fact that this group was lacking in extracts from malted wheat or from moistened kernels might be explained as an effect of dilution. A somewhat surprising observation is that all fractions of β-amylase were not equally sensitive to heat when separated on the agarose gels. This is observed, for example, in Fig. 3, where the most apparent fraction farthest from the origin was still partly active after the slide had been heated to 70°.

Table I gives the α-amylase activity of the two sections in slides A1 and B1 of Fig. 3. Three parallels were analysed and the activity was expressed in arbitrary units of $k(\alpha)$. In this experiment a trial was made to study the distribution of α-amylase activity in section 2 by dividing this section into two subsections, 2(a) and 2(b), each consisting of one of the two fractions that were generally found in this group.

The results in Table I confirm that the two fractions in section 2 really consist of α-amylase, whereas the clear bands in section 3 consist of β-amylase. It is clear that the main activity of the α-amylase was found in that fraction on the slide that had the greatest mobility. The fact that some activity was also found in two of the parallels in section 3 might be due to small differences in migration rate on the different slides, which makes the division of the agarose layer hazardous.

The zymogram in Fig. 3 and the results presented in Table I show that the α-amylases in unripe grains constitute a fairly well-defined group of enzymes. No α-amylase activity can be

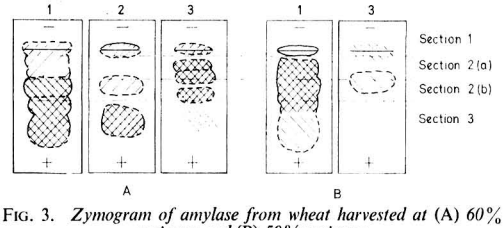

FIG. 3. *Zymogram of amylase from wheat harvested at* (A) *60% moisture and* (B) *50% moisture*

1, Original extract; 2, original extract – slide heated to 70°C after electrophoresis; 3, extract heated to 70°C before electrophoresis

TABLE I

Activity of α-amylase in different sections of the agarose slides of Fig. 3, k (∝) units

Variety A ('Prins') was harvested at nearly 60% moisture content; variety B (Sv 65,500), at 50%

	Section	Slide No. 1	2	3
Variety A:	1	0·0	0·0	0·0
	2 (a)	0·5	0·4	0·4
	2 (b)	1·8	1·8	1·3
	3	0·0	0·0	0·4
Total:		2·3	2·2	2·1
Variety B:	1	0·0	0·0	0·0
	2 (a)	0·1	0·0	0·0
	2 (b)	1·1	0·6	1·0
	3	0·0	0·1	0·0
Total:		1·2	0·7	1·0

found in section 1 in extracts from green kernels. For a comparison, the corresponding results of the kinetic constants from extracts of sprouted wheat (Fig. 4) are given in Table II. The results were expressed in the same arbitrary units as those in Table I.

In this case most of the activity was concentrated in the fractions round the basin and in the negative direction. An important problem was how to explain the activity in sections 1 and 2. In extracts from unripe kernels, the α-amylase activity was found in section 2 exclusively. This activity disappeared more or less completely during ripening and, in extracts from highly malted kernels the α-amylase activity occupied both section 1 and section 2. This might be explained as an effect of the synthesis of a wide range of enzyme molecules during prolonged malting. However, the activity in section 2 was most likely a result of the reactivation of the 'green' amylases. As will be discussed in more detail later, the germination might be regarded as a two-step process– the first step being a reactivation of the primary α-amylases that are formed during the development of the grain, while the second step is a real synthesis of new enzymes. A certain degree of proteolytic action stimulated by germination cannot, however, be excluded; and it is reasonable to suppose that this should cause some variations also in the electrophoretic pattern. It was observed that the longer the malting proceeded the more pronounced was the concentration in the negative direction, i.e. in section 1. The separate bands of amylase were also more distinct with less overlapping. The α-amylase activity shown in Table I was not thus, as a rule, found in normal wheat. When the grains approached the ripe stage, the α-amylases in section 2 continuously disappeared and the $k(\alpha)$ values found in the agarose bands remained on a very low level – 0·2 or below. The results confirmed, however, that it is not justifiable to neglect the influence from green kernels on the average falling number and the baking quality even when the grains reach the normal harvesting time.

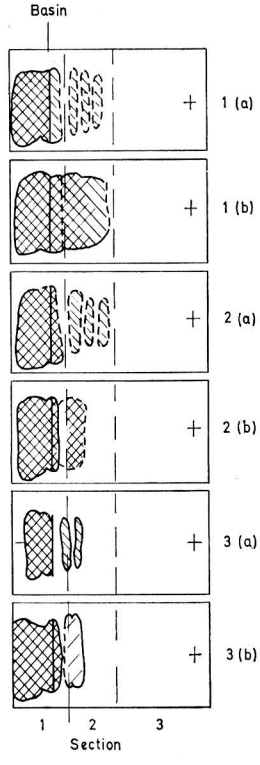

FIG. 4. *Zymogram of amylases from malted wheat*

1 (a) Original extract; 1 (b) original extract and slide heated to 70°C for 15 min; 2 (a) extract heated to 60°C for 15 min before electrophoresis; 2 (b) as for 2 (a) and slide heated to 70°C for 15 min; 3 (a) extract heated to 70°C for 15 min before electrophoresis; 3 (b) as for 3 (a) and slide heated to 70°C for 15 min after electrophoresis

TABLE II

Activity of α-amylase in the different sections of the zymogram from malted wheat (cf. Fig. 4)

Section	Slide No.	
	1	2
1	4·9	4·2
2	2·4	1·6
3	0·2	0·1

Drying generally reduces the enzymic activity. In agriculture and in the grain trade, this is manifested through the increase in the falling number, observed in years with late maturity when normal drying in the field is retarded. The effect of drying on the α-amylases pattern is illustrated in Table III. In this Table the kinetic constants $k(\alpha)$, in sections of the zymogram of extracts from dried kernels of the same sample as that in Fig. 3 and Table I, are presented. A considerable reduction was observed owing to the effect of drying and storage; but also under normal conditions when the wheat had reached a later stage of ripening, improvement was often observed.

The $k(\alpha)$ values of the original undried grains were nearly 200 (variety A) and 90 (variety B), calculated on a dry weight basis. In the corresponding dried grains, the activity had decreased to 160 and 45, respectively. This reduction of activity in the kernels was more pronounced on the electrophoretic slides.

The continuous variations in enzymic activity and falling number observed during ripening are thus associated with the α-amylases in section 2 in the experiments reported above. The range of variations illustrated in Tables I and III might well explain the variations observed in practice. If the activity of the 'green' amylases is of a reversible nature and associated with the natural drying process in the grains or the redistribution of moisture, it should be possible to reactivate

TABLE III

Activity of α-amylase in different sections of the zymogram from dried wheat of the same samples as those in Table I

	Section	Slide No.		
		1	2	3
Variety A:	1	0·3	0·1	0·1
	2	0·3	0·2	0·2
	3	0·2	0·0	0·0
Variety B:	1	0	0	0
	2	0	0·2	0
	3	0	0	0

the enzymes in section 2 by moistening the grains artificially without germination. In order to test this idea and to support the hypothesis mentioned above, the following experiment was performed.

Ripe and dried kernels that did not manifest any α-amylase activity either in sections 1 or 2 were steeped in water for about 1 h and then placed on moist filter paper at 4°. After 3 days, the kernels were crushed in a mortar in a small volume of water. The suspension was centrifuged and analysed in the usual manner. Fig. 5 shows the electrophoretic pattern of this extract. The broad zone of enzymes appearing in section 2 was also composed of at least two fractions. The treatment on the moist filter paper evidently exerted a strong influence on the amylases previously characterised as typical for immature, unsprouted wheat. The enzymes that appeared on the slide as a result of this reactivation have the same physical properties as the original α-amylases appearing in section 2. The obvious similarity between the enzymes in section 2 from extracts of unripe, non-dried kernels and the enzymes reactivated through moistening the dried kernels support the assumption that normal ripening is characterised by a continuous and reversible inactivation of the primary enzymes. The variations in α-amylase activity caused by disturbances in the drying process from the stage of yellow ripeness can explain a variation of 50–100 sec in the falling number from day to day. It is possible that this difference in electrophoretic pattern of α-amylase from reactivated or unripe grains on the one hand, and the malted grains on the other hand might be used to characterise the initiation of true sprouting.

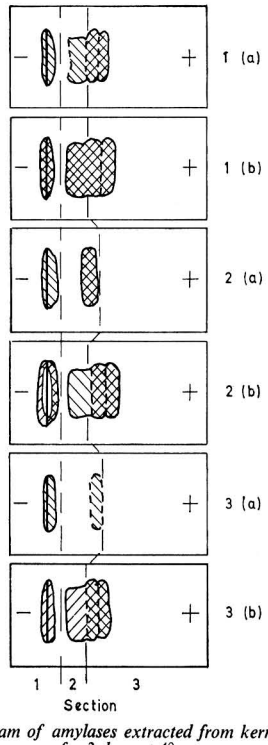

FIG. 5. *Zymogram of amylases extracted from kernels moistened for 3 days at 4°C*
1 (a)–3 (b) as for Fig. 4

That serious damage might appear as a result of reactivation of the primary α-amylase system in wheat, independent of germination, is illustrated in Table IV. In this experiment, the kernels were cut transversely and the germ halves were discarded. The endosperm halves were steeped in water for about 1 h and then stored on moist filter papers for 1 or 2 days at the temperature indicated. The endosperm halves were crushed in a mill and extracted with a 0·2% calcium chloride solution. The activity of the extract was determined by the method described earlier.

Values above 20 on this arbitrary scale indicate serious damage to the baking quality. The results presented in Table IV show that serious 'sprouting damage' might occur without sprouting in the ear; moreover, these results confirm the results shown in Fig. 2, where important declines in quality were observed without sprouting. There was significant effect of the time length as well as of the temperature, which is in agreement with the experiences that a period of moist weather and not too low temperatures might cause a decrease in the falling number even before the grain is fully ripe. The zymograms of extracts from wheat kernels moistened without the germ (Fig. 6) are very similar to zymograms of extracts from whole grains moistened for several days. In this experiment both whole-wheat grains from unripe wheat in the late stages of ripening and halves without the germ were treated on moist filter paper at 4° for 5 days and then crushed in a mortar with a small volume of water. There was an evident similarity between the slides in extracts from whole grains and those from wheat halves (Figs 5 and 6). It is therefore evident from this experiment that the activity of the primary α-amylase is not controlled by a direct influence from the germ and the gibberellin system, which controls the malting process.

From the results reported here, it seems reasonable to accept the hypothesis that two different types of α-amylase exist in cereal grains. The 'malt' amylase appears as a result of DNA-controlled synthesis during germination – a process in which the gibberellins play a central role. In the unripe stages, and when the moisture evaporation is retarded, another form of α-amylase exists. These α-amylases originate as a result of synthesis during the growing period of the grain, but their activity decreases rapidly as the grains approach physiological maturity. The extension of this activation depends on the moisture equilibrium in the grain, which is not identical with the moisture content. Therefore, a temporary increase in α-amylase activity might be stimulated by an increase in humidity at any level of development of the

TABLE IV

Effect of moisture treatment on the α-amylase activity of endosperm halves

Time of treatment, days	Temperature, °C	*k* (α) Sample No. 1	2	3	Mean value
1	3	2·0	1·6	1·6	1·7
2	3	2·8	0·8	0·8	1·5
1	12	0·8	1·6	1·6	1·3
2	12	66·2	58·8	7·7	44·2
1	20	34·0	29·6	29·6	31·1
2	20	31·2	27·2	26·0	28·1

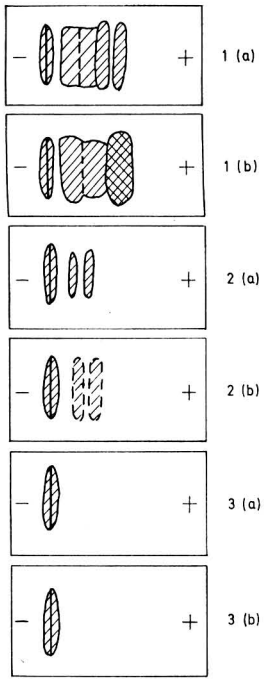

FIG. 6. *Zymogram of amylases from ripe wheat halves without the germ, steeped 3 days at 4°C*
1 (a)–3 (b) as for Fig. 4

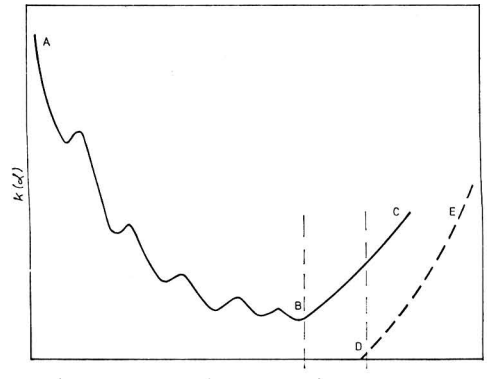

FIG. 7. *Diagram showing the variations in α-amylase activity in unripe wheat grains during ripening and the appearance of α-amylase from malting*
AB, Kernel development until full ripeness. Variations in α-amylase activity caused by changes in moisture distribution; BC, 'primary' α-amylases are re-activated by delayed drying; DE, synthesis of α-amylases during malting
The period between B and D is characterised by a high amylase activity and low falling numbers without the appearance of visible sprouts

wheat. While the 'malt' α-amylase most often shows a thousand-fold increase in activity through a process that probably is of an irreversible nature, the range of activity of the 'green' amylases generally remains on a fairly low level during normal growing conditions and can be continuously activated and inactivated in ripening grain.

Even very small variations in α-amylase activity influence the falling number significantly, especially at the high levels of activity. A $k(\alpha)$ value of 5 on the arbitrary scale used here indicates normal grain with negligible damage. Values above 20 signify that the sample of wheat in question has a very limited value from the milling point of view. It is thus clear that a small inclusion of green kernels might cause apparent deterioration to the quality, irrespective of the fact that no sprouting is observed.

A $k(\alpha)$ value below 1 corresponds to a falling number of 300 or more. With $k(\alpha) = 2$, the falling number decreases to about 250. On the other end of the scale, an increase in $k(\alpha)$ from 5 to 10 reduces the falling number only by 20 or 30 units, or from 150 to 120. The Falling Number Method, therefore over-estimates differences in the upper region. The reliability of the Falling Number Method in its actual form is not the same over the entire range of variation of amylase activity. The results obtained here affirm that it might be unjustified to pay too much attention to the upper part of the scale. If the variation of α-amylase activity in the pre-germination stages is controlled by factors other than sprouting, e.g. the water distribution that in its turn depends on the environmental conditions, the farmer has no real opportunity to harvest at a time of optimum falling number. Over-ambition to harvest early might result instead in an increase in the concentration of 'green' α-amylase. These disadvantages are not generally serious in comparison with those caused by sprouting, but they may still be an annoyance if they cause an amplitude in the variation of the falling number between 50 and 100 units when the nature of this variation is not understood by the farmers and millers.

Finally, in Fig. 7 no attempt has been made to summarise the interrelationships between 'green' α-amylase and 'malt' α-amylase during the final stages of ripening and the start of sprouting in the ear. The solid line represents the variation in the primary, 'green' α-amylase, resulting from changes in water content. The broken line represents the 'malt' α-amylase, which appears as a result of germination. During the time between stages B and D, a very bad correlation is to be expected between the amylase activity and the number of germinated kernels. During this period, the activity of the primary enzyme system, induced by moistening the grains, is the principal cause of damage. Only during the second phase of germination when sprouting begins, is a correlation between the number of visible sprouts and α-amylase activity to be expected.

The effect of drying and storage on the falling number is understandable if it is accepted that two types of α-amylase exist. By harvesting with combines and subsequent drying, the 'green' α-amylase is partly inactivated. The remaining activity is, however, sufficient to cause a variation in the falling number. During storage the reduction in the activity of the 'green' amylases very often continues and the falling number increases.

Communication from The Swedish Seed Association No. 372.

Swedish Seed Association,
Svalöf,
Sweden

Received 24 November, 1969

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ERRATA

In the paper by Anderson & Jackson, *J. Sci. Fd Agric.*, 1970, **21**

Page 231 right hand column, line 7 *for* 5·3% *read* 6·6%

Page 232, Table VI, True protein of silage 3 *for* 1·12 *read* 3·12
Starch equivalent of silage 6 *for* 8·49 *read* 48·49
Crude protein of silage 8 *for* 88·34 *read* 8·34
Total digestible nutrients of silage 8 *for* 69·59 *read* 67·59

Page 233, Table VIII, Cellulose (glucosan) of silo 5 *for* 4·5 *read* 14·5

FOOD AND AGRICULTURE ABSTRACTS

JULY, 1970

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1970

1.—AGRICULTURE AND HORTICULTURE

Soils and Fertilisers

Soil Formation, Classification, Constituents

Mineralogical composition of clay fractions of soils formed from boulder clay of various ages. S. UZIAK (*Annls Univ. Mariae Curie-Sklodowska, Agric. E*, 1968, **23**, 77–86. Pol., 25 ref.).—Glaciated clays of central and eastern Poland were studied by *X*-ray analysis. The main constituents are illite and montmorillonoids.
G. Chinnick.

Allophanic soils in New South Wales. J. BRADLEY and I. VIMPANY (*J. Aust. Inst. agric. Sci.*, 1969, **35** (4), 268–270. 6 ref.).—Soils known to 'fix' large amt. of added P were tested for allophane. These allophanic soils were restricted to areas associated with sub-tropical conditions. M. J. Rawlins.

Study of peaty and semi-peaty soils used in banana culture in the Tamatave region. J. KILIAN (*Fruits d'outre mer*, 1970, **25** (1), 35–45. Fr., 15 ref.).—A detailed description of the soils in this warm, humid region, is given. Banana yields, 5–6 yr after planting, were almost nil. Possible causes are: periodical swamping which, despite drainage, kills the plants; settling of soil after trenching to correct depth; movements of acids, bases and exchangeable Al, necessitating use of phosphate or lime. M. T. Rawnsley.

Physical Properties of Soils

Problems of sandy soils in their initial period of reclamation. Y. ABD-EL-MALEK and Y. Z. ISHAC (*Biotechnol. Bioengng Symp.*, 1967, *No 1, 2nd int. Conf. global Impacts appl. Microbiol.*, 1969, 293–298. 1 ref.).—Heavy dressings of slowly decomposing org. substances could be used to combat low moisture, poor ion-exchange capacity and scarcity of C and N. Addn. of sawdust to sand silts, over 120 days, raised H_2O-holding and cation exchange capacities 3–4-fold and benefited N content. Breakdown products of cellulose of the sawdust provided an energy source for *Azotobacter* which increased proportionately to the increase in sawdust.
M. J. Rawlins.

'L-value' determination under paddy soil condition. S. LARSEN (*Pl. Soil*, 1969, **31** (2), 282–286. 4 ref.).—The L values of 4 paddy soils and a U.K. soil growing rice under water-logged conditions attained const. levels during the growing period, indicating that isotopic equil. was achieved between added ^{32}P-labelled PO_4^{3-} and the soils. Under aerobic conditions with ryegrass, the equil. L values tended to be lower than those determined under water-logging. A. H. Cornfield.

Biological Aspects, Available Nutrients, Soil Analysis

Volatile factor in soil fungistasis. T. S. HORA and R. BAKER (*Nature, Lond.*, 1970, **225** (5237), 1071–1072. 15 ref.).—The sterile 'Cellophane' agar diffusion, the soil emanation agar (no contact between soil and discs), and the direct assay methods were used in a comparative study of volatile and diffusible fungistatic factors. Results revealed the presence in soils of a volatile inhibitory factor which contributed significantly to decreased response of fungal spores (*Trichoderma, Zygorhynchus, Penicillium* and *Aspergillus*) esp. in alk. soil. In general, a decline and steady state of inhibition was reached after 10–15 days, whilst the max. germination in acid or neutral soils reflected the influence of pH, esp. in recently moistened soils. Contact with soil was unnecessary for inhibition of germination. Alk. loams and sandy loams (1·5–4·7% org. matter) contained a lower concn. of volatile factor than did clay loams (0·8–2·2% org. matter).
W. J. Baker.

Non-biological reduction of nitrite in soil. L. A. BULLA, JUN. C. M. GILMOUR and W. B. BOLLEN (*Nature, Lond.*, 1970, **225** (5233), 664. 17 ref.).—Expt. reveal that NO, N_2 and N_2O are the products of the non-biol. decomp. of NO_2^- (as $Na^{15}NO_2$ and/or $Na^{14}NO_2$) in dry sterile silt-clay-loam soils at 25° in O_2-free He atm. Evolution of N_2 and NO from alk. and acid soils suggests that other factors (possibly metal ions, org. matter and clay) as well as pH are concerned in the pattern of gas production. Autoclaving had negligible effect on the decomp. W. J. Baker.

Study of denitrification in soil, especially in the presence of buried straw, using nitrogen-15 as tracer. G. GUIRAUD and Y. BERLIER (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (14), 1000–1007. Fr., 8 ref.).—An argilo-calcareous soil was used. Tests were done with soil + 2% wheat straw under Ar or air, and with soil + Italian rye-grass + KH_2PO_4 + $Ca(NO_3)_2$ with 5·15% of ^{15}N, with or without 1% straw. All were lab. expt. Results show that straw in soil has a depressive effect on the harvest, because of abnormal losses of N by denitrification. Results are not necessarily indicative of field conditions, as other factors, e.g., green fertiliser and heavy rains, may have similar effects. M. T. Rawnsley.

Some properties of the nitrogen fixing particles from *Azotobacter vinelandii*. M. S. NAIK and D. J. D. NICHOLAS (*Indian J. Biochem.*, 1969, **6** (3), 111–114. 14 ref.).—The N-fixing small particles (P6), prep. by differential centrifugation up to 144,000 g for 6 h, from cell-free extracts of *Azotobacter vinelandii* have higher $NADH_2$ oxidase activity than the P1 particles (up to 100,000 g for 1 h); the oxidase activity is partially insensitive to CN^-. The P1 and P6 particles contain most of the ATP-dependent hydrogenase, $NADH_2$ dehydrogenase, and $NADH_2$ viologen reductase. The P6 particles also contain an active ATPase, NADP transhydrogenase, and lipoamide dehydrogenase. Enzyme and respiratory activity of these intracellular substances were examined.
J. N. Ashley.

Nitrogen transformations and movement in soils. A. S. J. REID, G. R. WEBSTER and H. R. KROUSE (*Pl. Soil*, 1969, **31** (2), 224–237. 15 ref.).—A portion of the NH_4NO_3 (^{15}N-labelled NH_4^+) added to a chernozem and a podzol was immobilised initially as amino acid and acid-sol. humin, but was subsequently re-mobilised as mineral N. The chernozem immobilised more of the applied N in active forms (amino acids and acid-sol. humin) and less in inactive forms (amino sugars and hydrolysable org. N) than did the podzol. Response of barley to applied N was greater on the chernozem than on the podzol. The chernozem adsorbed more applied NH_4^+ than did the podzol in the A horizons, but the reverse was true in the C horizons. A. H. Cornfield.

Importance of the participation of the sub-soil for the establishment of organic carbon and nitrogen balance of soils. M. D. COLLIER (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (15), 1097–1109. Fr., 10 ref.).—Results from further tests caused the author to revise his previous opinions (*ibid.*, 1966, 1094–1102 and other work). M. T. Rawnsley.

Changes in extractable organic phosphorus in soil in presence and absence of plants. G. S. SEKHON and C. A. BLACK (*Pl. Soil*, 1969, **31** (2), 321–327. 6 ref.).—Moist incubation of 6 soils decreased extractable org. P, whilst cropping the soils with 4 successive crops (with removal of roots after each crop) decreased extractable org. P to an even greater extent. A. H. Cornfield.

Fertilisers

Influence of ameliorative ploughing and deep organic fertilising on the content and distribution of organic material and on some chemical properties of a sandy soil. S. NAWROCKI and T. KESIK (*Annls Univ. Mariae Curie-Sklodowska, Agric. E*, 1968, **23**, 97–107. Pol., 13 ref.).—The effects of deep (45 cm) ploughing, and of treatments with farmyard manure, peat and clay, on the content of org. matter, total N, available P, and K in a podzol soil, were studied over 6 yr.
G. Chinnick.

Influence of ameliorative ploughing on the content and distribution of the root mass, non-decomposed plant remains, P_2O_5 and K_2O in a sandy loam soil. S. NAWROCKI and K. SZYMANKIEWICZ (*Annls Univ. Mariae Curie-Sklodowska, Agric. E*, 1968, **23**, 109–122. Pol., 19 ref.).—Deep (45 cm) ploughing, with and without applications of farmyard manure, considerably increased the org. content, K_2O and available P_2O_5 in the lower layers of the soil.
G. Chinnick.

Influence of intensive fertilising and irrigation on some crops grown on loess soil. L. MALICKI (*Annls Univ. Mariae Curie-Sklodowska, Agric. E*, 1968, **23**, 123–149. Pol., 45 ref.).—The effects of three irrigation regimes and two levels of mineral fertilising on yields of cereals and legumes and on soil analysis were studied for 3 yr on podzolic and brown soils. Treatment must be adjusted to suit weather conditions and the variety of crop which is being cultivated. G. Chinnick.

Superphosphate on wheat: influence of previous level of application on current response. V. F. MCCLELLAND (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 622–624. 7 ref.).—With the cycle wheat–oats–fallow, the effect of current superphosphate decreased with increased previously applied superphosphate. This was not the case with wheat–pasture–pasture–fallow, presumably due to a soil P factor. M. T. Rawnsley.

Responses by pasture to current and previous applications of superphosphate. I. H. CAMERON and A. A. MCGOWAN (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 617–621. 12 ref.).—Two harvests were taken, in order to follow seasonal variations. Residual effects from 1 cwt of superphosphate per acre were almost negligible, but heavier dressings, previously applied, can supply a 'bank' of phosphate. M. T. Rawnsley.

Extractability of zinc granulated with macronutrient fertilisers in relation to its agronomic effectiveness. J. J. MORTVEDT and P. M. GIORDANO (*J. agric. Fd Chem.*, 1969, **17** (6), 1272–1275. 14 ref.).—The fertilisers (*F*) were extracted by various reagents and % recovery of the total Zn by each extractant was correlated with agronomic effectiveness of Zn in these *F*. The % recovery of Zn from *F* by de-ionised H_2O, 0·01 and 0·001 N-HCl, an acidic K_2SO_4 soln., and soln. contg. chelating agents in each case was highly correlated with agronomic effectiveness; the correlation was highest with H_2O. The soln. pH of orthophosphate *F* was inversely related to the agronomic effectiveness of Zn in the *F*. There was no relationship between total Zn in a *F* and its immediate availability to plants. I. Dickinson.

Studies on the availability of MnO_2 added to soils. K. C. MISHRA and S. G. MISRA (*Boln. Inst. nac. Invest. agron., Madrid*, 1969, **29** (1), 103–113. Engl., 19 ref.).—Lab. studies of the effect of addn. of $CaCO_3$ (0·4 and 4%), glucose (2%) and $FeSO_4$ (4 and 8%) on availability of MnO_2, 30–180 days after addn. to black, red and alkali soil, are reported. Availability was generally increased by addn. of glucose or $FeSO_4$ and reduced by $CaCO_3$.
E. C. Apling.

[A] Effect of some fertilisers and micronutrients on added molybdenum in soils of Uttar Pradesh (India). [B] Total and available molybdenum in soils of Uttar Pradesh (India). S. G. MISRA and K. C. MISHRA (*Boln. Inst. nac. Invest. agron., Madrid*, 1969, **29** (60), 85–92. 4 ref.; 93–101. 8 ref. Engl.).—[A] Lab. studies of the effect of addn. of $(NH_4)_2SO_4$, superphosphate, humic acid and various micronutrients on retention of added sol. Mo in black, red and alkali soils are reported. Mo retention was generally decreased by addn. of superphosphate (except in red soils) and increased by $(NH_4)_2SO_4$ and humic acid. Mo was completely converted to insol. form by addn. of Zn^{2+} or Al^{3+}, but retention was generally reduced by addn. of Cu^{2+} or Fe^{2+}, while Mn^{2+} reduced retention in red soils but increased it in black and alkali soils.

[B] Availability was studied in 27 surface samples and in soil profiles from 7 black, red and alkali soils. Total Mo varied from 0·87 to 7·80 ppm, generally decreasing with depth of profile. The amt. of org. Mo was generally highest in black, and least in alkali soils. E. C. Apling.

Properties and value of 1,1-diureido isobutane (IBDU) as a long-lasting nitrogen fertiliser. O. R. LUNT and S. B. CLARK (*J. agric. Fd Chem.*, 1969, **17** (6), 1269–1271. 7 ref.).—Hydrolysis of sol. IBDU to urea and then to NH_3 occurs readily under most soil conditions. Particle size influences dissolution rate; smaller particles dissolve more readily. Plants effectively use IBDU at pH ranges from below 5 to above 8. Conversion of IBDU to urea and NH_4^+ or NO_3^- does not occur readily under alk. conditions, but the effect is not large enough to present a problem in using IBDU in soils up to at least pH 8·3. Up to 32 lb of N/1000 ft^2 can be incorporated into soils without important detrimental effects. Plant response indicates a steady supply of N from IBDU for one or two months, or longer, if large applications are made or relatively coarse granules are used. I. Dickinson.

Sulphur coated urea. TENNESSEE VALLEY AUTHORITY (*Sulphur Inst. J.*, 1968, **4** (3), 2–6).—Based on the work of Rindt and Blouin (*J. agric. Fd Chem.*, 1968, **16** (5), 773, and U.S. Pat. 3,295,950 and 3,342,577) the production of this fertiliser (*SCU*) is described. Bermuda grass, grown for 16 weeks and clipped at 2 week intervals was used and comparative results are discussed. *SCU* reduced the toxicity of sol. N to plants, promoted more uniform growth of grasses and reduced 'luxury' consumption of N. Large scale autumn application of *SCU* may become suitable in humid areas without large winter loss of N by leaching or denitrification. The use of *SCU* for sugar-cane and crops with long growing periods which require repeated application of sol. N sources is discussed. The economics of *SCU* use are presented. C. V.

Basic slag [fertiliser]. FISONS FERTILIZERS LTD. and STEEL CO. OF WALES LTD. (Inventors: G. G. BROWN and D. C. HARPER (Br. Pat. 1,179,246, 14.10.66).—The P-content of a molten L.D. steel-making basic slag is enriched by adding $\geq$ 80% by wt. of a phosphate rock containing Al phosphate, e.g., the pseudowavellite (CaAl phosphate) rock from the Thies mine in Senegal. It may also be necessary to add some CaO or SiO_2 to the slag to obtain a final product containing gehlenite (2CaO, Al_2O_3, SiO_2) and nagelschmidtite (7CaO, $2SiO_2$, P_2O_5). J. A. Sugden.

Liquid fertiliser suspension containing ureaform. ALLIED CHEMICAL CORP. (Br. Pat. 1,180,884, 30.5.68. U.S., 6.6.67).—The non-resinous product obtained by heating urea and HCHO (1:1–2:1) in presence of NH_3 is diluted with water to a concn. of 35–65% by wt., the pH reduced to < 5 with, e.g., H_3PO_4, and the mixture heated at 30–80° until the water-insol. N content is 4–10%. Finally an alkaline material (NH_4 salt) is added to raise the pH above 5. S. S. Chissick.

[Fertiliser] composition comprising a solid petroleum hydrocarbon. BRITISH PETROLEUM CO. LTD., (Inventors: A. GALANAKIS and A. BELOT) (Br. Pat. 1,177,627, 18.2.66).—The compn. is prep. by mixing a petroleum hydrocarbon (asphalt) at a temp. between its m.p. and its decomp. temp. with $CaCO_3$, dolomite, bentonite or clay particles or granules, and cooling the product.
J. M. Jacobs.

Fertilisers and their use. BRITISH PETROLEUM CO. LTD. (Inventors: C. GUDIN and D. A. B. LLEWELYN) (Br. Pat. 1,177,077, 13.12.65).—The fertiliser consists of, or contains, a mixture of solid or liquid hydrocarbons ($\leq$ 10 C) (**I**) and the resting cells of living micro-organisms (mould, yeast or bacteria), e.g., *Azobacter vinelandii*, adapted for growing on **I**. S. S. Chissick.

Plant Physiology, Nutrition and Biochemistry

Light, Air and Water Relationships

Relationship between moisture stress, internal processes and growth in sugar-cane plant. A. H. KHAN and R. SAMANIEGO (*W. Pakistan J. agric. Res.*, 1969, **7** (2), 13–20. Engl., 7 ref.).—The degree of stress is shown to be an index of plant growth. Stomatal size remained unaffected by changes in relative turgidity, but the rate of photosynthesis fell when relative turgidity was < 80%, and stopped at 70%. The presence of growth hormones may be a controlling factor. M. T. Rawnsley.

Plant Nutrition and Metabolism

Uptake of applied selenium by agricultural plants. I. Influence of soil type and plant species. B. BISBJERG and G. GISSEL-NEILSEN (*Pl. Soil*, 1969, **31** (2), 287–298. 15 ref.).—The uptake of Se from 6 soils (representing most of the Danish agricultural soils) was, on average, 8 times greater where Se was applied at SeO_4^{2-} than as SeO_3^{2-}. Uptake decreased with increasing soil clay content. The Se% in plants varied with stage of development and plant part. Seed was usually higher than straw in Se%. Four species of cereals absorbed less Se than did clovers, radish, mustard and ryegrass. A. H. Cornfield.

Mechanism of grain contamination by radionuclides. W. NIERLE and H. D. OCKER (*Getreide Mehl*, 1970, **20** (2), 9–13. Ger., 15

ref.).—Studies of the uptake and localisation of ^{85}Sr, ^{54}Mn, ^{60}Co and ^{134}Cs by grain following application to leaves or roots of summer and winter wheat plants at various stages of growth are reported. All nuclides were taken up more by outer layers of the grain than by the endosperm, but relative contamination of endosperm with ^{85}Sr and ^{134}Cs was reduced and of ^{54}Mn and ^{60}Co increased as date of application was delayed (from flowering to yellow ripe). E. C. Apling.

Morphological and physiological effects of palladium on Kentucky bluegrass. W. G. Benedict (*Can. J. Bot.*, 1970, **48** (1), 91–93. Engl., 5 ref.).—Seedlings were grown in nutrient soln. to which $PdCl_2$ (at pH 5) was added in concn. of 0·5, 3, 10, 100 or 500 mg/l. Above 3 mg/l, transpiration ceased, but toxicity occurred at 10 mg/l. Cell changes included aberrant stomatal histogenesis, inhibition of nodal meristem development, changes in chloroplast structure etc. M. T. Rawnsley.

Carbohydrate movement in pea plants in relation to axillary bud growth and vascular development. I. F. Wardlaw and D. C. Mortimer (*Can. J. Bot.*, 1970, **48** (2), 229–237. 20 ref.).—Dormancy of the bud in the axil of leaf 4 of pea plants was broken within 4 h of decapitation of the shoot apex. After 24 h, there was an increase of ^{14}C-labelled assimilates by the bud, following assimilation of $^{14}CO_2$ by the lamina of leaf 4 and an increase in total sugar per bud. A similar increase in starch was noted. Vascular differentiation was complete 48 h after decapitation. It is suggested that apical dominance was not acting because of inadequate development of the bud vascular system. M. J. Rawlins.

Colonisation, isolation and cultural descriptions of *Thelephora terrestris* and other ectomycorrhizal fungi of shortleaf pine seedlings grown in fumigated soil. D. H. Marx, W. C. Bryan and L. F. Grand (*Can. J. Bot.*, 1970, **48** (2), 207–211, 13 ref.).—19 fungal symbionts were isolated from ectomycorrhizae of 7–9 month old seedlings. There were 5 distinct cultural groups; one was culturally identical with isolates of *Thelephora terrestris*. The other 4 groups belonged to different species. $HgCl_2$ (100 ppm) and $CuSO_4$ (2·5 and 5%) were successful as surface sterilants, in attempts to isolate symbionts. M. J. Rawlins.

Germination, Growth Regulation, Senescence

Gibberellins and inhibitors in ripening barley seeds. A. Rejowski (*Bull. Acad. pol. Sci. Sér. Sci. biol.*, 1969, **17** (10), 641–644. Engl., 12 ref.).—Giberellins and inhibitors appear during maturation and vary considerably in their activity, there being two peaks at embryo formation and at accumulation of storage substances. M. T. Rawnsley.

Action of the retardant CCC and of gibberellin on growth and flowering of lemon and peach plants. M. Kh. Chaĭlakhyan and T. V. Nekrasova (*Dokl. Akad. Nauk SSSR*, 1969, **189** (4), 905–908. Russ., 11 ref.).—Tests were conducted on *Prunus persica* and *Citrus limon* using gibberellin (gibberellic acid) 0·01% and the retardant CCC (chlorocholine HCl) 0·1–2·0%. Treatment of cuttings with a mixture of betaindolyl-butyric acid (10 mg/l) and CCC (0·5%) resulted in budding, absent on controls. Gibberellin and CCC function in opposite ways in activating generative growth of cuttings in lemons and peaches. Gibberellin raises the tendency to vegetative growth while CCC increases generative growth, in both cases by indirect action. L. A. Haddock.

Role of some inhibitors in potato dormancy and sprouting. M. Bielińska-Czarnecka and J. Domańska (*Bull. Acad. pol. Sci. Sér. Sci. biol.*, 1969, **17** (10), 635–639. Engl., 7 ref.).—Three varieties (early, quick sprouting; late, very quick sprouting; and late, difficult sprouting) were used. Tubers were soaked in a soln. of 50 ppm of K salt of gibberellic acid at 26° for 24 h, or in 0·3% soln. of maleic hydrazide for 24 h at room temp. Half were then stored at 2°, half at 8° for 5 months. Results showed that acceleration of sprouting was always connected with inhibitor concn. and with decrease of abscisic acid. Gibberellic acid enhanced growth of sprouts in all tubers but had varied effects on sprouting. Maleic hydrazide was not successful. M. T. Rawnsley.

Effect of maleic hydrazide on the growth of suckers in tobacco. M. Afzal and M. Rashid (*W. Pakistan J. agric. Res.*, 1969, **7** (2), 34–37. Engl., 5 ref.).—A 3-yr expt. on 6 concn. of maleic hydrazide (0·00, 0·25, 0·50, 0·75, 1·00 and 1·25%) used after 24 h of initial topping, is described, using T-17 variety. Lower concn. depressed sucker growth and enhanced leaf yield, but higher concn. depressed sucker growth and reduced tobacco yield. Cell enlargement and division were also affected. M. T. Rawnsley.

Floral inducing extract from *Xanthium*. H. K. Hodson and K. C. Hammer (*Science, N.Y.*, 1970, **167** (3917), 384–385. 12 ref.).—Flower formation was initiated in *Lemna* by acetone extracts of flowering crocklebur (*Xanthium*). These extracts also initiated flower formation in *Xanthium* when supplemented with gibberellic acid. Further effects are described. C. V.

Brassins—new family of plant hormones from rape pollen. J. W, Mitchell, N. Mandava, J. F. Worley *et al.* (*Nature, Lond.*, 1970. **225** (5237), 1065–1066. 6 ref.).—These hormones were prep. in pure state (as an oil) by extraction of the pollen, followed by t.l.c. on SiO_2-gel, location, removal and ethanolic extraction of the active zone and further t.l.c. The 'brassins' induced marked elongation of bean plants in the second internode test, the response being histologically different from that induced by gibberellic acid. The n.m.r. spectrum resembles that of fatty acid esters, with indications of a glyceride structure. Gas chromatog. and mass spectrometry reveal components with mol. wt. 250–580, whilst gel chromatog. with CH_2Cl_2 yielded five active components distinguishable by u.v. absorption at 254 nm. Similar hormones were obtained from older pollen. W. J. Baker.

Ethylene, natural regulator of leaf abscission. M. B. Jackson and D. J. Osborne (*Nature, Lond.*, 1970, **225** (5237), 1019–1022. 6 ref.).—Accumulation of C_2H_4 in lab. incubation tests has hitherto precluded accurate interpretation of abscission results. By continuous removal of the C_2H_4 by absorption in 0·2 M-$Hg(ClO_4)_2$–0·2M-$HClO_4$, it is shown by flask expt. that leaf-fall is initiated by increased production of C_2H_4 in senescing cells close to abscission zones, the C_2H_4 controlling the necessary biochem. stages. With explants of *Phaseolus vulgaris* and *Prunus serrulata senriko*, this increased concn. of C_2H_4 occurred after ~150 h., the rate of production decreasing when abscission was complete. The potentially interfering effect of wound C_2H_4 does not influence abscission, which is not regulated by any C_2H_4 present before the elapse of ~24 h. W. J. Baker.

Crops and Cropping

Field Crops

Effects of weather on wheat yields. J. Lee and M. J. Connaughton (*Ir. J. agric. Res.*, 1969, **8** (3), 349–357. 16 ref.).—Correlation and regression methods were used to investigate relationship between yield fluctuations and weather variables in 3 Irish counties from 1947–65. Strongest associations were found between the fluctuations and (*a*) rainfall in May–July and (*b*) sunshine and temp. in April and May. When yields were predicted outside the study period, results were satisfactory for 1966, but in 1967, when acreage under a new wheat variety increased, large discrepancies were apparent. M. J. Rawlins.

Influence of some leguminous crops (spring vetch, field pea, grass pea and flatpod pea vine) on the yield of winter wheat. B. Styk and T. Przybysz (*Annls Univ. Mariae Curie-Sklodowska, Agric. E*, 1968, **23**, 87–95. Pol., 5 ref.).—The effect of these legumes on the yield of a following crop of winter wheat was studied for 3 yr. The newly introduced grass pea and flatpod pea appear to give as good results as the traditional crops, but results were considerably influenced by a very large variation of rainfall from one season to another. G. Chinnick.

Response by wheat to phosphorus and nitrogen with particular reference to 'haying off'. P. R. Dann (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 625–629. 8 ref.).—Four pastures, some with high fertility, and some with recent superphosphate, were fallowed to make beds for Heron wheat. Various applications of $(NH_4)_2SO_4$ and superphosphate were made. The only site to be affected by harvest time was one of low fertility and no previous fertiliser. More shoots occurred at this site, but no. of grains per ear was smaller. In general, response to P was higher than to N. Induced soil moisture deficiency due to vegetative growth (caused by increased P) is possibly a contributing, but not controlling, factor causing 'haying-off'. M. T. Rawnsley.

Optimal application of N fertilisers to bread grains. E. Saalbach (*Getreide Mehl*, 1969, **19** (10), 77–80. Ger., 15 ref.).—Results of studies of the effect of various levels of N on yield and N content of wheat and rye are reviewed. Results showed that no general optimum level of application could be prescribed, since optimum level was affected by application of CCC, the weather and application of other fertilisers. Level of N application must depend on location, variety and management. E. C. Apling.

Effect of growth location and nitrogen fertilisation on the quality of barley, wheat and malt. R. SCHILDBACH (*Mschr. Brau.*, 1969, **22** (12), 361–369. Ger.).—The cereals were grown under normal farming conditions in fields in widely different locations with different fertiliser rates. Weather conditions and quality of crop were recorded. In general, crops from different locations varied more than different varieties grown in the same location. Barleys, differing by 3% in protein content, could be obtained from different regions with identical fertilisation. In a given region, increase in applied N gave improved yields; the protein of the grain increased and the protein modification, extract difference and attenuation were adversely affected. These changes were reversed at high altitude. It was observed that farming procedures could mask some of these trends. It is concluded that the best growing areas should be reserved for wheat, with barley being grown in the intermediate areas where its suitability for brewing is greatest.
J. B. Woof.

Changes in some rye quality factors during ripening. D. WEIPERT (*Getreide Mehl*, 1969, **19** (11), 81–86. Ger., 14 ref.).—Studies of changes in 1000-corn wt., contents of total and sol. protein and pentosans, total starch, diastatic activity (α-amylase, falling no., maltose fig., amylogram max.), dough η and η of water extract during the last month of ripening are reported for rye crops at two locations in 1968. During ripening the gross compn. changed only slightly, but total sol. material and enzymic activity increased and η fell with increase in 1000-corn wt. E. C. Apling.

Nutrient concentration curves for oats and barley at different times of the growth period. E. BOKEN (*Pl. Soil*, 1969, **31** (2), 311–320. 13 ref.).—When harvested during the early stages of growth, the Mn% and P% of oat and barley varieties, grown on Mn- or P-deficient soils treated with varying levels of Mn^{2+} or PO_4^{3-}, increased rapidly with dry matter yields and level of applied Mn or P. When harvested at the later stages of growth, Mn% and P% of the plant tissue decreased and dry matter yields increased with increasing initial levels of application of Mn or P, but both increased with further rates of application of either nutrient. Thus the ability of forecasting dry matter yields from tissue nutrient content during growth will vary depending on the stage of growth at which the plant is analysed. A. H. Cornfield.

Effects of heat waves on grain sorghum at the stage of head emergence. D. PASTERNAK and G. L. WILSON (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 636–638. 2 ref.).—Moist and dry heat waves were simulated, and it was found that heat, not moisture, was responsible for killing heads. Generally, only enclosed flowers were affected. Pollen was only slightly reduced.
M. T. Rawnsley.

Effect of a legume on soil nitrogen mineralisation and percentage nitrogen in grasses. H. F. BIRCH (*Biotechnol. Bioengng Symp.*, 1967, *No. 1, 2nd int. Conf. global Impacts appl. Microbiol.*, 1969, 299–303).—An effect of *Desmodium uncinatum* on 3 associated grasses was studied. The grasses were cut back at the start of the rains (Phase 1), the period of scattered showers (Phase 2) and the period of heavier rainfall (Phase 3). Soil and grass samples were taken and analysed for N after the phases. % N was increased significantly in the grasses after the first cutting back.
M. J. Rawlins.

Effect of density and fertility on the competitive interactions of diploid and tetraploid ryegrasses during early growth. J. NORRINGTON-DAVIES and J. G. CROWLEY (*Ir. J. agric. Res.*, 1969, **8** (3), 359–374. 7 ref.).—3 each of diploid and tetraploid varieties of *Lolium perenne* were grown in all possible pair combinations under different conditions of density and soil fertility, for 6 months. Mixtures of diploid varieties had no advantage over diploid pure stands, nor tetraploid mixtures over pure stands of tetraploids. In some cases, diploid/tetraploid mixtures outyielded mixtures of both tetraploids and diploids. Further expt. have been designed to test whether these same interactions would occur on a field plot scale. M. J. Rawlins.

Enzymic browning and free tyrosine in potatoes as affected by level of treatment with pentachloronitrobenzene (PCNB). J. P. SWEENEY (*J. agric. Fd Chem.*, 1969, **17** (6), 1412–1413. 7 ref.).—Three varieties were grown in soil treated with PCNB at 25 lb/acre. PCNB at that level tended to lower free tyrosin in the potatoes. In Pungo potatoes, reduction in enzymic browning was also indicated. Decreases in both free tyrosine and enzymic browning in potatoes treated with PCNB at 25 lb/acre were not as great as those previously reported for potatoes grown in soil treated with PCNB at 50 lb/acre. I. Dickinson.

Degeneration of seed potatoes in the north-eastern region of the Lublin Voivodship. J. SKALSKI, (*Annls Univ. Mariae Curie-Sklodowska, Agric. E*, 1968, **23**, 339–362. Pol., 26 ref.).—In this area, during a 4 yr trial, two susceptible potato varieties remained immune to leaf-roll and mosaic virus, independently of the fertiliser treatment applied. The effect of infection on the amino acid content of the tubers is discussed. G. Chinnick.

Prevention of boron deficiency in beets by means of fertiliser sprays. B. VAN LUIT (*Landbouwvoorlichting*, 1969, **27** (9), 321–324. Dut., 3 ref.).—Results show a connection between % of B in the soil and in the leaf, relative yield and existence of heart rot. Sixteen, 16–8 and 4 kg of borax/ha are required when soil B concn. are 0·20, 0·20–0·30 and 0·30–0·35 ppm, resp.
J. C. T. Niewenhuis.

Comparative trials of sugar-beet varieties. Seasons 1959 to 1963. E. PRIETO HERAUD, J. CARBONELL ANTOLI, and J. J. MATEO MARTINEZ (*An. Inst. nac. Invest. agron., Madrid*, 1969, **17** (3), 461–767. Span.).—Results of field trials at 14 stations during five seasons, covering 60 varieties (generally 25 per station) are reported in detail. E. C. Apling.

Horticultural Crops

Trace metal deficiencies in orange trees. II. Response of trees to corrective treatments for deficiencies of zinc, manganese and boron. J. M. CARRASCO, P. CUÑAT and E. PRIMO (*Revta Agroquim. Tecnol. Aliment.*, 1969, **9** (4), 554–563. Span.).—A study of the response of orange trees to various forms of treatment, applied in the spring during blossom, is reported. Foliar treatment with 0·7%-$ZnSO_4$, neutralised with CaO or Na_2CO_3, was more effective than application of $ZnSO_4$ alone. Mn deficiency was also successfully treated by foliar spray, and annual treatments are recommended. Spraying with 0·1%-H_3BO_3 or $Na_2B_4O_3$ or fertiliser application of 100 g of boric acid per tree were effective in reducing the proportion of gum pockets in fruits from trees showing low initial foliar B. E. C. Apling.

Achene spacing of strawberries as aid to calculating potential yield. A. J. ABBOTT and R. A. WEBB (*Nature, Lond.*, 1970, **225** (5233), 663–664. 3 ref.).—From an equation relating berry fresh-wt., achene spacing and achene no., it is possible to calculate the shortfall in yield due to sub-max. receptacle development. The results for achene-spacings of 6–10 achenes/cm² show up to 40% gap between actual and theoretical yield and emphasise the need for improved environmental control during the final stage of development. Method should be applicable to other similar fruits.
W. J. Baker.

Some effects of gibberellic acid and Cycocel on grapevines. B. M. EL-ZEFTAWI and H. L. WESTE (*J. Aust. Inst. agric. Sci.*, 1969, **35** (4), 274–276. 5 ref.).—Small field trials of gibberellic acid (GA) and Cycocel CCC were made on 3 grape varieties. The treatments, applied by spraying the whole vine, involved various combinations of the 2 hormones. 100 berries from each vine were harvested, and berry wt. and sugar content of juice were detd. In trial I on Sultana, the GA effects overrode those of CCC. In trial II on Gordo Blanco, GA (1 ppm and 5 ppm) gave fewer berries/bunch and increased berry wt. Trial III on Ophanez showed that GA sprays at anthesis were associated with reduced berry no. and increased berry wt. M. J. Rawlins.

Root morphology of the vine. Relationships with production, density and arrangement of the vineyard. L. HIDALGO and M. R. CANDELA (*Instituto Nac. Invest. agron., Madrid*, 1969, 101 pp. Span., 26 ref.).—Extensive studies of root morphology in a vineyard planted in 1951 are reported. Results show that amt. of root system per vinestock, and strength of stock, reduced progressively with planting *d*, but that total root *d* (root system/m² of soil) and production increased. Optimum production was obtained with 2500 stocks/ha, planted with row spacing adequate for cultivation.
E. C. Apling.

Influence of 2,4,5-T on the maturation and marketing of Canino apricots. A. ALBERT B. and J. M. MARTÍNEZ J. (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18** (1), 87–107. Span., 9 ref.).—Studies of the effects of treatments with 25, 40 or 50 ppm of 2,4,5-T, applied 35 days after petal fall, on fruit maturation and properties are reported. After treatment with 50 ppm of 2,4,5-T, fruit reached similar maturity index, juice content and texture 4–5 days earlier than untreated controls and weighed 15% more without deformations or increased wt. of stone. Treated fruits suffered greater loss in wt. and were more subject to bruising than untreated controls and were particularly subject to an external greying of

fungal origin during refrigerated transport. All these effects were minimised by immersion treatment with *o*-phenylphenol before air cooling. E. C. Apling.

Plantation Crops

Direct determination of fibre in cane. P. A. PRINCE (*Proc. S. Afr. Sug. Technol. Ass. 43rd Ann. Congr.*, 1969, 144–145. Engl.).—A homogeneous sample contg. fibres ~ 0·25 in long was added to water and then extracted with a high speed extractor for ~ 4 min to give very small particles. These were drained under vac. on a sintered glass funnel. The fibre mat obtained was broken up and dried in a moisture teller for 1 h at 240°F, and then weighed. Reproducibility is within ~ 1% of the mean value.
M. T. Rawnsley.

Effect of row and plant distances on the yield and quality of Virginia tobacco. M. A. AZIZ, M. SIDDIQ and A. RASHID (*W. Pakistan J. agric. Res.*, 1969, **7** (2), 21–26. Engl., 4 ref.).—A 3-yr expt. on Delcrest TS–12 showed that 18-in spacing in 36-in wide rows is optimum. M. T. Rawnsley.

Influence of transplanting on growth of the tobacco crop. J. M. HOPKINSON (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 639–643. 6 ref.).—Careful expt. on transplanting at various stages of seedling growth showed that, despite damage to upper or lower leaves, or roots, compensatory growth always occurred. Neither cured-leaf yield nor total leaf yield decreased, and it is considered that too much emphasis is placed on transplanting precautions.
M. T. Rawnsley.

Forest Crops

Soluble and slow-release PK-fertilisers for seedlings and transplants of *Picea sitchensis* (Sitka spruce) and *P. abies* (Norway spruce) in two English nurseries. B. BENZIAN, J. BOLTON and G. E. G. MATTINGLY (*Pl. Soil*, 1969, **31** (2), 238–256. 18 ref.).—Phosphate and K^+ were leached less and nutrients absorbed more efficiently by seedlings where KPO_3 than where KCl + superphosphate was applied to acid sandy soil. The best growth was attained when KNO_3 was applied 3 times during the summer to plots receiving KCl + superphosphate initially. A. H. Cornfield.

Sulphite pruning agents. UNIROYAL INC. (Br. Pat. 1,176,100, 3.12.68. U.S., 22.12.67).—Compn. useful for killing meristematic buds of plants contain compd. (2×10^3–1×10^5 ppm) of formula $R^1OSO{\cdot}OR^2$ where R^1 is alkyl, alkenyl, cycloalkyl, alkoxyalkyl (or a halo deriv.) and R^2 is alkynyl, alkenyl (or a halo deriv.). As an example of prepn., tridecyl chlorosulphinate is added slowly to a mixture of propargyl alcohol, pyridine and xylene and the temp. maintained at < 8°; the product, propargyl tridecyl sulphite, is useful for pruning, e.g., azaleas, chrysanthemums and tobacco plants. S. S. Chissick.

Sulphonium compounds. BADISCHE ANILIN- & SODA-FABRIK A.-G. (Br. Pat. 1,179,907, 31.5.67. Ger., 1.6.66).—The plant growth regulators have the formula $R^1R^2S^+.R^3X^-$ where R^1 is C_{1-4} aliphatic radical; R^2 is alkyl, hydroxyalkyl, or alkenyl; R^3 is halomethyl, Bu^t, alkynyl or $-CHR^4.CHR^5.R^6$, where R^4 and R^5 are H, alkyl, Ph, CN, -COOH or carbalkoxy, R^6 is, e.g., H, Me, Cl, OH, halomethyl, or $\cdot CH_2CR^7{:}CR^8.R^9$, where R^7 is Me, Cl or Br, R^8 and R^9 are H or Cl; X^- is an anion. An example is β-hydroxyethyl-n-butylmethylsulphonium iodide.
S. D. Huggins.

Sugar-cane [of increased sugar content]. MONSANTO CO. (Br. Pat. 1,176,728, 25.9.67. U.S., 27.9.66).—The desired effect is attained by applying to maturing cane a non-herbicidal amount of a compd. (I) of formula $NH_2.CO.NH.SO_2R$ or $M[NH_2CO.\overline{N}{\cdot}SO_2R]_n$ wherein R is Ph optionally substituted by halogen, alkyl, alkoxy, alkenyl, alkynyl, NO_2, NH_2, NO, CN, CHO, or acyl; M is cation of valency *n*. I is, e.g., Ca salt of 1-(*p*-nitrobenzenesulphonyl)isourea. F. R. Basford.

Animal Husbandry

Feedstuffs

Microbiological and physiological principles in the biosynthesis of protein and fatty substances from petroleum hydrocarbons. N. B. GRADOVA, A. P. KRUCHKOVA, G. S. RODIONOVA *et al.* (*Biotechnol. Bioengng Symp., 1967, No. 1, 2nd int. Conf. global Impacts appl. Microbiol.*, 1969, 99–104).—Work involved the selection of productive yeast cultures capable of ensuring high cell yields when cultivated on media contg. hydrocarbons of the paraffin series, as their sole C-source. A detailed study of their physiol. properties was made and enabled the organisation of large scale production of fodder yeast on n-paraffins. The majority of successful yeast strains in the study were of the genus *Candida*.
M. J. Rawlins.

Production and utilisation of BP protein concentrate. I. Production. B. LAINÉ. **II. Use of hydrocarbon-grown yeasts in commercial type rations for pigs and poultry.** C. A. SHACKLADY (*Biotechnol. Bioengng Symp., 1967, No. 1, 2nd int. Conf. global Impacts appl. Microbiol.*, 1969, 71–76, 77–97).—I. The production of a protein-rich byproduct by micro-organisms using n-paraffins was studied. A non-aseptic fermenter, and varied equipment enabling good aseptic control of fermentation parameters including harvesting were used. Rat feeding trials were carried out throughout the work. The end product was evaluated for net protein utilisation, digestibility and biol. value, which were low but were improved when supplemented with methionine.

II. Work was directed toward the manufacture of a major feed ingredient of protein concentrate, produced by yeast. Short term effects such as production of broilers and bacon of pork pigs, were studied, also the effect over a laying cycle of a bird and long term effect over 3 generations. It was found satisfactory to replace from 40–80% available high protein materials in poultry feeds by BP protein concentrate. In pig rations greater replacement can be made. M. J. Rawlins.

Dried animal feed produced from cheese whey and spent brewer's yeast. D. L. GIBSON and R. P. ANEJA (*Can. Dairy Ice Cream J.*, 1968, **47** (12), 13–15).—Addn. of ≠ 25% yeast was necessary to ensure a satisfactory drying of the whey. The mixture was condensed to 30% solids, and lime added to obtain pH 6·7. The final product was 4·6 moisture, 22·1 protein, 1·2 fat, 10·4 ash and 52·8% lactose. C. V.

Effect of pressure, moisture and cooking time on susceptibility of corn [maize] or sorghum grain starch to enzymic attack. Y. T. LIANG, J. L. MORRILL, F. R. ANSTAETT *et al.* (*J. Dairy Sci.*, 1970, **53** (3), 336–341. 11 ref.).—The susceptibility of starch to enzymic attack increased as pressure was increased from 1·8 to 6·0 kg/cm². Addn. of > 16% water, prior to cooking for 10 min, caused a sharp decrease in maize and sorghum grain starch digestibility. When cooking time was 1 min, 18% added water was required to give the same effect. M. O'Leary.

Effects of fertiliser N additives and season on silage fermentation in laboratory silos. R. K. WILSON (*Ir. J. agric. Res.*, 1969, **8** (3), 307–318. 19 ref.).—A high or low level of $(NH_4)_2SO_4$ was used to fertilise grass from a permanent pasture. At 3 dates the grass was ensiled in lab. silos (capacity 600 g) with 7 additive variations: control, formic acid, molasses, and a sodium nitrite–hexamine mixture, in triplicate. After 100 days, silages and effluents were examined. Comparison of farm and lab. silages showed the production of similar silage. Increasing compaction pressure in lab. silos increased effluent but did not effect silage quality.
M. J. Rawlins.

Yield, chemical composition, *in vitro* digestibility and voluntary intake of Irish perennial and A.1 ryegrasses and of S.143 cocksfoot at progressive stages of maturity. W. SHEEHAN (*Ir. J. agric. Res.*, 1969, **8** (3), 337–342. 12 ref.).—The freshly cut herbage was offered daily to sheep. *In vitro* digestibility (*D*), leaf %, N content and voluntary intake (*VI*) declined, and crude fibre content (*CFC*) increased during the expt. *VI* was related to *in vitro D* (r = 0·75) and *CFC* (r = 0·81). Herbage *D* was closely related to *CFC* (r = 0·93) and to a lesser extent to N content (r = 0·75).
M. J. Rawlins.

Sulphur stimulates starch digestion. L. G. KENNEDY, G. E. MITCHELL JUN. and C. O. LITTLE (*Sulphur Inst. J.*, 1968, **4** (1), 8–9).—Addn. of low level S ($Na_2S_2O_3$, Na_2SO_4 and methionine) to the simple diet medium resulted in significant stimulation of starch digestion over the basal medium, while high levels resulted in significant rise. C. V.

Estimation of digestible energy intake from forages by ruminants. R. C. KELLAWAY (*Aust. J. exp. Anim. Husb.*, 1969, **9** (41), 578–583. 17 ref.).—Van Soest's method (*J. Ass. Off. agric. Chem.*, 1963, **46**, 829) for detn. of lignin used as a marker in faeces, was shown to be better than previously published methods. It should, however, be used with caution. M. T. Rawnsley.

Efficiency of total collection and chromic oxide techniques in short-term digestion trials. G. L. HATTAN and F. G. OWEN (*J.*

Dairy Sci., 1970, **53** (3), 325–329. 11 ref.).—A comparison was made of digestion coeff. det. by a 7-day total collection with (*a*) 1- and 3-day total collections and (*b*) the chromic oxide method using either 1-day or 3-day total collection, or a 1-day collection of six rectal grab-samples at 4-h intervals. Results suggest that the length of digestion trials may be reduced from the conventional 7- to 10-day period for trials in which animal no. can be increased and where comparative rather than absolute values are acceptable.
M. O'Leary.

Poultry manure and meat meal as a source of dietary nitrogen for sheep. J. LEIBHOLZ (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 589–593. 18 ref.).—Poultry manure can be used as a main supplementary source of N when sheep are fed low protein, poor quality roughage. 15% sawdust can also be tolerated. The manure is not sterilised as this reduces the digestibility. The possibility of infection must always be considered in this case.
M. T. Rawnsley.

Stability and effect of energy metabolism of thiamine in poultry rations. L. S. S. GUO and J. D. SUMMERS (*Poult. Sci.*, 1969, **48** (4), 1471–1478. 28 ref.).—Addn. of thiamine-HCl at 0·002 g per kg of a semi-purified diet supported normal energy metabolism of mature cockerels, even though there was a considerable loss of thiamine (**I**) during autoclaving. K_2HPO_4 added to the diet was more destructive to **I** during autoclaving than was $CaHPO_4$. Naturally-occurring **I** in maize and soyabean was quite stable under the conditions of commercial steam pelleting.
A. H. Cornfield.

Use of raw and extruded soyabeans in layer diets. P. W. WALDROUP, D. R. SLOAN and R. F. DAVENPORT (*Poult. Sci.*, 1969, **48** (4), 1481–1486. 15 ref.).—In 2 trials 13–17% protein diets containing 12–27% extruded soyabeans were equal in feeding value to isonitrogenous diets containing soyabean meal + soyabean oil. Diets containing raw ground soyabeans were not effective, even when supplemented with methionine and lysine to the N.R.C. standard.
A. H. Cornfield.

Influence of dietary particle size and composition on phosphorus availability to chicks. M. GRIFFITH (*Poult. Sci.*, 1969, **48** (4), 1255–1261. 18 ref.).—Addn. of 7% coarse soyabean hulls, ground maize, bagasse, or wood to the diet of chicks resulted in better utilisation of P than did addn. of corresponding finely powdered materials to diets based on soyabean or cottonseed meal, or blood fibrin. The utilisation of P by chicks on diets based on casein or maize gluten meal was unaffected by the addition of coarse or fine materials.
A. H. Cornfield.

Metabolisable energy, and protein and fat digestibility evaluations of fish solubles in diets of young turkeys. A. B. CHU and L. M. POTTER (*Poult. Sci.*, 1969, **48** (4), 1169–1174. 8 ref.).—In two expt. with young turkeys the *ME* of lyophilised fish solubles was 3·967–4·351 kcal per g of dry matter. The coeff. of digestibility of the protein was 65·9–68·4% and of the fat 96·5–98·9%.
A. H. Cornfield.

Effects of Diet and Environment on Livestock

Comparative acceptability and nutritive value of barley, wheat mixed feed, and a mixed concentrate ration in meal and pelleted forms for lactating cows. D. E. WALDERN and G. CEDENO (*J. Dairy Sci.*, 1970, **53** (3), 317–324. 34 ref.).—The results of feeding trials with lactating cows showed that digestibility of dry matter, energy, and total digestible nutrient content of wheat mixed rations were lower than those of barley or a control mixed concentrate ration. Wheat mixed feed meal was less acceptable than all other grain rations ($P < 0·05$). Cows fed pelleted grain rations produced more milk, protein, solids-not-fat, fat, and fat-corrected milk than those fed meal rations ($P < 0·10$). Wheat mixed feed, fed in pelleted form, was comparable to barley or a mixed grain ration in meal or pelleted forms as the only concentrate for lactating cows.
M. O'Leary.

Consumption of corn [maize] silage dry matter by bred heifers and its correlation with subsequent first-lactation production. G. W. BRANDT, C. C. BRANNON, W. E. JOHNSTON and W. C. COOK (*J. Dairy Sci.*, 1970, **53** (2), 215–219. 10 ref.).—Second and third generation crossbred heifers showed heterosis for the consumption of silage. Results indicate that the amt. of silage dry matter eaten during a test period by a crossbred heifer is a poor indication of the amt. of milk and milk constituents subsequently produced in the first lactation.
M. O'Leary.

Digestibility of dry matter and nitrogen and the nitrogen balance of three skimmilk diets fed to Holstein calves. A. S. WOOD, J. D. DONKER and J. B. WILLIAMS (*J. Dairy Sci.*, 1970, **53** (2), 221–226. 20 ref.).—18 Holstein bull calves were used to determine the apparent digestibility of dry matter, N, and N retention of low-temp.-treated skimmilk (71·1° for 16–20 sec), high-temp.-treated skimmilk (85–87·7° for 20 to 30 min), and fresh skimmilk as the sole diet during 4 to 13 and 14 to 23 days of age. The resp. diets contained 77, 44, and 102 mg whey-protein per 100 g of milk. There were no significant differences among diets or between periods in the digestibility of dry matter, total N, or N retention. There was, however, a slightly better over-all apparent digestion and N retention of the fresh skimmilk diet. There was also a tendency towards a greater frequency of diarrhoea among calves fed diets contg. the smallest amt. of whey protein. As dry matter % in the faeces decreased, apparent digestibilities of dry matter and N decreased in all diets.
M. O'Leary.

Relationship of pearl millet to milk fat depression in dairy cows. II. Forage organic acids as influenced by soil nutrients. B. A. SCHNEIDER, N. A. CLARK, R. W. HEMKEN and J. H. VANDERSALL (*J. Dairy Sci.*, 1970, **53** (3), 305–310, 17 ref.).—The nutrients and org. acids of pearl millet (which causes milk fat depression) and those of Sudangrass (which does not affect milk fat levels) were compared. Pearl millet had a higher total org. acidity than Sudangrass and also higher concn. of oxalate and succinate. Succinate tended to vary inversely with milk fat levels. The influence of these and other observations on milk fat depression are discussed.
M. O'Leary.

Supplemental corn [maize] silage or baled hay for correction of milk fat depressions produced by feeding pellets as the sole forage. W. CHALUPA, G. D. O'DELL, A. J. KUTCHES and R. LAVKER (*J. Dairy Sci.*, 1970, **53** (2), 208–214. 37 ref.).—Small quantities of supplemental conventional forage alleviated the milk fat depression caused by feeding pellets as the main forage. The major factor influencing milk fat changes was shown to be an alteration in adipose tissue metabolism probably caused by increased propionate in the rumen. The increased unsatn. of milk fat produced during milk fat depressions was related to the defaunating effects of pelleted forages.
M. O'Leary.

Effect of feeding on composition of cows' milk in late winter–early spring. J. P. WALSH (*Ir. J. agric. Res.*, 1969, **8** (3), 319–327. 39 ref.).—The cows were fed indoors for 28 days, followed by outside grass feeding for 28 days. During late winter, concentrate supplementation of the hay diet increased ($P < 0·01$) milk yield by 13·4 lb daily and milk solids-not-fat, protein and lactose contents by 0·68, 0·35 and 0·16% units, resp. Milk fat content was reduced ($P < 0·01$) by 0·56% units. Hay supplementation of the grass diet did not markedly effect milk yield or the concn. of milk constituents with the exception of fat.
M. J. Rawlins.

Stability of the caseinate complex in milk. II. Influence of certain feeding treatments on the stability of milk. J. H. A. LABUSCHAGNE and J. S. A. LANDREY (*S. Afr. J. Dairy Technol.*, 1969, **1** (1), 31–37. Engl., 13 ref.).—The influence on the stability of milk of (*i*) dry feed ration, (*ii*) low protein content in the ration and (*iii*) ration contg. no bonemeal was examined. The influence of these feeding treatments on mineral component compn, (esp. those associated with the stability of the casein complex in milk) was also detd.
W. J. G.

Relations between the amount of forage units consumed by Charollais cows at the end of gestation and the body weight after calving, the birth weight and the mortality of calves. J. DARDILLAT, M. BROCHART and P. LARVOR (*C.r. hebd. Séanc. Acad. Agric, Fr.*, 1969, **55** (14), 1016–1020. Fr., 1 ref.).—Correlations derived from 13 sets of results on 704 cows confirm previous work, showing that the maternal wt. has a strong influence on the birth wt., and that undernutrition of mothers at the end of gestation has an adverse effect on the mortality of calves. Maternal wt. seems to depend more on the contribution of forage units in the total forage than on the total forage, possibly because of a qual. difference between base forage and cereals.
M. T. Rawnsley.

Digestive utilisation of whey proteins by pre-ruminant fattening calves. R. TOULLEC, C.-M. MATHIEU, L. VASSAL and R. PION (*Ann. Biol. anim. Biochim. Biophys.*, 1969, **9** (4), 661–664. Fr., 8 ref.).—A milk substitute, with only whey proteins as source of N, was fed to two calves kept in balance cages between the ages of 7 and 96 days. Appetite, growth, efficiency of feed utilisation and health were all satisfactory. Apparent digestibility of the constituents of the substitute was satisfactorily high (org. matter 97, N 93, fat 94%).
E. C. Apling.

Incidence of abnormally crimped fleeces among merino ewes in relation to nutrition early in life and to the number of lambs produced.

R. E. CHAPMAN and T. F. REARDON (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 574–577. 6 ref.).—It was found that the level of nutrition early in life and lambing performance are unlikely to affect the frequency of abnormally crimped fleeces.
M. T. Rawnsley.

Partial and total replacement of wheat and soyabean meal by pollard in a wheat–soyabean meal grower-pig ration. R. M. BEAMES and W. J. NATOLI (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 594–598. 16 ref.).—Pollard in amt. of 48·4, 67·8 and 96·9% of ration, with suitable vitamin etc. supplements, was shown to be suitable for replacement of some grain and protein. Difficulties encountered were prolonged feeding time because of dryness of feed, and reduction of dressing %. Backfat thickness in carcasses on the higher pollard amt. decreased. M. T. Rawnsley.

Direct *versus* indirect estimation of feed efficiency as a measure of performance of pullets and hens. A. W. NORDSKOG, H. FRENCH and S. L. BALLOUN (*Poult. Sci.*, 1969, **48** (4), 1303–1310. 7 ref.).—Feed efficiency (*FE*) (ratio of feed for production to feed for body wt. maintenance) was estimated (*i*) indirectly, from data on egg mass and body wt. and (*ii*) directly, from data on egg mass, body wt. and feed consumption. The av. error variance ratio of the two methods in 2 expt. showed that the statistical efficiency of (*ii*) was only 35% of that of (*i*), indicating that the latter method should be more satisfactory for measuring *FE*. A. H. Cornfield.

Metabolisable energy content and feeding value of mandioca meal in diets for chicks. D. W. OLSON, M. L. SUNDE and H. R. BIRD (*Poult. Sci.*, 1969, **48** (4), 1445–1452. 13 ref.).—Mandioca (*Manihot utilissima*) meal (3·2% protein, 82·7% *NFE*) showed a metabolisable energy of 3·44 kcal per g in chick trials. Addn. of > 30% mandioca meal (replacing ground maize and made isocaloric and isonitrogenous with animal fat and soyabean meal) to the diet had no effect on wt. gains and feed efficiency to 4 weeks of age. A. H. Cornfield.

Weight gain in chickens in relation to intake of growth ration. J. KELLER (*Ann. Biol. anim. Biochim. Biophys.*, 1969, **9** (3), 393–404. Fr., 14 ref.).—An exptl. study of the maintenance and growth ration requirements of growing chicks is reported. 50 day-old New Hampshire chicks were fed (by forced feeding) a composite ration in amt. increasing by 1·05 g of dry matter per day, and sacrificed in groups of 5 at 7 day intervals for detn. of body compn. (total dry matter, total N and total ash). For detn. of the relation between maintenance requirement and body size, 10 further groups of 5 chicks were fed rations which were initially increased at the same daily rate for varying periods and then held const. until wt. gain had ceased for 5 days, when the chicks were sacrificed for analysis. Results showed that the maintenance requirement was proportional to total body N and that the growth ration was related to daily gain in total body N by a second order equation. The growth rate estimated to correspond to optimum utilisation of nutrients was 50 g per day. E. C. Apling.

Low-protein cage-layer diets and amino acids. E. J. NOVACEK and C. W. CARLSON (*Poult. Sci.*, 1969, **48** (4), 1490–1497. 8 ref.).—Egg production, feed conversion, and egg wt. were improved by addition of methionine and lysine to a 9·4% protein maize–soyabean meal diet of caged layers. The protein requirement for a 2-kg hen at 60% egg production was 11·3 g and of total S-amino acids 0·46 g per day, with 0·32 g supplied as methionine.
A. H. Cornfield.

Use of amino acid imbalanced and low-protein starting rations for the rearing of egg production-type pullets and subsequent performance of these pullets when placed on laying rations of varying protein levels. J. D. SUMMERS, W. F. PEPPER and E. T. MORAN, JUN. (*Poult. Sci.*, 1969, **48** (4), 1351–1358. 8 ref.).—Birds reared on floors or in cages were considerably lower in wt. where a feather meal (20% protein) and somewhat lower in wt. where a 14% protein (maize–soyabean meal) diet than where 20% protein (maize–soyabean meal or rapeseed) diets were fed to 8 weeks of age. Although all birds were placed on a common growing ration at 8 weeks of age, the feather meal and 14% protein groups were lower in body wt. at 25 weeks of age. Subsequent laying house performance was not much different due to type of diet supplied to 8 weeks of age, except that with the feather meal diet, egg production and egg wt. were somewhat lower than with the other diets.
A. H. Cornfield.

Fish meal as the sole source of protein for the growing chick. I. Effect of different supplements on growth and feed efficiency. G. SCHUMAIER and J. MCGINNIS (*Poult. Sci.*, 1969, **48** (4), 1462–1467. 10 ref.).—Growth was relatively poor on a glucose–fish meal basal diet (22% protein). Addn. of penicillin, 5% cellulose or 3·75% maize oil to the diet did not improve growth. Addn. of 40% maize or increasing the fish meal so as to give 32–34% protein in the diet caused similar increases in wt. gains, whilst addn. of maize ash (≡ 40% maize) had no effect. Autoclaving fish meal did not improve its feeding value. A. H. Cornfield.

Use of lysine-imbalanced feeds for starting or growing chicks. D. H. SHERWOOD, C. D. CASKEY, J. E. JONES *et al.* (*Poult. Sci.*, 1969, **48** (4), 1319–1328. 9 ref.).—Limiting feed intake during the growing period resulted in lower feed cost to maturity than feeding a low-lysine diet in either the starting or growing periods. Subsequent egg production was higher where birds had been reared with controlled feed intake than with low-lysine diets, but the latter system was equal or superior to full feeding of a conventional diet.
A. H. Cornfield.

Processed feather and hog hair meals as sources of dietary protein for the laying hen. E. T. MORAN, JUN., W. F. PEPPER and J. D. SUMMERS (*Poult. Sci.*, 1969, **48** (4), 1245–1251. 13 ref.).—Hen performance on a 10% protein basal diet was improved by addn. of 5% protein as feather or hog hair meal, but supplemental methionine was required for max. production and egg wt. The observation that egg wt. was reduced with a methionine, but not with a cystine, inadequacy was related to the needs of protein for feather maintenance and whole egg synthesis.
A. H. Cornfield.

Protein requirements of laying hens as affected by strain. S. L. BALLOUN and G. M. SPEERS (*Poult. Sci.*, 1969, **48** (4), 1175–1188. 11 ref.).—Requirements were studied in relation to strain of hen, dietary energy level and degree of crowding in cages.
A. H. Cornfield.

Effect of varying levels of dietary 1,3-butanediol (1,3-BD) on growing chickens. R. F. DAVENPORT and M. GRIFFITH (*Poult. Sci.*, 1969, **48** (4), 1365–1371. 9 ref.).—The metabolisable energy value of 1,3-BD for the chick was 6·6 kcal per g. Wt. gains, feed consumption, and feed efficiency to 4 weeks of age were not affected by 5% 1,3-BD, but decreased with increasing levels up to 20% in the diet (all diets were made isocaloric and isonitrogenous by replacing cellulose and sucrose with 1,3-BD). Effects on carcass and liver fats are also discussed. A. H. Cornfield.

Effect of dietary calcium carbonate on feed intake and conversion in laying hens. S. HURWITZ, S. BORNSTEIN and A. BAR (*Poult. Sci.*, 1969, **48** (4), 1453–1456. 8 ref.).—Addn. of $CaCO_3$ to increase dietary Ca from 3·0 to 4·5%, decreased feed intake and body wt., increased feed efficiency with respect to egg production, but had no effect on egg production or egg size. Depression of feed intake was greater with pptd. $CaCO_3$ than with limestone or oyster shell. A. H. Cornfield.

Comparison of phosphorus assay techniques with chicks. V. Influence of supplemental magnesium on performance of soft phosphate and monosodium phosphate. B. L. DAMRON and R. H. HARMS (*Poult. Sci.*, 1969, **48** (4), 1328–1331. 6 ref.).—Addn. of 2000 ppm Mg (MgO) to diets containing soft phosphate tended to decrease, whilst addn. to diets containing NaH_2PO_4, tended to increase chick wt. to 3 weeks of age. Mg treatment decreased tibia ash % when soft phosphate was added, but had no effect when NaH_2PO_4 was added to the diets. A. H. Cornfield.

Magnesium nutrition of the hen: influence on retention of magnesium, calcium and nitrogen, and on ration metabolisable energy value. J. L. SELL (*Poult. Sci.*, 1969, **48** (4), 1437–1442. 20 ref.).—Egg production decreased when the hen's diet contained ≤ 270 ppm of Mg ($MgSO_4$); 52–170 ppm Mg^{2+} decreased feed consumption and hypomagnesemia also occurred, but retention of dietary Ca and N and metabolisable energy of the diet were not affected. Retention of dietary Mg was somewhat higher where 170–270 ppm than where 370 ppm Mg was supplied.
A. H. Cornfield.

Effect of EDTA on intestinal absorption of zinc and manganese in turkey poults. P. VOHRA and N. GONZALES (*Poult. Sci.*, 1969, **48** (4), 1509–1510. 6 ref.).—The presence of 200 ppm Na_2-EDTA in the diet of Zn-deficient or Zn-sufficient chicks preferentially stimulated the absorption of Zn over Mn by the small intestine.
A. H. Cornfield.

Effect of feeding various levels of lead on performance of broilers. B. L. DAMRON, C. F. SIMPSON and R. H. HARMS (*Poult. Sci.*, 1969, **48** (4), 1507–1509. 9 ref.).—Addn. of 100 ppm Pb (as acetate) to the diet of broilers, from 4 to 8 weeks of age, had no effect on wt. gains, feed efficiency, or feed consumption. 1000 ppm Pb in the feed decreased wt. gains (by 4–38%), feed efficiency and feed consumption. A. H. Cornfield.

Effect of a high level of dietary ascorbic acid on egg quality. R. B. HERRICK and C. F. NOCKELS (*Poult. Sci.*, 1969, **48** (4), 1518–1519. 8 ref.).—Addn. of ascorbic acid (2·6 g per kg of feed) to a commercial maize-soyabean meal diet, supplied to pullets for 28 weeks starting at 25 weeks of age, increased Haugh unit score of fresh eggs. The difference due to treatment was maintained after 1 week of storage. The treatment had no effect on egg production, egg wt., shell thickness or feed efficiency with respect to egg production. A. H. Cornfield.

Flavour comparisons in gnotobiotic and conventional broiler chickens. M. K. LONSDALE, M. WOODBURN and W. J. STADELMAN (*Poult. Sci.*, 1969, **48** (4), 1261–1264. 6 ref.).—Broilers reared to 6 weeks of age in a germ-free environment (gnotobiotic) tended to have more chicken flavour and fewer off-flavours than did birds raised in a normal environment. A. H. Cornfield.

Heat and moisture production of broilers. I. Summer conditions. F. N. REECE, J. W. DEATON and C. W. BOUCHILLON (*Poult. Sci.*, 1969, **48** (4), 1297–1303. 6 ref.).—A study was made of heat and moisture production and associated performance of broiler chickens grown on litter under moderately high temp. and high r.h.
A. H. Cornfield.

Analysis and Other Aspects

Fluoride content of commercial dairy concentrates and alfalfa [lucerne] forage. J. W. SUTTIE (*J. agric. Fd Chem.*, 1969, **17** (6), 1350–1352. 16 ref.).—The F content of 168 samples of 16% dairy feed and 107 samples of lucerne hay from different locations were determined. Dairy feeds ranged from 3 to 296 ppm of F with a mean of 21 ppm of F and a median of 15 ppm F. Only 7% of the samples exceeded the 90 ppm limit set by the American feed control officials. Lucerne hay from areas thought to be free from F pollution contained from 0·8–36·5 ppm of F, with a mean of 3·6 and a median of 2·0 ppm. No relationship between lucerne F-content and geographic location was detected.
I. Dickinson.

Microbiological assay of monensin in chicken rations. R. M. KLINE, R. E. STRICKER, J. D. COFFMAN *et al.* (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 49–53. 3 ref.).—The antibiotic is extracted with MeOH–H_2O (9 : 1) and, after clean-up on an Al_2O_3 column, microbiol. activity is measured with *Bacillus subtilis* (ATCC 6633).
D. I. Rees.

Gas-liquid chromatographic and colorimetric determination of ipronidazole in finished feeds. M. OSADCA, W. MERGENS, M. ARAUJO and E. DE RITTER (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 35–39. 5 ref.).—The feed ($\equiv > 0{\cdot}003\%$ of ipronidazole used in the control of blackhead in chickens and turkeys) is extracted into 0·2 N-HCl soln. In the g.l.c. method, the acid extract is made alk., extracted with benzene and an aliquot of the latter is analysed on a packed column contg. 5% of Carbowax 20M TPA at 180° with electron capture detection. In the spectrophotometric method, the acid extract is purified on a column contg. a mixture of MgO, diatomaceous earth and asbestos, the eluate is made alk., extracted with $CHCl_3$ and the absorbancy measured at 318 nm.
D. I. Rees.

Analysis for carotenes and xanthophylls in dried plant materials. F. W. QUACKENBUSH, M. A. DYER and R. L. SMALLIDGE (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 181–185. 9 ref.).—Carotenes and xanthophylls were extracted from corn products, alfalfa meal or mixed feeds with hexane–acetone–ethanol–toluene (10:7:6:7), separated by SiO_2 gel and chromatog. into total carotenes and two xanthophyll fractions (monohydroxy and dihydroxy pigments). The amt. present were detd. spectrophotometrically. A procedure is described for calculating the 'dihydroxy pigments equivalent' to serve as a measure of pigmenting effectiveness for avian skin and yolks. D. I. Rees.

Xanthophylls and carotenes in feeds and feed materials: collaborative study. F. W. QUACKENBUSH (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 186–189. 5 ref.).—The method of Quackenbush *et al.* (*ibid.*, 1970, **53** (1) 181) was collaboratively studied. Of the 11 collaborators, 8 showed satisfactory agreement. D. I. Rees.

Evaluation of clays as binding agents for reduction of radionuclides in milk. I. Binding properties of natural and hydrogen-form clays with strontium and essential cations in artificial rumen and in simulated abomasal and intestinal fluids. II. Binding properties of clays with ^{134}Cs in artificial rumen and in simulated abomasal and intestinal fluids and uptake of ^{134}Cs by rumen microflora. III. Effect of Belle Fourche bentonite on excretion of ^{134}Cs in lactating goats. J. BARTH, A. N. MIKALIS (III) J. Y. HARRIS (III) and B. H. BRUCKNER (*J. agric. Fd Chem.*, 1969, **17**, (6) 1340–1349. 42 ref.).—None of the clays tested removed Sr^{2+}, Ca^{2+}, Mg^{2+}, K^+ or Na^+ from the rumen juice or simulated digestive fluids to any considerable degree. Natural Belle Fourche bentonite (Na montmorillonite) (**I**) removed 82% of the radioactivity from the rumen juice. There was dissociation of the clay Cs complex with some of the ^{134}Cs returning into the abomasal and intestinal fluids. Less than 3% of the total radioactivity was associated with the microflora and other sediment. I effectively reduced the secretion of a single oral dose of ^{134}Cs in the milk of lactating goats when added to the ration at levels of 4 and 8% of the concentrate. Blood levels and urinary excretion of ^{134}Cs were reduced and the faecal excretion of ^{134}Cs was increased considerably. I. Dickinson.

Effect of udder washing time on milk production. B. ZACCHI and A. BORDI (*Produz. Anim.*, 1968, **7** (4), 297–299. It., 2 ref.).—Effect of changes from 1 to 4 min washing time (and *vice versa*) on milk production and rate of machine milking were studied with two groups of 7 Friesian cows. Increasing udder washing time decreased milk yield by 1·23 kg per day (1·32 kg of milk converted to 4% butterfat basis) and decreased milking rate (difference not significant). Decreasing washing time also decreased yield, but increased milking rate (both differences non-significant).
E. C. Apling.

Responses of dairy calves to aflatoxin-contaminated feed. G. P. LYNCH, G. C. TODD, W. T. SHALKOP and L. A. MOORE (*J. Dairy Sci.*, 1970, **53** (1), 63–71. 25 ref.).—Aflatoxin-induced changes in the calf at the clinical biochem. and tissue levels were shown to be similar to those produced in other species. Tissue changes at dose levels of $\geqslant 0{\cdot}02$ mg of B_1 included fatty infiltration of the livers corresponding to a loss of glycogen, disorganisation of liver lobules, and an invasion of the lobules by reticular fibres.
M. O'Leary.

Comparison of the body composition of male castrated pigs and females at slaughter weight (90–100 kg). M. LEROY (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (14), 1030–1035. Fr., 3 ref.).—Reference to Danish and French work shows that females form more nitrogenous substance and less fatty substance, than castrated males, wt. for wt. and age for age. Females generally have a better appreciation value. It is suggested that standards for nitrogenous feeds for pigs should be revised. M. T. Rawnsley.

Main routes of calcium metabolism in growing pigs. P. BESANÇON and L. GUÉGUEN (*Ann. Biol. anim. Biochim. Biophys.*, 1969, **9** (4), 537–553. Fr., 42 ref.).—The main parameters of Ca metabolism were measured in 10 growing pigs (30–50 kg wt.) after a single intravenous injection of 2–3 mC of ^{45}Ca. Interpretations of the curve of disappearance of specific activity of Ca in the blood in terms of bone parameters by the methods of Bauer *et al.* (*Anim. Metabolism*, 1961, **1B**, 609) and of Aubert and Milhaud (*Biochim. Biophys. Acta*, 1960, **39**, 122) are compared and discussed. It is concluded that the method of Bauer gives a systematic overestimation of the rate of incorporation of Ca in bone. True digestive utilisation of Ca in the feed was 45%; loss of endogenous Ca represented 17% of total faecal loss of Ca; urine excretion of Ca was negligible. E. C. Apling.

Effects of nitrate in the drinking water on poult and turkey growth. A. W. ADAMS, J. L. WEST and A. J. KAHRS (*Poult. Sci.*, 1969, **48** (4), 1222–1229. 8 ref.).—Poults tolerated up to 3325 ppm NO_3^- in the drinking water up to 7 weeks of age, but 3990 ppm NO_3^- caused high mortality. 1485 ppm NO_3^- in the drinking water of birds up to 24 weeks of age had little effect on wt. gain, feed and water consumption, mortality and blood haematocrit values.
A. H. Cornfield.

Calcium reserves in bones of laying hens: their presence and utilisation. S. HURWITZ and A. BAR (*Poult. Sci.*, 1969, **48** (4), 1391–1396. 14 ref.).—The ability of the hens to utilise Ca from cortical bone decreased with age. Because of limited bone reserves of Ca early in lay, the young hen is more sensitive to Ca restriction than is the older hen. A. H. Cornfield.

Toxicity of high levels of zinc-65 to avian embryos. F. R. MRAZ (*Poult. Sci.*, 1969, **48** (4), 1460–1462. 12 ref.).—Most of the $^{65}Zn^{2+}$, injected into the embryo at 3 days of incubation, was incorporated during the last week of incubation. Injection of > 200 μCi $^{65}Zn^{2+}$ at 4, 8 or 14 days of incubation usually resulted in death within a few days. Results are discussed in relation to contamination of the hen's food and water with $^{65}Zn^{2+}$.
A. H. Cornfield.

Effects of prednisolone on growth and nucleic acid metabolism of Pekin ducks. G. D. BOTTOMS, M. D. MCCRACKEN and W. W. CARLTON (*Poult. Sci.*, 1969, **48** (4), 1420–1425. 16 ref.).—
A. H. Cornfield.

Effect of reserpine on reproductive performance of Japanese quail. J. C. GILBREATH and RU-CHIUNG KO (*Poult. Sci.*, 1969, **48** (4), 1316–1319. 7 ref.).—Addn. of reserpine (0·004 g per kg of feed) to the diet of quail from 5 to 11 weeks of age decreased fertility and egg production, but had no effect on body wt., hatch of fertile eggs, or hatch of total eggs set. A. H. Cornfield.

Improved method for separate collection of urine, faeces, and expiratory gases from the mature chicken. G. D. PAULSON (*Poult. Sci.*, 1969, **48** (4), 1331–1336. 7 ref.).—An improved method for surgical modification of the chicken, apparatus for collection of urine and faeces, and a metabolism unit for collecting respiratory CO_2 are described. A. H. Cornfield.

Non-protein nitrogenous compositions. IMPERIAL CHEMICAL INDUSTRIES LTD. (Inventors: N. M. MORSS, J. PARK and I. THOMSON) (Br. Pat. 1,181,285, 2.6.67).—The compn. which are mixtures of urea, biuret, NH_4 sulphate or phosphate with water-sol. hydroxypropyl methyl cellulose (**I**), are useful as animal feeds or additives.
S. S. Chissick.

Animal feeding stuffs. ISAAC SPENCER & CO. (ABERDEEN) LTD. (Inventors: H. J. GREGSON and F. A. GIBSON) (Br. Pat. 1,176,196, 6.9.67).—A cattle 'lick' consists of a water-sensitive setting agent ($CaSO_4$) (< 15%) and nutrients (vitamins, minerals) or medicaments. S. S. Chissick.

Ruminant feed supplement. AMERICAN CYANAMID CO. (Inventor: R. L. GILBERT JUN.) (Br. Pat. 1,176,315, 26.3.68. U.S., 4.4.67).—The compn. contains urea and NH_4 phosphates [NH_3:H_3PO_4 = 1:1–1·4:1, NH_3-N:urea-N < 2·0:8·75 and N:P = 3:1–10:1], carbohydrates and proteins, the H_3PO_4 being defluorinated wet-process acid. S. D. Huggins.

Isorenieratenes. SOCIETÀ FARMACEUTICI ITALIA (Br. Pat. 1,181,609, 11.11.68. It., 14.5.68).—A carotenoid-producing strain of *Streptomyces mediolani* is grown aerobically in submerged culture, in a liquid medium containing assimilable sources of C, N and mineral salts, at pH 6–8 and at 24–37° for 72–160 h. The pigments extracted from the mycelium are used as additives to animal foodstuffs. S. S. Chissick.

[Preparation of] lactone derivatives. COMMERCIAL SOLVENTS CORP. (Br. Pat. 1,177,695, 18.5.67. U.S., 29.6.66, 3.3.67).—The lactones are oestrogenic growth promoting agents for meat-yielding animals, and have the formula 3,5,1,2-$(OR)_2C_6H_2R^{II}R^{II}$ (**I**) wherein R is H, alkyl, or CH_2Ph; the 2 R^{II} together represent a chain, viz., $A[CH_2]_3CR^I_2[CH_2]_3CHMe{\cdot}O{\cdot}CO$, where A is $[CH_2]_2$ or CH:CH, and R^I is halogen or one of them is H. They are prep. from compd. F.E.S. (fermentation oestrogenic substance). Thus, F.E.S. 2,4-dimethyl ether in ether is added to PCl_5 at 0°, to give **I** (R = Me; R^I = Cl; A = CH:CH). F. R. Basford.

2.—FOODS AND CROP CONVERSION

Cereals, Flours, Starches, Baking

Modern analytical methods and their use in cereal chemistry. H. D. JODLBAUER (*Getreide Mehl*, 1969, **19** (10), 73–77. Ger., 19 ref.).—Principles involved in the techniques of measurements of optical rotary dispersion and circular dichroism and in analytical and preparative disc electrophoresis on polyacrylamide gels are briefly outlined and some recent applications to the study of cereal proteins are reviewed. E. C. Apling.

Quality evaluation of wheat grains suffering from black point disease caused by *Helminthosporium sativum*, P.K. and B. and *Alternaria tenuis* Nees. A. K. GOSWAMI, and K. L. SEHGAL (*J. Fd Sci., Technol.*, 1969, **6** (1), 35–37. 8 ref.).—Wheat samples contg. 0, 6, 12, 25, 50 and 100% of severe black point diseased grains were prep. Protein, ash, Ca and P contents were not significantly different with respect to black point infection for each variety. Chapati test of the samples revealed a limited departure of the varieties from their general properties. Varying % of infected grains slightly changed the dough character, puffing character and taste. I. Dickinson.

Effect of artificial drying on tocopherols and fatty acids of corn [maize]. C. K. CHOW and H. H. DRAPER (*J. agric. Fd Chem.*, 1969, **17** (6), 1316–1317. 11 ref.).—Samples of maize were dried at temp. ranging from ambient to 290°F until the moisture content was reduced from ~ 25 to 15%. No effect on either the fatty acid or vitamin E content was discernible. I. Dickinson.

Some chemical elements in flour and bran. N. A. IL'VITSKY and Z. I. ASMAYEVA (*Pishch. Tekhnol.*, 1969, [2 (69)], 34. Russ.).
C. V.

Influence of different fats on gluten quality. N. I. KOSIN and T. A. SAPRYKINA (*Pishch. Tekhnol.*, 1969, [2 (69)], 36. Russ.).—
C. V.

Influence of heat treatment of maize on its extractability. M. V. ROSHAK, N. V. ROMENSKY and V. A. YAKOVENKO (*Pishch. Tekhnol.*, 1969, [2 (69)], 44. Russ.).— C. V.

Influence of water-insoluble endosperm pentosans on the dough and baking properties of soft wheats. J. P. J. CASIER, H. STEPHAN, G. de PAEPE and L. NICORA (*Brot Gebäck*. 1969, **23** (11), 231–236. Ger., 25 ref.).—Insol. pentosans were separated from two Belgian wheat varieties (Leda—winter wheat; Gaby—spring wheat) and the effects of varying addn. on the properties (Farinograph, Extensograph, bread vol., bread score, staling rate) of flours from three soft wheat varieties (Starke, Carsten VI and Gaby) were studied. Addn. of insol. pentosans (1–3%) markedly increased water absorption, dough development time and stability, bread vol., bread score and shelf-life. It is concluded that, in view of their effect in improving baking quality, attention should be paid to the water-insol. pentosan content of the endosperm of soft wheats.
E. C. Apling.

Water binding in dough. H. HUBER and W. BLUM (*Brot Gebäck*, 1970, **24** (1), 8–12. Ger., 8 ref.).—Studies of the effects of flour extraction rate, dough temp., dough mixing rate, addn. of yeast and salt, and resting time on the bound water content of doughs are reported. The amt. of water inseparable by ultra-centrifugation decreased with dough temp. and increased with dough resting time and the other factors studied. Change in bound water content with resting time was greater in yeasted than in unyeasted doughs.
E. C. Apling.

Gas production and retention during proofing of bread doughs. C. J. MAREK, W. BUSHUK and G. N. IRVINE (*Cereal Sci. Today*, 1968, **13** (1), 4–6, 13).— C. V.

Observations on medium chain fatty acids in yeast pre-ferments. H. D. OCKER (*Brot Gebäck*, 1969, **23** (11), 205–209. Ger., 4 ref.).—G.l.c. studies of the development of C_4–C_{10} fatty acids and their esters in glucose/mineral salt pre-ferments with and without addn. of flour and/or amylases and proteases are reported. One C_9 acid (probably α-Me-α-ethylcaproic acid) predominated, its content increasing with fermentation time. During 5 h fermentations, the no. of components detected increased with time, and only minor differences in the fatty acid spectrum resulted from incorporation of flour and enzymes in the ferment.
E. C. Apling.

Practical hints on the use of different qualities of yeast. A. SCHULZ (*Brot Gebäck*, 1970, **24** (2), 28–31. Ger.).—The use of new highly-fermentative yeast prepn. in straight dough and sour-dough practice is discussed. E. C. Apling.

Methods for the production and retention of crisp crust in rolls. G. MORGENSTERN (*Brot Gebäck*, 1969, **23** (11), 217–224. Ger., 4 ref.).—An extensive study of factors influencing crust quality is reported. Crust crispness is shown to be most adversely affected by the use of short baking times, very tight doughs, or flours of high maltose figure. Favourable conditions for production and retention of crust crispness were: use of low or medium quantities of yeast; a dough water absorption of 55%; extensive kneading at comparatively high dough temp.; short bulk fermentation; addn. of dough additives, e.g., fat; long baking time (< 20 min), the use of flour contg. ~ 25% wet gluten and a maltose figure of 2·0%; storage at 20–25° and low r.h.; and packaging in water-permeable materials. E. C. Apling.

Relative importance of protein, wheatmeal fermentation time and sedimentation values as indices of loaf volume. A. AUSTIN, DALJIT SINGH and RAMACHANDRAN NAIR (*J. Fd Sci., Technol.*, 1969, **6** (1), 33–34. 10 ref.).—Multiple regression technique was used to study the relationship between the loaf volume and sedimentation, protein and wheatmeal fermentation time. Loaf vol. was expressed as a linear function of the other three characters. Standard partial regressions were also computed to study the relative

importance of the different characters in the detn. of loaf vol. It is concluded that a knowledge of protein alone is sufficient for prediction of loaf vol. I. Dickinson.

High-protein supplements in breadmaking. Y. POMERANZ (*Baker's Dig.*, 1969, **43** (5), 39–40. 17 ref.).—Wheat flour glycolipids were found to improve loaf vol., crumb grain, and freshness retention of bread. Comparison of the effects of various synthetic glycolipids of the type present in wheat flour indicated that hydrophobic and H bonds were important for the improvements in the bread. Wheat flour enriched with plant and animal proteins and supplemented with natural or synthetic glycolipids could be valuable in producing low priced, protein enriched breads, where nutritious but inexpensive foods are a major need. M. J. Rawlins.

Fermentation flavours of white bread. S. J. JACKEL (*Baker's Dig.*, 1969, **43** (5), 24–28, 64. 18 ref.).—More than 100 org. compd., produced by yeast and/or bacterial fermentation in bread, were isolated by vapour phase and g.l.c. trapping and the formation of deriv. Bread leavened chemically, omitting fermentation, differed substantially in flavour from normal white bread. When fermentation was increased, so also was bread flavour. Yeast grown without bacteria and then used to leaven bread, produced less flavour. The fermentation products of yeast and bacteria, together with ingredients, mechanical and/or biochemical degradations and thermal reaction products contribute to bread flavour. M. J. Rawlins.

Food law aspects of butter bread for toasting and technical problems of its production. W. SEIBEL and H. STEPHAN (*Brot Gebäck*, 1970, **24** (2), 21–28. Ger., 5 ref.).—It is argued that the general legal standard for butter-contg. bakery products (10%, flour basis) is not applicable to butter bread, where toasting characteristics must be an essential criterion. The suggested standard is 5 parts of butter per 100 parts of flour, with the permitted addn. of up to 0·3% (flour basis) of emulsifier (mono- and di-glycerides of natural fatty acids). An extensive investigation into the effects of recipe and production methods on product quality is reported, and the technical recommendations include: use of flour contg. 12–12·5% protein; 4—6% fat and up to 0·5% emulsifier (flour basis), with up to 6–8% fat where the bread is intended for eating solely as toast; up to 2–4% of sugar, malt or dried milk; use of 6–8% of yeast (to limit mould problems) with conventional dough handling, but using short fermentation times and comparatively tight doughs. E. C. Apling.

Explanation of ionic sequences in various phenomena. X. Protein-carbohydrate interactions and the mechanism for the staling of bread. S. R. ERLANDER and L. G. ERLANDER (*Stärke*, 1969, **21** (12), 305–315. Engl., 61 ref.).—Previous work has shown that fats and alcohols inhibit the staling of bread by forming complexes with starch, thus preventing its retrogradation. It is suggested that proteins can complex in a similar manner. High protein bread was prep. with eggs and milk to enhance the protein and staling properties were then evaluated organoleptically. Importance of the protein–starch ratio was demonstrated and, by applying the ionic sequence method, it was shown that the amide groups of the gliadin, glutenin and possibly the albumin are H-bonded to the C_2 and C_3 hydroxyls of the starch. Unlike concanavalin A, the interaction does not involve terminal sugar units. Models are proposed for the starch–protein interaction. J. B. Woof.

Sodium stearyl fumarate: Effects in cake formulation. P. F. SCHAMBERGER, JUN. and C. P. HETZEL (*Cereal Sci. Today*, 1968, **13** (1), 31–33, 40).— C. V.

Role of release agents in the bakery. E. KUCHINKE and A. MENGER (*Brot Gebäck*, 1970, **24** (1), 1–7. Ger.).—Properties and uses of release agents (oils and fats, waxes, silicones and Teflon, and paraffin) are described and their relative advantages and disadvantages for particular bakery requirements are reviewed. E. C. Apling.

Utilisation of the continuous dough mixing process for the production of sweet goods. E. R. HAYES and W. C. HURLEY (*Baker's Dig.*, 1969, **43** (5), 44–49. 7 ref.).—A lab. continuous dough-making unit was used to determine the possibility of sweet dough production by continuous mixing procedures. Factors studied included mixing requirements, shortening systems, levels of dough conditioners and general dough-handling characteristics. Cinnamon rolls produced by this method compared very well with commercially produced rolls. Characteristics observed were vol., grain, texture, heating and keeping qualities. M. J. Rawlins.

Calculation of starch manufacturing processes with the help of data processing machines. B. I. DAHLBERG (*Stärke*, 1969, **21** (10), 270–273. Engl.).—Mathematical models are developed, the method being based on the iteration principle. The system provides total flexibility regarding equipment and flow pattern, and handles 4 material components at the same time. G. B. Woof.

Genetic variations in maize: Effect on properties of starches. R. M. SANDSTEDT, B. D. HITES and H. SCHROEDER (*Cereal Sci. Today*, 1968, **13** (3), 82–85, 88, 90–92, 94, 156. 19 ref.).— C. V.

Investigations on pure wheat starches. II. Viscosity. G. HAMPEL (*Getreide Mehl*, 1969, **19** (12), 89–91. Ger., 15 ref.).—Viscosity characteristics are reported for 55 samples of wheat starch of German and foreign origin. No relationship was found between η of separated starch and the baking quality of the parent wheat variety, but there was a close relationship between the susceptibility of the native starch to attack by β-amylase and η coeff. (η of a 45% gel). A weak correlation was also noted between η coeff. and final Viscograph η. E. C. Apling.

Determination of gelatinisation property of highly concentrated starch suspension by Brabender Plastograph. II. Examination of the gelatinisation curve obtained. F. GOTO (*Stärke*, 1969, **21** (10), 267–270. Engl., 7 ref.).—The method was applied to different species of starch in comparison with Brabender amylograms and photopastegrams. The plastogram can be used to measure the gelatinisation ranges of highly conc. starch slurries and also the intrinsic properties of starches on gelatinisation. J. B. Woof.

Pre-gelatinisation of wheat starch in a drum drier. R. TAKAHASHI and T. OJIMA (*Stärke*, 1969, **21** (12), 318–321. Engl., 4 ref.).—Phys. properties of the pre-gelatinised starch were measured. To achieve max. swelling and a paste of high η, a water content of 70% was required. At < 60% water content, dextrinisation occurred. Because of the reconstitution of starch micelles and inter-mol. H-bond formation, reheated starch showed lower swelling and digestibility. Results suggest that gelatinisation, dextrinisation and retrogradation occur simultaneously in a drum drier. J. B. Woof.

Spinnability of starch pastes. R. TAKAHASHI, T. OJIMA and M. YAMAMOTO (*Stärke*, 1969, **21** (12), 315–318. Engl., 6 ref.).—An apparatus is described in which spinnability is indicated by the length of starch paste thread spun by a steel ball driven by a synchronous motor. Spinnability increased with rate of increase of velocity of the ball; reproducibility of results was satisfactory. Unmodified starch pastes of the same η, gave thread lengths decreasing in the following order: potato > tapioca > waxy corn > wheat corn. The degree of spinnability increased with relaxation time and decrease in structural η. Introduction of hydrophilic groups, which decreased the gelatinisation temp. and increased the degree of gelatinisation, increased the degree of spinnability. J. B. Woof.

Digestibility of starches and phosphatised starches with pancreatin. C. J. JANZEN (*Stärke*, 1969, **21** (9), 231–237. Ger., 13 ref.).—Starch and modified starch samples were digested at 37° in phosphate buffer, pH 6·9, with pancreatin. The dextrose and maltose equiv. of the products were detd. and the sugars present investigated by chromatog. Modification of the substrate with 0·05 and 0·1% $POCl_3$ had no effect on digestibility but at 0·5 and 1·5% it was inhibited. At high crosslinking levels, hydrolysis depended on the swelling induced by autoclaving at 120°. In starch degraded by acid treatment, degree of crosslinking had little effect. Extended digestion yielded maltose (50%) and glucose (8%) and some polymeric material. J. B. Woof.

Distribution of proteolytic and α-amylase activity, damaged starch and ash content during roller milling and air classification. J. F. DE LA GUERIVIERE, Y. AUDIDIER, Y. SEINCE and K. BENOUALID (*Getreide Mehl*, 1969, **19** (11), 86–88; (12), 92–94. Ger., 15 ref.).—Analyses of air-classified fractions are reported and discussed. In general, damaged starch and protein particles from the central endosperm (low in enzymic activity) were conc. in the fine fraction; protein from the outer layers (enzymic activity and ash content higher than for whole flour) conc. in the middle fraction, and protein-starch agglomerates from the intermediate zone of the grain conc. in the coarse fraction. E. C. Apling.

Enzymic preparation of starch hydrolysates. F. E. KNUDSEN and J. KARKALAS (*Stärke*, 1969, **21** (11), 284–291. Ger., 7 ref.).—Methods available for starch hydrolysis include acid treatment, acid–enzyme systems and ones in which both liquefaction and saccharification are carried out with enzymes. Each of these processes

is discussed in detail with flow diagrams, and conditions and analyses of the products at each stage. Commercial enzymes available are specified and a detailed analysis is quoted for the final products obtained from different starches under the different conditions. J. B. Woof.

Sugars, Syrups, Confectionery

Process development on production of sugar from palm juice. RAMZAN ALI (*Un. Burma J. Sci. Technol.*, 1969, **2** (1), 173–180).—A process for production of sugar from palmyra palm juice is described. Major process variables are optimised. The quality of sugar produced is comparable with cane sugar, and yields of 5·6% by wt. of juice are obtained. Social and economic factors important to the local jaggery-producing community of central Burma are discussed. L. MacQuisten-Wallace.

Determination of suspended solids in mixed juice. P. A. PRINCE (*Proc. S. Afr. Sug. Technol. Ass.*, 43rd Ann. Congr., 1969, 141–143, Engl., 3 ref.).—A sample of mixed juice is taken hourly and treated with a juice preservative, e.g., $HgCl_2$. At the end of the sampling period a composite juice sample is mixed and weighed. A filter aid is added. The juice is then filtered under vac. through a weighed filter paper, pre-coated with filter aid. The filter paper and contents are washed and dried to const. weight. M. T. Rawnsley.

Rapid direct analysis of zinc in sugar products and molasses. J. M. L. MEE and H. W. HILTON (*J. agric. Fd Chem.*, 1969, **17** (6), 1398–1399. 2 ref.).—Addn. of citric acid at 10 g/100 ml makes possible the direct analysis of Zn in sugar-contg. soln. by atomic absorption spectrophotometry. All but 2 to 3% of the Zn in sugar-factory juices is pptd. by the practice of treating the hot juice with $Ca(OH)_2$ to pH 7·5 to 8·0. I. Dickinson.

Use of starch hydrolysates in the preparation of liquid sugar. G. TINTELNOT (*Stärke*, 1969, **21** (9), 237–243. Ger.).—A review dealing with the economics, technology and application of mixtures of liquid sugar products and starch hydrolysates. The essential criteria for production of mixtures are discussed. J. B. Woof.

Crude sugar liquor defecation process. H. E. BODE (Br. Pat. 1,179,913, 17.4.67. U.S., 18.4.66).—A mixture of the liquor (e.g., non-screened sugar-cane juice), a phosphated starch (**I**) and an alkaline earth metal defecating reagent, e.g., oxides, hydroxides or carbonates of Ca or Ba, at pH $\geqslant$ 7·2 is heated ($\geqslant$ 180°F) and the resulting ppt. of **I**-metal deriv. is separated. S. S. Chissick.

Confectionery syrup. CORN PRODUCTS CO. (Br. Pat. 1,181,650, 14.1.69. U.S., 17.1.68).—The sucrose-free syrup, useful as a sweetening agent in confectionery, has a D.E. < 60 (and contains levulose (3–16), dextrose (10–35), maltose (10–25) and oligosaccharides (15–40%). When used in soft candies it maintains a satisfactory water balance, so that the products do not harden or become too soft. S. S. Chissick.

Sweet non-crystallising syrup and its preparation. CORN PRODUCTS CO. (Br. Pat. 1,177,701, 2.3.67. U.S., 3.3.66).—The syrup, which has a dextrose equivalent (D.E.) < 55 and contains dextrose, ketose (levulose) and maltose, is prep. by hydrolysis of liquified starch (**I**) of D.E. $\geqslant$ 20. **I** is treated successively with a malt enzyme and a microbial (fungal) enzyme and then passed over a strongly basic anionic exchange resin at ~ 65° (2–4 h). S. S. Chissick.

Malting, Brewing and Alcoholic Beverages

Effect of temperature on the formation of higher alcohols by culture yeasts. T. ÄYRÄPÄÄ (*Brauwissenschaft*, 1970, **23** (2), 48–55. Ger., 35 ref.).—A 10·9° Plato decoction wort was fermented with a strain of *S. carlsbergensis* in 20-l Al bottles at 7·5, 12·5, 20, 28 and 35°. For comparison, synthetic media and semi-synthetic substrates contg. wort components and labelled amino acids were also fermented. In wort, the biosynthesis was more temp. dependent than the catabolic Ehrlich pathway esp. with regard to the major component 3-Me-butanol. The effect of temp. is largely dependent on the nature and concn. of nitrogenous constituents. Max. yield of higher alcohols occurred at 20°. The max. rate of formation occurred at ~ 28° where the fermentation and growth were also at a max. J. B. Woof.

Use of chromatographic methods for investigating dextrins and fermentable sugars in wort. G. TRÉNEL and M. JOHN (*Mschr. Brau.*, 1970, **23** (1), 6–10. Ger., 5 ref.).—The gel permeation method on Bio-gel P2 permits resolution of G1 to G15 oligosaccharides in a mixture like wort using a sample of only 25 μl and requiring no preliminary sample prepn. In conjunction with g.l.c. of the trimethylsilyl ethers of the G1, G2 and G3 fractions it permits complete analysis of carbohydrate constituents. The method is used to demonstrate the presence of G1 to G8 oligomers in the hydrolysis products of amylose using α-amylase. β-amylase yields only maltose. Analysis of worts with different attenuation limits, produced by different mashing procedures, showed that glucose and maltose only were significantly affected; maltotriose and dextrin remained almost const. In a wort produced with barley and Brew-n-zyme, the dextrins were normal but the maltose level was increased whilst the glucose fell to half its normal level. J. B. Woof.

Differentiation of viscosity in wort and beer. P. KOLBACH (*Mschr. Brau.*, 1970, **23** (1), 1–5. Ger., 9 ref.).—The contributions of dextrin, maltose and alcohol to the total viscosity (η) of wort is considered and a table is constructed from Höpplers η showing the contribution to be expected from each over the normal range of concn. These three compd. account for ~ 55% of total η. Congress worts were prep. from Wisa barley germinated for 4 to 7 days. As germination proceeded the glucan content decreased and its contribution to η fell from 35·5 to 16·8%. Over the same range, η accounted for by the known constituents decreased from 79·6 to 73%. J. B. Woof.

Wort fine 'break' in the fermentation and colloidal stability of beers. ST. MANTSCHEV and G. DSCHATOV (*Brauwissenschaft*, 1970, **23** (2), 56–61. Ger., 13 ref.).—Hot worts directly from the hop strainer were found to contain 450–660 mg/l dry wt. of break material. A centrifuge reduced this to 15% and a Whirlpool system to 25% of the original level. After removal of hot break and cooling to 5–6°, 150–300 mg/l of fine cold break is present. This can be reduced to 90–200 mg/l by either system. Kieselguhr filtration of the cold wort removed high mol. wt. protein and polyphenol and gave the resultant beer greater colloidal stability. Fermentation was more rapid and the attenuation limit was higher in the presence of 'break' and the fact that a similar effect could be obtained with kieselguhr in suspension suggests that surface phenomena may be involved. J. B. Woof.

Head retention problems. G. KRAUSS (*Mschr. Brau.*, 1970, **23** (2), 26–32. Ger., 53 ref.).—A review of the literature indicates that coagulable and $MgSO_4$-precipitable N gums, bittering substances and low pH improve head retention whilst low mol. wt. nitrogenous compd., fats and EtOH have a deleterious effect. Anthocyanogen content is negatively correlated with head but no direct evidence is available. Whilst barley and hop variety does not affect head, old hops and unkilned or over-modified malt are detrimental. In general, brewing procedures are only significant in so far as they affect the concn. of the above components. Flocculent yeasts are better than non-flocculent and the final CO_2 content is important. J. B. Woof.

Investigation of the filterability of beers. I. Large scale evaluation. F.-W. SCHIMPF, W. RINKE and H.-F. EHRKE (*Mschr. Brau.*, 1969, **22** (12), 353–361. Ger., 8 ref.).—Large scale filterability tests were carried out on 75,000 hl of beer brewed commercially with 5 malts (unblended). Malt and beer analyses, and compn. of the residue remaining on the kieselguhr filter, were then related to filtration capacity. There were very wide variations in behaviour which could only be related significantly to β-glucan content. Tank sediment controlled turbidity (T) of the beer entering the filter and its capacity. It is suggested that T and viscosity of the beer should be used to gauge filterability; T can be related to kieselguhr dosage rates. J. B. Woof.

Investigation of the filterability of beers. II. Laboratory investigations. F.-W. SCHIMPF and W. RINKE (*Mschr. Brau.*, 1970, **23** (2), 21–25. Ger., 5 ref.).—Lager beers, taken after primary filtration and just before filtration (30 brews), were tested in the lab. for filterability and at the same time analysed for total- and sol.-N, viscosity and turbidity. In immature beer, both insol.-N and viscosity were correlated with filterability but in mature beer only the latter affected filtration. It is suggested that the amt. of sludge at dropping and the viscous substances in mature beer indicate subsequent filtration behaviour. Hop resin, ether sol. components, trub, yeast and β-glucan extracted from beer or wort were added back to beers and the effect on filterability detd. Glucan had a marked effect as did yeast but the other substances had little effect on filtration. J. B. Woof.

Proteolytic treatment of beer. Control and study of enzyme preparations and their use in beer filtration. R. SCRIBAN, M.

STIENNE and B. STROBBEL (*Brasserie*, 1969, **24** (272), 581–615. Fr., 24 ref.).—The use of a no. of commercially available proteolytic enzymes in the stabilisation of beers against chill haze is considered. It is necessary to standardise (*i*) prepn. and (*ii*) effectiveness of beer treatment. For (*i*), the Codex français which estimates the wt. of fibrin solubilised under standard conditions, the method of Ayre and Anderson with haemoglobin as substrate or the Am. Pharm. Ass. method (casein substrate) are suitable. The methods are discussed and the correlations between them established. Temp.-activity relations for the prepn. at different pH's were established and also in beer. Detn. of optimum dose rates and conditions for beer treatment are discussed and the action mechanism considered. Ultimate effectiveness in the beer is detd. by measuring the rate of turbidity formation under standard conditions. J. B. Woof.

[Separation of] *cis* and *trans*-tetrahydroisohumulones. W. J. G. DONNELLY and P. V. R. SHANNON (*J. chem. Soc. C*, 1970, (4), 524–530. 23 ref.).—Alternate acylation and reduction treatment of phloroglucinol yielded optically inactive tetrahydroisohumulone. The racemates were separated by silicic acid chromatog.; the ratio of *cis* to *trans* isomers was found to be 2:3. V. Rolfe.

Gas chromatographic analysis of beer. M. BENARD (*Brasserie*, 1969, **24** (273), 663–683. Fr., 14 ref.).—A review of the principles of g.l.c. and its application to alcohols, esters, acids, carbonyls, sulphur deriv., phenols and non-volatile acids. The effect of these compd. on flavour is considered and methods of head space analysis and extraction are described. J. B. Woof.

Determination of diacetyl content of beer. K. D. ESSER and C. KREMKOW (*Mschr. Brau.*, 1970, **23** (1), 11–14. Ger., 12 ref.).—A modification of the methods of Hetzel and Gjertsen is described. The beer sample is steam distilled in a Parnas apparatus for N detn. The distillate is treated with acid *o*-phenylenediamine and the colour measured at 335 nm. Results agree well with the Owades/Drews methods ('t' test) and are more reproducible than the original Hetzel method. J. B. Woof.

Influence of different concentrations of sugar on the formation of side products of fermentation by some strains of wine yeast. Part I. Formation of higher alcohols and esters. S. BUJAK and E. PODGÓRSKA (*Annls Univ. Mariae Curie-Sklodowska, Agric. E*, 1968, **23**, 363–371. Pol., 17 ref.).—The concn. of Et, Bu^i and isoamyl alcohols, EtOAc and total esters produced in samples of apple cider, with and without additional sugar, by six yeast strains, were detd. 2, 4 and 6 weeks after inoculation. G. Chinnick.

Observations on enzymes in fermenting palm wine. S. A. VISSER and O. BASSIR (*J. Fd Sci., Technol.*, 1969, **6** (1), 23–28. 9 ref.).—Changes in the activities of 12 enzymes were followed during the first 9 days of fermentation of palm wine (the fermented sap from various species of palm trees). Three distinct groups of enzymes were distinguished, (*i*) those which reached max. activity at the first to second day of fermentation, (*ii*) those which showed a steady increase in activity during the first 9 days of fermentation and (*iii*) those which showed a steady decline in activity during the first 9 days. Origin of the various enzymes could be traced to the original palm sap, to micro-organisms, or to both. I. Dickinson.

Avoiding decoloration during the production of red wine. H. HAUSHOFER, W. MEIER and E. BAYER (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1969, **19** (5), 344–360. Ger., 11 ref.).—Expt. carried out over 2 yr indicated that yeast and lees adsorb tannin and colouring compd. from red wine. Preliminary centrifugal clarification by reducing the amt. of these substances, gives wine with better colour values and permits regulation of the tannin content to some extent. J. B. Woof.

Aroma substances of wine in relation to the maturity of the grapes. F. PRILLINGER and A. MADNER (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1969, **19** (5), 361–368. Ger., 9 ref.).—Pentane–ether extracts from wines made from 4 different grapes harvested at different times were subjected to g.l.c. on a capillary column of Carbowax 1540. Benzyl alcohol was used as internal standard. Bar charts were used to follow changes in 13 known and 2 unknown components. Changes were found in the levels of various components but only in the case of β-phenylethanol was there a consistent increase with degree of maturity of all the grapes tested. J. B. Woof.

Contribution to the analytical study of Spanish wines. E. FEDUCHY M., J. A. SANDOVAL P., A. M. MARCILLA C. and T. HIDALGO Z. (*Boln. Inst. nac. Invest. agron., Madrid*, 1969, **29** (60), 1–42. Span., 17 ref.).—Results of detn. of Cl, Na, K, tartaric acid, P, Cu, Ca, Mg, F and Br are reported for a large no. of 1966 Spanish wines. The analytical methods used are described in detail. E. C. Apling.

Determination of free and SO_2-bound acetaldehyde in wine. H. REBELEIN (*Dt. LebensmittRdsch.*, 1970, **66** (1), 6–11. Ger., 7 ref.).—Wine which has been decolorised with active charcoal is mixed with Na nitroprusside and piperidine. Absorption at 570 nm is measured and the AcH content detd. from a calibration curve. AcH-bound SO_2 is also detd. Good agreement is obtained with the distillation method of Jaulmes and Espezel. In wines with residual sugar, the bound SO_2 increases linearly with the amt. of sugar remaining in the wine. In wines made from rotting fruit, the increased SO_2 is almost all residual and cannot be controlled by cellar treatment. J. B. Woof.

Determination of ethanol in wine by chemical oxidation: 1969 studies. A. CAPUTI, JUN. (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 11–12. 2 ref.).—The dichromate oxidn. method described by Caputi and Wright (*ibid.*, 1969, **52**, 85) was used in the analysis of 14 different wine types; the EtOH contents found ranged from 11 to 23% with standard deviations of 0·04 to 0·07%. D. I. Rees.

Determination of malic acid in wines and grape juice. Comparison between the enzymic procedure and the method of Rebelein. D. OLSCHIMKE, W. NIESNER and CH. JUNGE (*Dt. LebensmittRdsch.*, 1969, **65** (12), 383–384. Ger., 2 ref.).—Malic acid content of several wines was detd. by the Rebelein method involving a colour reaction with chromotropic acid. The enzymic method with which it was compared involved conversion of the malic acid to oxalacetic acid using the enzyme malate dehydrogenase in the presence of nicotinamide adenine dinucleotide (NAD). The oxalacetic acid was trapped with hydrazine and the NADH formed detd. by the change in adsorption at 334, 340 or 366 nm. Values obtained in this way were generally below those detd. by the Rebelein method. If the wine sample was pre-treated by hydrolysing it with alkali under reflux for 30 min, the two methods gave excellent agreement. J. B. Woof.

Investigation of musts and white wines by gel chromatography. D. ZAKOW, B. MESROB, TSCH. IWANOW *et al.* (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1969, **19** (6), 437–447. Ger., 13 ref.).—A no. of different samples were chromatographed on a 25 × 480 mm column of G25 Sephadex. Absorption of the eluate fraction was measured at 280 nm and peptides were detected by the Folin reaction. In this way some peaks could be attributed to proteins and others to tryptophan and polyphenols. Relative peak sizes from the different samples are discussed and changes brought about by heat treatment at 40 and 60° and by bentonite and $K_4Fe(CN)_6$ treatment are considered. J. B. Woof.

Determination of lead, zinc, copper, iron, calcium and magnesium in grape musts and wines by atomic absorption spectrophotometry. M. C. POLO, M. D. GARRIDO, C. LLAGUNO and J. GARRIDO (*Revta Agroquim. Tecnol. Aliment.*, 1969, **9** (4), 600–605. Span., 9 ref.).—Detn. in 21 samples each of Levante grape must, West Andalusian and La Rioja wines are reported. Levels of Fe, Zn and Ca varied considerably with region; high levels of Pb (up to 4 ppm) were found in several samples. E. C. Apling.

Determination of iron in alcoholic beverages. M. K. MEREDITH, S. BALDWIN and A. A. ANDREASEN (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 12–16. 3 ref.).—Atomic absorption was used in the detn. of Fe (0 to 17 ppm) in wines and spirits, due to its speed and accuracy; direct colorimetry with use of 2,4,6-tripyridyl-*s*-triazine (**I**) eliminated the need for ashing but did not give accurate results for brandy; wet oxidn. with use of NaClO and H_2O_2, followed by colorimetry with use of **I**, gave satisfactory results for brandy. The standard deviations obtained for the first two methods were $< \pm 0{\cdot}05$ (except sherry wine $\pm 0{\cdot}30$). D. I. Rees.

Thermo-gas chromatographic investigation of the correlations between fusel alcohols in plum brandy. K. PFEILSTICKER (*Dt. LebensmittRdsch.*, 1970, **66** (1), 13–19. Ger., 40 ref.).—Samples of a no. of plum brandies, new and after ageing, were subjected directly to g.l.c. on a column of polypropylene glycol. MeOH, 2-BuOH, Bu^{i}OH, Bu^{n}OH, amyl alcohol and amyl acetate peaks were measured in each case. Detailed statistical evaluation of the results indicates a high correlation between MeOH and Bu^{n}OH which is considered to be connected with the secondary fermentations brought about by moulds and fungi. The Bu^{i}OH/amyl alcohol correlation would be expected as a result of normal fermentation. J. B. Woof.

Gas chromatographic investigation of spirits. II. Fractional distillation and aroma intensity of plum brandy. W. KAIN and F. BANDION (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1969, **19** (6), 457–473. Ger., 2 ref.).—Fractions of Sliwowitz, obtained by fractional distillation, are examined by g.l.c. and the aroma intensity assessed. The 80–100° fraction contained the major part of the fusel oil and aroma fraction. Difficulties in obtaining reproducible results are discussed.
J. B. Woof.

Investigation of commercial kirsch. S. NOSKO (*Dt. Lebensmitt-Rdsch.*, 1969, **65** (12), 384–388. Ger., 5 ref.).—Investigation of many samples confirms that both the MeOH content and i.r. absorption (13/25 μ) follow Gaussian distributions and the two parameters are correlated. Using this relationship and the i.r. spectra it is possible to detect adulteration with spirit (with or without MeOH), fruit juice and cherry essence. J. B. Woof.

Fermenting and ageing a malt beverage. JOS. SCHLITZ BREWING CO. (Inventors: J. A. KOZULIS and P. D. BAYNE) (Br. Pat. 1,179,482, 13.4.67. U.S., 18.4.66).—A beer of sp. gr. < 5° Plato, produced by agitating a fermentable yeast-containing wort at temp. T° (45–70°F), is aged by agitating at (T + 4)°F. An apparatus for the process is described (1 diagram). S. S. Chissick.

Malt liquor products having very low protein, sugar and real extract contents with distinctive vinous flavour. C. GLUEK (Br. Pat. 1,177,475, 22.1.68).—The product contg. 3·2–5% of EtOH is prep. by mixing a cooker mash (made from malt, cereal and water, the starches then being liquefied) and a boiling main mash (from malt and water, sol. proteins being extracted), followed by withdrawing and boiling the wort, adding hops and infusion liquid (**I**) and fermenting the wort 13–18 days in presence of **I**, which is rich in enzymes and is obtained from the main mash. The final wort is mixed with brewing water of pH 4·2–5·5, an emulsifier, hop extract etc., and impregnated with CO_2. S. S. Chissick.

Adsorbent for treating fermented or fermentable liquids. A.-G. FÜR BRAUEREI-INDUSTRIE (Br. Pat. 1,181,435, 7.2.67. Ger., 11.2. and 12.12.66).—The adsorbent is an acid-treated swollen silicate-type laminar material of residual water content < 20% and pore vol. 0·3–8·0 ml/g (dry material), e.g., HCl-treated montmorillonite. S. S. Chissick.

Production of alcoholic beverages. HIRAM WALKER AND SONS (SCOTLAND) LTD. (Inventors: J. W. LAWRIE and A. A. CUNNINGHAM) (Br. Pat. 1,181,382, 27.4.67).—A process and apparatus for the economic recovery of grain solids from spent liquor are described. (6 diagrams). S. S. Chissick.

Fruits, Vegetables and Their Products

Gas-liquid chromatographic determination of organic acids in fruits as their trimethylsilyl derivatives. E. FERNANDEZ-FLORES, D. A. KLINE and A. R. JOHNSON (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 17–20. 11 ref.).—Pb salts of the acids are pptd. from a 95% ethanolic extract (with glutaric acid as internal standard) of the fruit by addn. of satd. Pb acetate soln. The residue is washed with 85% ethanol, Et_2O and dried for 1 h at 100°. The Pb salts are directly silylated by addn. of 1·5 ml of a mixture of pyridine–hexamethyldisilazane–trimethylchlorosilane (9:3:1) and, after allowing to stand 15 min at 45°, an aliquot is analysed by g.l.c. with thermal conductivity detection. Comparative analyses of *l*-malic acid in oranges showed that the g.l.c. method gave slightly higher results than those of a polarimetric method. Contents of various acids detd. in 27 types of fruits are given. D. I. Rees.

Effects of physiological maturation and storage on physical and biochemical changes in some stone fruits. I. Apricots (*Prunus armeniaca* L.). P. B. DESHPANDE and D. K. SALUNKHE (*J. Fd Sci. Technol.*, 1969, **6** (1) 15–17. 9 ref.).—The effect of storage at different degrees of maturity at 21° and 85% humidity, and at 1·1° and 95% humidity is assessed. Storage at the lower temp. before ripening did not have any detrimental effect on the phys. and biochem. changes. I. Dickinson.

Prediction of maturity in plums for prune production. D. MCG. MCBEAN, N. L. OUTHRED, A. A. JOHNSON and G. G. COOTE (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 648–654. 7 ref.).—A hydrometer for detn. of sp. gr. of plums is described. A 5 lb sample is held in a mesh basket (0·25 in. mesh) hooked to a polystyrene cistern float (800 ml capacity). A scale for sp. gr. is calc. and marked on a Perspex tube fastened to the float. A calc. technique for prediction of harvest date is given (based on sol. solids) but must be field-tested before routine use.
M. T. Rawnsley.

Heat-induced compounds in strawberries. J. L. SLOAN, D. D. BILLS and L. M. LIBBEY (*J. agric. Fd Chem.*, 1969, **17** (6), 1370–1372).—Puréed strawberries were heated to 120° and Me_2S, AcH, isobutyraldehyde, furan, 2-furaldehyde, 2-acetylfuran and Et furoate were produced. Shorter heating periods were sufficient to produce the first three compd. With the exception of AcH, none of the heat-induced compd. were detected in unheated, puréed strawberries. Identification was based on g.c. retention time, reactivity with specific reagents and mass spectrometry. Low-boiling compd. were separated and quantitated directly by an on-column trapping g.c. procedure. High boiling compd. were isolated from the purée by low-temp. (< 30°) vac. steam distillation. Concn. of the identified compd. in heated strawberry purée are in excess of published flavour threshold values. I. Dickinson.

Detection of adulterations in citrus juices. XVI. Characteristic chromatograms of amino acids from the juices of several species and varieties of citrus fruits. A. ARANDA, A. CASAS and J. ROYO IRANZO (*Revta Agroquím. Tecnol. Aliment.*, 1969, **9** (4), 586–599. Span., 20 ref.).—Identification by two-dimensional t.l.c. of the amino acids present in the juices from Comuna, Cadenera, Sanguina, Valencia, Navel Late, Salustiana and Verna oranges, Comuna, Clementina and Satsuma mandarines, Marsh grapefruit and Verna lemon is reported. Chromatograms were all similar, enabling the normal range to be established. Adulteration of juices by addn. of commercial sucrose, citric acid, or commercial amino acid altered the chromatograms sufficiently for the detection of adulteration; this method of detection is particularly effective where amino acids have been added to the adulterated juice in amt. sufficient to balance the HCHO-index. E. C. Apling.

Forced maturation and colour development in fruits and vegetables. I. Forced maturation of Canino apricots using elevated temperatures and dip treatment with naphthalene-acetic acid. A. ALBERT B., A. REIG F. and C. PÉREZ-NIEVAS A. (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18** (1), 9–20. Span., 2 ref.).—Preliminary studies are reported in detail. Immature apricots, harvested in two lots 8 days apart, were treated by immersion dipping in soln. of naphthalene-acetic acid (**I**) for 2 min followed by storage for 7–12 days at two different temp. (18 and 23°) and humidity levels (85–90 and 90–95%). Best colour development resulted from treatment with 200 ppm of **I**, storage at 23° and 90–95% r.h. Storage losses were low, and, although loss of yield was excessive with early picking and the later picking showed rapid loss of texture, the process should be economically satisfactory under more appropriately chosen conditions. E. C. Apling.

Forced maturation and colour development in fruits and vegetables. VI. Experiments with new techniques of de-greening citrus fruits. A. REIG F., A. ALBERT B. and J. CUQUERELLA C. (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18** (1), 21–38. Span., 9 ref.).—Commercial application of the technique of modified atm. storage with thermal leaps to colour development of Marsh Seedless grapefruit, Satsuma mandarins and Navelina, Washington Navel and Salustiana oranges were studied. Storage chambers permitted treatment of up to 37,000 kg of fruit, and provided for gas injection (O_2 and C_2H_4), absorption of CO_2 and programmed temp. cycling between 10 and 25°. CO_2 removal was by the Bonomi system, employing diethanolamine soln. (40–60% in water) as a sorbent, with provision for thermal regeneration, and changes in sp. gr. Treatment cycles varied from 48 to 72 h according to variety, with O_2 42–52, C_2H_4 1·0–1·6 and r.h. 90–96%. Results were very satisfactory, with accelerated colour development generally continuing for 48 h after removal of fruit from the chamber. E. C. Apling.

Determination of the oil content of olives by indirect complexometry with magnesium. R. GARCÍA-VILLANOVA and M. C. LÓPEZ MARTÍNEZ (*Grasas aceit.*, 1969, **20** (6), 283–286. Span., 8 ref.).—The pulped olives (~ 10 g) are saponified by heating with 8%-alc. KOH for 15–20 min; the mixture is cooled, filtered and washed with EtOH. NH_3/NH_4Cl buffer of pH 10 is added and the acids pptd with 0·02 M-$MgSO_4$. The ppt. is filtered off, washed with H_2O and the excess Mg^{2+} in the filtrate titrated with 0·02 M-EDTA soln., with Eriochrome Black T indicator. The results are a little lower than obtained by Soxhlet extraction, which may extract matter other than oil. L. A. O'Neill.

Effect of treatment with hot water on ripening and lycopene content of tomatoes. N. S. SINGH, M. S. KRISHNAPRAKASH, N. V. N. MOORTHY *et al.* (*J. Fd Sci. Technol.*, 1969, **6** (1), 18–20. 11 ref.).—

Dipping tomatoes at breaker and turning stages of maturity in hot water at 41–42° for 10 min resulted in accelerated rate of ripening with increased lycopene content. Tomatoes treated at turning stage ripened in 6 days, with a ripening index > 90, while treated fruits at breaker stage took 8 days for the same degree of ripening.
I. Dickinson.

Quality in canned peas. P. G. Goose (*Fd Wld*, 1970, **5** (2), 9–12).—A review including: ripening of peas; measurement of maturity; classification by tenderometer readings; effect of heat processing on texture; vining operations and quality; improvement in natural colour; other quality factors and the assessment of quality. W. J. G.

Texture of carrots. P. L. Howard and D. E. Heinz (*J. Texture Stud.*, 1970, **1** (2), 185–195. 2 ref.).—Carrots, stored at ~ 0° and conditioned at 21° and 65% r.h. for ≯ 24 h prior to use, are evaluated for shear (**I**) and compression (**II**) strengths using an Instron Universal Testing Machine. Correlation between the results and those obtained by judgement of sensory hardness are low in the case of **I** and high in the case of **II**. The coeff. of **II** is useful for predicting textural quality of carrots.
S. S. Chissick.

Preparing cooked potato products. Canadian Patents & Developments Ltd. (Br. Pat. 1,176,897, 30.4.68. Can., 9.6.67).—The surface starch is removed from sliced raw potatoes, which are then cooked for 3–15 min., air cooled, mashed, mixed with edible binder (cellulose Me ether), and after adjusting the solids content to 20–35% either dehydrated or formed into a product. Optionally $CaCl_2$, egg white, etc. are added at various stages.
S. S. Chissick.

Non-alcoholic Beverages

Soluble tea production processes. N. Pintauro (*Fd Process. Rev.*, 1970, (11), 182 pp.).—The review is based on 50 U.S. patents and 25 British and Canadian patents relating to production processes for sol. tea. These include: withering and rolling; fermentation, firing and sorting; extraction; recovery of aroma; tannin–caffeine ppt. (cream); filtration and concentration; dehydration; and agglomeration and aromatisation. W. J. G.

Milk, Butter, Other Dairy Products, Eggs

Determination of lactose in milk. Adaption of the colorimetric method of Somogyi and Nelson. H. A. Ainciburu (*Revta Fac. Agron. Univ. nac. La Plata*, 1968, **44** (1a), 65–72. Span., 4 ref.).—Application of the colorimetric method for glucose (*J. biol. Chem.*, 1944, **153**, 375) to the detn. of lactose in milk and dairy products is described in detail. The method requires only 0·5 ml of sample and is applicable in the presence of up to 20% of added sucrose.
E. C. Apling.

Determination of phosphorus, potassium, and calcium in milk by the rapid soil analysis method of Marino R.R. Zaffanella. N. C. A. Sanchez (*Revta Fac. Agron. Univ. nac. La Plata*, 1968, **44** (1a), 97–106. Span., 3 ref.).—The methods of Zaffanella (*Revta de Invest. Agric.*, 1956, **10**) are shown to be unsatisfactory for application to the rapid detn. of the mineral constituents of milk.
E. C. Apling.

Use of 2,4-dinitrophenylhydrazine and of 2,6-dichlorophenol-indophenol for determination of vitamin C in raw and in heat-treated milk. J. Toothill, S. Y. Thompson and J. Edwards-Webb (*J. Dairy Res.*, 1970, **37** (1), 29–45. 30 ref.).—The 2,6-dichlorophenolindophenol (DCP) method, with H_2S as reducing agent, and the 2,4-dinitrophenylhydrazine (DNPH) method, without chromatog., were found to be satisfactory for detn. of vitamin C in raw and mildly heated milks but not in strongly heated milks. With the latter, use of *E. coli* instead of H_2S for reduction improved the DCP method. However, only the DNPH method with column chromatog. and t.l.c. of the DNPH deriv. was absolutely specific for vitamin C. M. O'Leary.

Simplified method of detecting antibiotics in milk. G. Letourneur, Y. Potin and M. Jardin (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (15), 1077–1081. Fr., 8 ref.).—Wright and Tramer's method (*J. Soc. Dairy Technol.*, 1961, **14**, 85) was modified using *Streptococcus thermophilus* instead of *Bacillus subtilis*. Lyophilised discs, impregnated with *S. thermophilus* culture, and in a nutritive medium contg. triphenyltetrazolium chloride, were used. Control samples of milk were placed in a water bath for several min at 85°. One ml of milk was then added to each of 16 test tubes and reactive discs. These were placed on a water bath at 42° and examined after 3·5–4 h. Each disc showed an end-point of red colour of varying depth, if antibiotic was absent. Discs remaining clear were contaminated. Detection thresholds are: penicillin 0·005 I.U./ml, erythromycin 0·1 μg/ml, terramycin 1 μg/ml, neomycin, streptomycin, spiramycin, soframycin, aureomycin, chloramphenicol, triacetyloleandomycin 10 μg/ml. M. T. Rawnsley.

Spectrophotometric determination of protein and fat in milk simultaneously. S. Nakai and Anh Chi Le (*J. Dairy Sci.*, 1970, **53** (3), 276–278. 7 ref.).—A method for the simultaneous detn. of protein and fat in milk is described. A clear soln. is obtained by adding 5 ml of 97%-HOAc to 0·05 ml whole milk and the protein is calc. from the absorbance at 280 nm. 2·5 ml of a soln. contg. 20% urea and 0·2% imidazole is then added and the fat is detd. from absorbance at 400 nm with a round cuvette.
M. O'Leary.

Pattern of release of free fatty acids from milk fat under the action of intrinsic and added lipases. E. B. Hemingway, G. H. Smith and J. A. F. Rook (*J. Dairy Res.*, 1970, **37** (1), 83–96. 11 ref.).—Rate of release of fatty acids from milk by porcine pancreatic lipase was found to be low initially and then to increase logarithmically before a final decline. Addn. of boiled milk, previously subjected to the action of lipase, enhanced fatty acid release as did an extract contg. monoglycerides from lipalysed milk. In the early stages of lipolysis, short-chain fatty acids were released preferentially and passed mainly into the aq. phase. Fresh milks, in which taint was induced by aeration, displayed a considerable release of fatty acids with a redistribution between the aq. and fat phases. M. O'Leary.

Effect of temperature on water vapour sorption by dried milk powders. E. Berlin, B. A. Anderson and M. J. Pallansch (*J. Dairy Sci.*, 1970, **53** (2), 146–149. 7 ref.).—The influence of temp. on the equil. water vapour sorption values for dried milk powders varied with the relative pressure P/P_o, at 14–34°; both positive and negative temp. effects on adsorption were observed. Thermodynamic treatment of the data indicate an initial high isosteric heat of adsorption for protein-water binding, followed by lower energy values, less than the heat of liquefaction of water, which are attributable to mobility of sorbed water mol. and solubilisation and crystallisation of lactose. M. O'Leary.

High efficiency filtration eliminates cream sediment problems. A. Catchick (*Mod. Dairy*, 1969, **48** (2), 25–26, 29).—85·5% of the samples tested (561) were acceptable, the sediment being 0·00–0·10 mg; in the 14·5% group of unacceptable samples, the sediment was > 0·15 mg/100 g cream. Within this group, 6·8% were difficult to filter. C. V.

Factors influencing photo-oxidation in butter. J. Foley, P. O'Flynn and W. Phelan (*Ir. J. agric. Res.*, 1969, **8** (6), 431–438. 25 ref.).—Butter granules were taken from the churn at the break point, and used to study the influence of treatments on their chem. oxidn. The effects were detd. of salt concn., available Cl concn. of the wash water and the washing of butter granules on the peroxide and thiobarbituric acid (TBA) values of butter exposed to fluorescent lighting. Salt and chlorine exhibited pro-oxidant activity which increased with increasing concn. Washed butters had lower TBA serum values and higher fat peroxide values than unwashed butters. M. J. Rawlins.

Colouring of butter by means of annatto and β-carotene. H. Lück, J. C. Novello and D. J. Van Zyl (*S. Afr. J. Dairy Technol.*, 1969, **1** (1), 15–24. Engl., 14 ref.).—It was found that different annatto extracts available varied considerably in quality and price per colour unit. Peroxide and free fatty acid values indicated loss of quality during storage of the extracts at 50°. β-Carotene soln. maintained their quality under similar conditions. Oxidative deterioration was also examined. β-Carotene was found to be preferable for use as colouring matter in dairy products. The possibility of using β-carotene for standardisation of the colour of butter is discussed. W. J. G.

Fat crystals and the flow rheology of butter and margarine. C. Parkinson, P. Sherman and S. Matsumoto (*J. Texture Stud.*, 1970, **1** (2), 206–213. 15 ref.).—Viscosities of fat crystals (*FC*), extracted from butter (**I**) and margarine (**II**) by continuously washing in thin layers with a 1% soln. of Aerosol O.T., were detd. with a Weissenberg Rheogoniometer at 25°. Results indicate that the *FC* from **II** are broader than the *FC* from **I**. Extrapolation of the results to very high shear rates could be useful for predicting the spreadability characteristics of **I** and **II**.
S. S. Chissick.

Influence of work softening on the viscoelastic properties of butter and margarine. P. SHERMAN (*J. Texture Stud.*, 1970, **1** (2), 196–205. 18 ref.).—The properties were studied by shearing in a parallel plate viscoelastometer and by compression between parallel plates. The creep compliance-time response (*CCTR*) for margarine (**I**) was found to increase to a much larger extent than that for butter (**II**); *CCTR* falls more rapidly with ageing for **I** than for **II**.
S. S. Chissick.

Modification of the plastic properties of butter to improve spreadability. J. J. STROZ (*Can. Dairy Ice Cream J.*, 1968, **47** (4), 19–20).—The lowest usable and most acceptable temp. ranges for butter and margarine are compared and discussed. The following are summarised: thermal treatment of Reinart and Nesbitt (*ibid.*, 1958, **38** (9), 54); the use of a homogeniser suggested by de Man and Wood (*ibid.*, 1959, **39** (2), 42); the addn. of crystal modifiers (0·1–0·5%) or blending the anhydrous butter oil with vegetable oil, thus resulting in chem. re-arrangement. C. V.

Search for new uses for whey powder and its byproducts by cheesemakers. J. KEAY (*Mod. Dairy*, 1969, **48** (5), 33–34, 39).—A general discussion. C. V.

Research developments and new processes for handling cheese whey. K. KEAY (*Mod. Dairy*, 1969, **48** (6), 19, 21).—The production of non-hygroscopic whey powder by crystallising the lactose before drying is discussed together with uses for condensed whey. Further work on yeast fermentation of whey is indicated together with the production of vitamin B_{12} (**I**) by growing *Propionibacterium shermanii* in whey. The cell mass contg. **I** can be separated either by centrifuging or spray drying, this latter being economically feasible for enriching feeds with a view to growth stimulation. Removal of water from whey by reverse osmosis technique is reviewed and this appears to be economically possible, attaining a four-fold reduction in total vol. and total solids to ~ 30–40%.
C. V.

Curd production in a continuous ribbon in automatic cheesemaking. ANON. (*Mod. Dairy*, 1969, **48**, (10) 19–21).—The process now operating in Minnesota is described; $1·0$ to $1·5 \times 10^7$ lb milk are processed per day producing 100,000–150,000 lb cheese and 65,000–85,000 lb powdered whey. C. V.

Cheddar cheese: comparative effects of raw and heated milk on quality and ripening. W. V. PRICE and A. O. CALL (*J. Milk Fd Technol.*, 1969, **32** (8), 304–311. 38 ref.).—Cheese was cured at 7°, scored at 1, 3, 6 and 12 months and analysed for total N, water sol. N and amino N at 3, 6 and 12 months. Milk pasteurised by the holder method or by heating to 71° produced better cheese than raw milk or milk treated for a short time < 71°. Raw milk cheese cured most rapidly and had most intense flavour but was the least stable on storage. Rate of curing and intensity of flavour decreased and storage stability increased with severity of heat treatment.
C. V.

One-sided defect of Swiss cheese. S. A. SCHNEIDER, E. G. HAMMOND, G. W. REINBOLD and E. R. VEDAMUTHU (*J. Dairy Sci.*, 1970, **53** (1), 30–37. 21 ref.).—Moisture, pH, lactic acid concn., dye-binding capacity, and rheological properties were measured on both the inside and outside of young and aged samples of normal Swiss cheese (**I**) and cheese exhibiting the defect of one-sidedness (**II**). In the young samples, the lactic acid in **II** was higher in the inside than the outside, whereas the reverse was true for **I**. In the aged samples, the elasticity and shear strength of **II** cheese was lower in the outside than the inside, whereas the reverse was true for **I**. M. O'Leary.

Gas chromatographic determination of heterocyclic bases in the volatiles of Russian cheese. R. V. GOLOVNYA, R. M. ABDULLINA, I. L. ZHURAVLEVA and G. A. MIRONOV (*Izv. Akad. Nauk SSSR Ser. Khim.*, 1969, (1), 2570–2572. Russ., 6 ref.).—Using samples of 4-month old cheese, the changes in the qual. and quant. compn. of the volatile base components were detd. during 10 months ageing at −3° and 85–87% r.h. From 500 g of original cheese, 23·0 mg of amine hydrochlorides were separated, while after ageing, 29·4 mg were separated. Primary, secondary and tertiary amines and heterocyclic bases present were separated and identified by g.l.c. using a flame ionisation detector. Qual. compn. of the components changed little during ageing but significant increases in concn. of piperidine and α-picoline occurred, while pyridine content decreased. Concn. of piperidine, α-picoline, pyridine and triethylamine were detd. by an abs. calibration method.
M. E. Traxton.

Utilisation of milk citrate by lactic acid bacteria and 'blowing' of film-wrapped cheese. T. F. FRYER, M. E. SHARPE and B. REITER (*J. Dairy Res.*, 1970, **37** (1), 17–28. 12 ref.).—When *Streptococcus diacetilactis* 1007 was grown in milk alone or with either or both *Streptococcus cremoris* 924 or *Lactobacillus casei* B142/C, more than 99% of the milk citrate was utilised within 5 days. *L. casei* B142/C alone and with *Str. cremoris*, utilised 57 and 14% of the citrate, resp. When *L. casei* C2 and *L. casei* C5 were grown in milk in which *S. cremoris* 924 had previously grown, 94 and 64%, resp., of the citrate was utilised in 7 days. It is suggested that when starters contg. active citrate fermenting strains of *S. diacetlactis* are used for cheese-making most of the citrate will be utilised before film wrapping. 'Blowing' after wrapping should not occur unless a strain of *S. diacetilactis* is used which does not ferment the citrate until after wrapping of the cheese. M. O'Leary.

Determination of moisture in dairy products by near infra-red absorption of methanol extracts. J. D. S. GOULDEN and D. J. MANNING (*J. Dairy Res.*, 1970, **37** (1), 107–112. 8 ref.).—The method employs absorbance measurements at the 1·93 μm water band on MeOH extracts. M. O'Leary.

Collaborative study on the gas–liquid chromatographic method and AOAC methods for the quantitative determination of lactic and succinic acids in egg. W. F. STARUSZKIEWICZ, JUN. (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 28–35. 5 ref.).—The study is a continuation of the one previously described (*Idem., ibid.*, 1969, **52**, 471) which involves detn. of the acids by analysis of their propyl esters by g.l.c. The method was found to be reliable when applied to both passable and decomposed egg. D. I. Rees.

Studies on the preparation of whole egg powder (chicken). J. R. IYENGAR, N. V. SRIPATHY and R. SURYANARAYANA RAO (*J. Fd Sci. Technol.*, 1969, **6** (2), 89–92. 20 ref.).—A process for the manufacture of whole egg powder in lab. and on pilot plant scale is described. The eggs were washed mechanically and by hand in H_2O contg. Tween 80, and then dipped in 2% bleach. They were broken, churned and filtered. Reactivated dry yeast at 0·25 or 0·5% of the egg liquid was added to the melange and incubated at 31 or 36°. The fermented melange was pasteurised at 61° then cooled and chilled to 8°; this almost completely eliminated *E. coli* and *Salmonella* growth. pH was then adjusted; and drying took place. The product was found to be acceptable with respect to texture, taste and flavour. M. J. Rawlins.

Preparation and storage of duck whole egg powder. J. R. IYENGAR, N. V. SRIPATHY and R. SURYANARAYANA RAO (*J. Fd Sci. Technol.*, 1969, **6** (2), 123–127. 27 ref.).—The manufacture of whole egg powder in lab. and in pilot plant was standardised. The egg melange was subjected to (*a*) acid stabilisation, (*b*) desugaring by yeast and (*c*) desugaring by yeast followed by acid stabilisation, before making the powder. Egg powder was also prep. without pre-treatment (control). The max. life of egg powder prep. without pre-treatment was 3 months at 22–25° and that of the yeast desugared sample was 1 yr at 37°. M. J. Rawlins.

Heat treatment of milk and milk products. A.P.V. CO. LTD. (Inventor: C. H. BRISSENDEN) (Br. Pat. 1,176,792, 12.10. and 2.12.65).—The milk/product is heated to > 132° and exposed to (ultra)sonic vibration in the frequency range 10^4–10^5 Hz. E.g., a good grade milk is obtained by treating the milk for 2·3 sec. with a power input of 4·9 W/gal. S. S. Chissick.

Production of milk clotting enzyme. KYOWA HAKKO KOGYO CO. LTD. (Br. Pat. 1,177,054. Jap., 14.3. and 1.12.67).—A microorganism belonging to the genera, *Irpex*, *Fomitopsis*, *Coriolus* or *Lenzites*, is cultured aerobically in an aq. medium at pH 2–7 and at 25–35°, and the enzyme recovered. S. S. Chissick.

Cheese product. BENCKISER-KNAPSACK GMBH (Inventors: H-A. ROHLFS, W. KOCH and G. SCHEURER) (Br. Pat. 1,180,716, 13.6.68. Ger., 13.6.67).—A processed cheese product with a reduced melting salt (**I**) content is prep. by adding a mixture of **I** and thickening agent to starting uncured cheese and melting the mixture. E.g., Na polyphosphate and phosphate-modified starch which has been dispersed in water and heated to 85°, are added to a mixture of Cheddar and Emmentaler cheeses, and the product melted and processed at 85° for 7 min with stirring to give a smooth, non-ropy product. S. S. Chissick.

Edible Oils and Fats

Effect of technological processing on the tocopherol content in rapeseed oil. A. RUTKOWSKI and L. MZYK (*Riv. ital. Sostanze grasse*, 1969, **46**, (11), 614–616. Engl., 12 ref.).—Changes in the

content of α- and γ-tocopherols (I and II) during extraction and refining of the oil were examined by techniques involving t.l.c. combined with re-chromatog. after saponification of the oil. There was no difference between the I + II content of expressed or extracted oil, or oil extracted at different temp. During refining, the I + II content decreases from 58 mg% to 43 mg% on neutralisation, to 26 mg% on bleaching and to 15 mg% on deodorisation. The ratio of I and II does not change during the processing. Concn. of I + II in the various waste products is highest in the deodorisation condensate. L. A. O'Neill.

Fluid shortening for cakes and cream icings. PROCTER & GAMBLE Co. (Br. Pat. 1,180,695, 15.11.68. U.S., 16.11.67).—The shortening comprises a liquid glyceride base oil (I) (cottonseed), a triglyceride hardstock (II) (hydrogenated soyabean oil), and a quaternary mixture of: (*a*) glycerol monoester, (*b*) polyoxyethylene sorbitan monoester, (*c*) a decaglycerol ester and (*d*) propyleneglycol monoester. The esterifying fatty acids in (*a*), (*b*), (*c*) and (*d*) have 12–22 C, 14–22 C, 14–22 C and 14–26 C atoms, resp.
S. S. Chissick.

Meat, Poultry, Fish

Fresh meat processing. E. KARMAS (*Fd Process Rev.*, 1970, (12), 236 pp.).—The review is based on ~ 105 U.S. patents since 1960, relating to processing of fresh meat. Processes for enhancing palatability are discussed with respect to (*i*) tenderness, considering feed additives, enzyme treatments, ageing at high temp., mechanical tenderising etc., (*ii*) flavour and tenderness, considering the simultaneous action of moulds and bacteria etc., (*iii*) flavouring by meat and bone hydrolysates and extracts, (*iv*) colour and (*v*) integral texture. Preservation processes are discussed including moisture retention, antimicrobial treatment, ionising radiation etc.
W. J. G.

Phosphoprotein phosphate content of some meat and sausage products. R. THALACKER (*Dt. Lebensmitt Rdsch.*, 1969, **65** (12), 373–377. Ger., 29 ref.).—The phosphoprotein (*PP*) content of veal, beef, pork, fatty tissue and offal was detd. Values are quoted in mg/g and as % apparent casein content. Values ranged from 15–25 mg/g for pork to > 200 mg/g in lamb and beef. The level in fatty tissue is low. Pig liver gave the highest offal value. To study the cellular distribution of *PP*, pig liver with low glycogen content was freeze dried, powdered and extracted. The homogenate was then suspended in org. solvent and separated by centrifugation. All the fractions contained appreciable amt. of *PP*.
J. B. Woof.

Collaborative study of a rapid electrophoretic method for fish species identification. II. Authentic flesh standards. R. J. LEARSON (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 7–9. 1 ref.).—The use of authentic fish standards in place of photographs of standard electrophoretic patterns as in Part I (*Idem., ibid.*, 1969, **52**, 703) resulted in the identification of 91% of the 10 unknown samples submitted to 11 collaborators. D. I. Rees.

Parameters of texture change in processed fish: myosin denaturation. G. H. CHU and C. STERLING (*J. Texture Stud.*, 1970, **1** (2), 214–222. 46 ref.).—Samples of white muscle from *Orthodon microlepidotus* were subjected to (*a*) freezing; (*b*) freezing and storage; (*c*) dehydration; (*d*) dehydration and storage; (*e*) cooking. Myosin from the samples was extracted and examined (solubility, viscosity, isoelectric point, ATPase activity, u.v. absorption, and optical rotatory dispersion) for evidence of denaturation. It was found that denaturation increases in the order fresh $< a < b < c < d < e$. S. S. Chissick.

Factors concerning green tuna. M. YAMAGATA, K. HORIMOTO and V. NAGAOKA (*Fd Technol., Champaign*, 1970, **24** (2), 198–199. 8 ref.).—The relationship between trimethylamine oxide (TMAO) and myoglobin (Mb) content of raw (minced) fish meat and the effects of cooking temp. and time were examined. Green was associated with high concn. of TMAO and Mb. If Mb was high a dark brown colour appeared. Increase in cooking temp. and time was essential to produce discoloration. M. J. Rawlins.

An improved method for obtaining good grade fish oil from oil sardines (*Sardinella longiceps*). K. VISWESWARIAH (*J. Fd Sci. Technol.*, 1969, **6** (2), 99–102. 9 ref.).—Dehydration of fish from 70% to 55–60% of moisture gave a good quality oil with max. yield and also fish meal of higher nutritive value, compared with the existing commercial process. The undesirable changes in stick liquor were controlled (by addn. of 1 ml of 40% formalin to 1 l of stick liquor) for ~ 20–22 days, giving sufficient time for the fishermen to undertake further processing. M. J. Rawlins.

Meat product. J. J. SCHROEDER (Br. Pat. 1,179,343, 24.4.67. U.S., 1.9.66).—The residues of high/low temp. rendered meat scrap or trim are acidified to pH 1–4 and comminuted for sufficient time to degrade the proteins as far as the proteose stage. The products are suitable for human or animal consumption.
S. S. Chissick.

Food Additives

Preservatives, Colouring Matter

A survey of the use of chemical preservatives for beer stabilisation. K. SILBEREISEN and B. WAGNER (*Mschr. Brau.*, 1970, **23** (2), 32–36. Ger., 61 ref.).—A literature review of the use of benzoic acid, *p*-hydroxybenzoic acid esters, dehydroacetic acid, diethylpyrocarbonate, gallic acid n-octyl ester, monohalogen acetic acid and esters, salicylic acid, sorbic acid and vitamin K_5 in beer processing.
J. B. Woof.

Antimicrobial food additives. B. A. BRACHFELD (*Baker's Dig.*, 1969, **43** (5), 60–65. 17 ref.).—The most common food preservatives, Ca propionate, Na propionate, sorbic acid, K sorbate, Na benzoate, and Na acetoacetate function by interfering with the growth of micro-organisms. A table of their recommended levels in baked goods was prep. Further precautions against contamination would be to ensure the absence of moisture on products before wrapping, personal hygiene amongst personnel, and maintaining a pH < 5 in the food product. M. J. Rawlins.

Chemical preservation of sardine fish to obtain a good grade fish oil. I. K. VISWESWARAIAH (*J. Fd Sci. Technol.*, 1969, **6** (2), 103–105. 10 ref.).—No single chemical was found to preserve fish, without affecting colour, smell and yield of processed oil. The combined effect of chemicals was investigated. Continuous dip treatment of sardines in satd. NaCl soln. contg. 0·4%-HCHO was found to be satisfactory. This method enabled fish to be held at room temp. for 24 h in good condition. M. J. Rawlins.

Sugar industry use for new preservative. G. R. BADGLEY (*Fd Technol. Champaign*, 1970, **24** (2), 138. 3 ref.).—n-Heptyl *p*-hydroxybenzoate (WS-7), successful in controlling yeast and Gram positive anaerobes, was selected to check yeast growth in stored sugar soln., together with Me *p*-hydroxybenzoate (MK) and Pr *p*-hydroxybenzoate (PK). Sugar soln. contg. 150 ppm and 300 ppm of WS-7, MK or PK were left for 3 days. Sucrose loss was then detd. A yeast count was made at 5 days. WS-7 (concn. 12 ppm) prevented loss of sucrose in sweetners stored for 72 h. Due to the wide range of effectiveness of WS-7 it is being considered as a sanitising agent. M. J. Rawlins.

Treatment of citrus fruit with sodium *o*-phenylphenate foam. A. RAJZMAN and A. APELBAUM (*Pestic. Sci.*, 1970, **1** (2), 59–62. 15 ref.).—Treatment conditions for reducing the incidence of rot in stored citrus fruit with sodium *o*-phenylphenate (SOPP) foam and the possibility of improving efficiency of the treatment were examined. Marked differences were noted among different packing houses with regard to ambient temp., temp. of the fruit surface, duration of treatment, SOPP content and pH of the solution, quantities of foam and of SOPP dispersed on the fruit, and between the *o*-phenylphenol (OPP) residues in fruit treated simultaneously. It should be possible to prolong the duration of treatment without increasing the OPP residues in the fruit above the tolerance limits of 10 ppm OPP in the whole fruit and without causing injury to the fruit. W. J. G.

Use of ascorbic acid in the production of preserved meats. R. GRAU (*Fd Wld*, 1970, **5** (3), 22–25. 5 ref.).—When nitrite curing salt is used, it is advantageous to use ascorbic acid. Technical advantages (e.g., time saving, better colour in cured meat, longer lasting colour in cold cuts and reduced tendency to rancidity) are discussed, together with physiol. and pharmacol. benefits (e.g., reduction in nitrite content). W. J. G.

The use of sorbic acid in salted fish. J. J. DOESBURG, E. C. LAMPRECHT, M. C. ELLIOTT and D. A. REID (*J. Fd Technol.*, 1969, **4** (4), 339–352. 24 ref.).—Halophilic bacteria and moulds cause spoilage of salted fish with a salt content > 10%. The most frequently occurring mould, *Hemispora stellata*, was isolated from salted snoek. The effects of sorbic acid (SA) and pH on its growth were studied. From exptl. evidence it was suggested that dipping

fish in salt–sorbate soln. was more efficient in combating *H. stellata* than mixing the SA with salt. M. J. Rawlins.

Gas-liquid chromatographic determination of butylated hydroxyanisole and butylated hydroxytoluene in breakfast cereals. D. M. TAKAHASHI (*J. Ass. off. analyt Chem.*, 1970, **53** (1), 39–43. 3 ref.).—Slight modifications to the method previously described (*ibid.*, 1968, **51**, 490) resulted in quant. recoveries of from 25 to 30 ppm of the antioxidants in breakfast cereals. D. I. Rees.

Volatile components of hardwood sawdust smoke. Components of phenolic fraction. A. O. LUSTRE and P. ISSENBERG (*J. agric. Fd Chem.*, 1969, **17** (6), 1387–1393. 27 ref.).—Thirty-one compd. were separated by g.c. on a variety of columns. They were identified by comparison of their retention times and mass and i.r. spectra with those of known authentic compd.; where reference materials were not available, degradative and synthetic reactions to known standard compd. were employed. Some of the identified compd. have not previously been reported. Condensates prep. from mixed hardwood sawdust and from hickory sawdust smoke were compared and found to contain the same compd.
I. Dickinson.

Polyamide–silica gel layer chromatography of yellow food dyes. HUNG-CHEH CHIANG and CHWAN-HWAI CHEN (*J. pharm. Sci.*, 1970, **59**, (2) 266–267. 7 ref.).—T.l.c. separation of five yellow food dyes and three toxic yellow dyes on mixed polyamide–SiO_2 gel layers developed with MeOH–2%-NH_4Cl soln.–$CHCl_3$ or Bu^tOH – 0·45%-NaCl soln. – EtOH was better and quicker than that obtained on polyamide or SiO_2 gel layers and sharper spots were formed. G. W. Flinn.

Use of synthetic carotenoids for colouring foods. H. KLAUI and F. HOFFMAN (*Fd Technol. Aust.*, 1969, **21** (12), 620–621, 623, 625, 627. 16 ref.).—The international status of carotenoid food colours within the context of permitted colours is discussed; β-carotene, β-apocarotenal, the ethylester of apocarotenoic acid and cantha-xanthin are found to be acceptable for use in foods. Chem. and phys. properties of the carotenoids are discussed with respect to their stability and solubility and the significance of such properties when used in food. Applications are discussed in terms of fat-based and water-based foods. Microcrystalline β-carotene was found suitable for the colouring of fatty materials, e.g., margarine, processed cheese, oil and shortenings. The colouring of butter with various forms of β-carotene is discussed in detail. Water dispersible carotenoid prepn. are described together with their application in the soft drinks industry. Other applications of water-sol. carotenoid powders are in cheese, tomato products, soup powders, sugar coated tablets etc. A. Gordon.

Spices, Flavours, Other Additives

Oleoresin pepper. E. S. NAMBUDIRI, Y. S. LEWIS, N. KRISHNAMURTHY and A. G. MATHEW (*Flavour Ind.*, 1970, **1** (2), 97–99. 12 ref.).—An analysis of 5 commercial grades of pepper shows that Malabar garbled grade is the preferred type for extraction, giving 3·4% of volatile oil, 9·2% of non-volatile ether extract and 61% of piperine in the extract. Prepn. of raw material and selection of solvents is considered; ethylene dichloride is the preferred solvent. Methods of extraction of spice-oleoresins are described, together with techniques of solvent stripping, and evaluation of the final extracts. G. R. Whalley.

Characterisation of some volatile constituents of bell peppers. R. G. BUTTERY, R. M. SEIFERT, D. G. GUADAGNI and L. C. LING (*J. agric. Fd Chem.*, 1969, **17** (6), 1322–1327. 16 ref.).—Steam volatile components of Californian green bell peppers were qual. analysed by conventional and capillary g.l.c. separation with characterisation by mass, i.r. and in some cases u.v. and p.m.r. spectra. The major components were identified in oil obtained by vac. steam-distillation–continuous extraction; additional components were detected in small amt. in the vac. isolated oil but in much larger amt. in oil isolated at atm. pressure. The identification of 24 components was confirmed by direct comparison of their spectral and g.c. retention data with those of authentic samples. Tentative identification was obtained for an additional 19 components from their mass spectra fragmentation patterns. Odour thresholds in water soln. were detd. for major components.
I. Dickinson.

Effect of modified salt on dough development. K. FORTMANN, H. WELCKER and F. BARRETT (*Baker's Dig.*, 1969, **43** (5), 50–52. 4 ref.).—Salt is necessary to the final flavour of bread, but hampers and extends the time of dough mixing. A process was developed to coat each salt granule with a high melting point fat, thus removing the salt from the system during mixing and fermentation of breadmaking. Hydroxylated lecithin may be added to assist removal of the fat, at the end of mixing. M. J. Rawlins.

Rapid and convenient salt measurement in meat, fish and cheese. L. J. VANDER WERF and A. H. FREE (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 47–48. 5 ref.).—Data are presented which show that results comparable with those from the Volhard titration are obtained with use of commercially available paper strips impregnated with $Ag_2Cr_2O_7$. The sample is stirred with hot H_2O, filtered, and the paper strip is placed in the filtrate.
D. I. Rees.

Quantitative analysis of synthetic culture flavour concentrates by gas chromatography. J. P. WALRADT and R. C. LINDSAY (*J. Dairy Sci.*, 1970, **53** (1), 94–97. 9 ref.).—A description is given of a quant. analytical method for the detn. of AcH, Me_2S, HOAc, diacetyl, and propylene glycol from a single g.c. injection.
M. O'Leary.

Oil of *Mentha piperita* (oil of peppermint). I and II. O. P. VIRMANI and S. C. DATTA (*Flavour Ind.*, 1970, **1** (1), 59–63; (2), 111–133. 684 ref.).—I. Characteristics and distribution of peppermint-type plants are reviewed, together with the effect on growth and oil yield of soil, climate, propagation and planting conditions. The effect upon growth of growth-regulators, temp., light, fertilisers, and the conditions of harvesting and drying upon the oil yield are also considered.

II. Distillation of peppermint oil is briefly considered and the oil content and oil yields from plants grown in other countries are presented in tabular form, together with economic aspects of home-grown and imported oils. Physico-chem. properties of all peppermint oils are also reported. G. R. Whalley.

Automated determination of cyclohexylamine in cyclamates. C. T. BERRY and R. J. CROSSLAND (*Analyst, Lond.*, 1970, **95** (1128), 291–295. 4 ref.).—Concn. down to 5 ppm in Na cyclamate, Ca cyclamate and cyclamic acid are detd., in the Technicon Auto-Analyzer, by coupling the cyclohexylamine with diazotised 4-nitro-aniline to form a red dye; the extinction of the dye is measured at 510 nm. Na borate buffer (pH 10·4) must be used instead of the usual K borate. The coeff. of variation is ~ 5% for 15–25 ppm and 20 samples can be analysed per h. Dicyclohexylamine does not interfere. W. J. Baker.

Food preservatives comprising sorbic acid. UENO PHARMACEUTICAL CO. LTD. (Br. Pat. 1,176,115, 26.3.68. Jap., 27.3.67).—Sorbic acid (**I**) particles (dia. 10–100 μm), coated with an edible hardened glyceride oil (**II**) (m.p. 40–90°) are prep. by dispersing the **I** in molten **II** at < 90° and spraying the melt into a cooled chamber. The preservative is suitable for use with, e.g., fish, meat and bread, and does not hinder bread yeast fermentation when added to the bread mixture. S. S. Chissick.

Food compositions. UNILEVER LTD. (Inventor: G. T. MUYS) (Br. Pat. 1,177,654, 28.3.66).—Glycerol (**I**) (5–30%) is incorporated in the aq. phase of the compn. which contains water, sugar, fat and optionally AcOH, to increase its resistance to attack by micro-organisms. In an example pasteurised fat and pasteurised sugar soln. containing 5% **I** and NaCl are mixed and emulsified. The product is stable for > 2 months against yeasts, moulds, etc.
S. S. Chissick.

Polyene compounds. N. V. PHILIPS' GLOEILAMPENFABRIEKEN (Br. Pat. 1,176,224, 30.3.67. Neth., 1.4.66).—Yellow to orange polyenes of structure $A{\cdot}CH{:}CH{\cdot}CMe{:}CX^1{\cdot}CX{:}CH{\cdot}CMe{:}CY^1{\cdot}CY(:CH{\cdot}CH{:}CMe{\cdot}CH)_2{:}CHA^1$ wherein X and X^1 are CO_2R (R is H, cation, or alkyl) and Y–Y^1 are H, or *vice versa*, and A–A^1 are $CH{:}CMe{\cdot}[CH_2]_2CH{:}CMe_2$, 2,6,6-trimethyl-3-R-4-$R^1$-cyclohex-1-enyl or -2-enyl, (R and R^1 are H, OH, OMe, or OEt) and their prepn. are claimed. The compd., e.g., β-carotene 14,15-dicarboxylic acid (**I**), are useful as intermediates, or as fat-sol. colouring substances *per se*. **I** is prep. by reacting retinal in EtOH soln. with NaOEt (added over 0·5 h) at 0°, followed by stirring at 20° for 1 h and boiling for 1 h. Aq. alc. KOH is added, and boiling continued for 2 h; extraction, acidification and further working up yields **I** of m.p. 148–150° (decomp.).
F. R. Basford.

Mixed crystals of glutamine and inosinic acid derivatives. KOYOWA HAKKO KOGYO K.K. (Br. Pat. 1,178,905, 7.4.67. Jap., 7.4.66).—An aq. soln. containing Na glutamate (**I**) and Na_2 inosinate is mixed with an aq. soln. containing **I** and seed crystals of **I** and the final mixture is concentrated. The product is a mixed seasoning.
S. S. Chissick.

Sweetening agents. MONSANTO CO. (Br. Pat. 1,176,246, 18.7.67. U.S., 19.7.66).—The agent comprises a mixture of ≮ 85 pt. by wt. of either a maize or a waxy-starch hydrolysate (D.E. 15–45 or 5–15 resp.) and ≯ 10 pt. by wt. of non-nutritive sweetener(s), e.g., saccharin, and has a bulk *d* of ≯ 30 lb/ft³, the physical appearance and sweetness of granulated sugar, and a low calorific value.
S. S. Chissick.

Food Processing, Refrigeration, Packaging and Storage

Food and vegetable processing in East Africa. B. N. GHOSH (*Fd Wld*, 1970, **5** (3), 9–11, 18).—A review. W. J. G.

Effect of oxygen uptake on quality of cooked, freeze-dried combination foods. J. M. TUOMY, L. C. HINNERGARDT and R. L. HELMER (*J. agric. Fd Chem.*, 1969, **17** (6), 1360–1363. 14 ref.).—Positive statistical correlations were found between O_2 uptake and flavour and odour ratings. Slopes for the regression lines for all 8 items (cooked freeze-dried combination items used in Armed Forces operational rations) were almost identical. No correlation was found between O_2 uptake and rehydration ratios. Several items exhibited antioxidant properties which suggests that further work on formulations combined with O_2 uptake studies will result in improved storage characteristics for this type of freeze-dried product. I. Dickinson.

Steaming of paddy for improved culinary, milling and storage properties. H. S. R. DESIKACHAR, C. M. SOWBHAGYA, C. S. VIRAKTAMATH *et al.* (*J. Fd Sci. Technol.*, 1969, **6** (2), 117–121. 8 ref.).—Freshly harvested paddy with moisture content 19–13% was steamed for 5–30 min. then shade dried. Studies were made of (*a*) milling and cooking qualities of rice, (*b*) development of free fatty acid (*FFA*) in bran during storage, and (*c*) *FFA* and peroxide value development during storage of undermilled rice and its organoleptic acceptability. There was a beneficial effect on milling, cooking and storage quality of rice as a result of steaming, besides a stabilising effect on the rice bran. M. J. Rawlins.

Continuous vacuum drying of whole milk foam. IV. Pilot plant. E. F. SCHOPPET, N. C. ACETO, J. C. CRAIG, JUN. and H. I. SINNAMON (*J. Dairy Sci.*, 1970, **53** (1), 56–62. 9 ref.).—The construction and operation of a pilot plant capable of producing an acceptable beverage-quality dry whole milk foam is described.
M. O'Leary.

Dehydration of bamboo shoots. DAW TIN TIN OO and U WIN AUNG (*Un. Burma J. Sci. Technol.*, 1969, **2** (1), 167–172. 1 ref.).—Expt. to determine optimum conditions for processing, dehydrating, packaging and storage of young bamboo shoots are described. The necessity for boiling off lethal amt. of cyanogens is emphasised. Sodium metabisulphite is used to preserve the finished product and develop a pleasant colour. It is shown that reduction in costs is achieved by replacement of a 2% steeping soln. of $Na_2S_2O_5$ by a soln. contg. an equiv. amt. of SO_2. L. MacQuisten-Wallace.

Bacteriological and whey protein denaturation aspects of heating processes used in the manufacture of low-heat skim-milk powder. F. O'CONNOR, B. M. MCKENNA and A. C. O'SULLIVAN (*Ir. J. agric. Res.*, 1969, **8** (3), 417–430. 18 ref.).—Bacterial counts and whey protein N levels at various manufacturing stages were monitored over 5 runs at 2 commercial processing plants for skim-milk powder. 5 preheating temp. were investigated. Low-heat powder was only produced with preheating conditions of 162°F × 30 sec, but bacterial counts were excessive. During all runs, the unsatisfactory bacterial level was due to sublethal effect of preheating rather than contamination. However increase in preheating treatment increased the whey protein denaturation during drying. M. J. Rawlins.

Pilot-plant concentration of cheese whey by reverse osmosis. F. E. MCDONOUGH and W. A. MATTINGLY (*Fd Technol., Champaign*, 1970, **24** (2), 194–197. 12 ref.).—The whey of Cheddar and cottage cheese was clarified, pasteurised and held at 40°F for no longer than 3 days prior to concn. Variables considered include pressure, membrane porosity, feed rate, temp. and pH. Concn. rates increased with increase of pressure and/or membrane porosity. Less than 1% of total solids was lost in the permeate through the tighter membranes and 3·7% through the loose membrane. Concn. of 4:1 would be practical but higher levels would be uneconomical and would cause clogging problems.
M. J. Rawlins.

New equipment for the thermal processing of canned foods. D. J. CASIMIR (*Fd Technol. Aust.*, 1970, **22** (1), 8–9, 11, 13, 15, 17, 19. 12 ref.).—Engineering aspects of thermal processing and their implications, as related to high speed processing, are reviewed. Equipment such as the Grover Howard retort system, Spiral-Type continuous reel cooker-cooler, Orbitort, hydrostatic cookers, hydrolock steriliser, 'flash 18' process and Ekelund hot-air steriliser are discussed. Flame sterilisation, with particular reference to the many benefits claimed for this process in a mushroom operation is considered in some detail. Also considered are the possible applications of microwave heating, recent procedures for obtaining time-temp. history in continuous operation, heating media costs and future trends. A. Gordon.

Combined effects of irradiation, storage and cooking on vitamins E and B_1 levels of foods. J. F. DIEHL (*Fd Irrad.*, 1970, **10** (1–2), 2–7. 4 ref.).—Vitamins E and B_1 were detd. in various foods before and after irradiation, after storage for periods of up to 8 months and after cooking and baking. Vitamin losses caused by combination of radiation, storage and heat were greater than those due to the additive effects of the individual treatments. W. J. G.

Packaging of fresh meat. I. Optimum packaging for fresh beef. R. HEISS and K. EICHNER (*Fleischwirtschaft*, 1969, **49** (6), 757–764. Ger., 28 ref.).—The red oxymyoglobin colour can be retained by using packaging materials with high O_2 permeability. The degree of CO_2 and O_2 permeability necessary were calc. for various packaging materials, considering chemistry of the muscle pigment, tissue metabolism and metabolism of the organisms causing spoilage. Optimum conditions for storage are discussed. (From Engl. summ.) W. J. G.

Evaluation and selection of flexible films for food packaging. E. G. DAVIS (*Fd Technol. Aust.*, 1970, **22** (2), 62–67. 17 ref.).—Properties of material and food requirements must be considered when choosing a package material. Variable foodstuffs tested ranged from freeze-dried to fresh produce. Methods are described for detn. of gas and vapour permeability of films and toxic and tainting effects on food by transfer of compd. from packaging. The variable sensitivity of foods to the uptake or loss of gases was studied together with the importance of moisture sensitivity of foods and its relation to water vapour transmission rates of the container. M. J. Rawlins.

Relationship between method of production and packaging [and quality of] oven-warm [wheat/rye] mixture bread. H. STEPHAN (*Brot Gebäck*, 1969, **23** (12), 236–239. Ger., 3 ref.).—Influence of production methods, packaging materials and storage conditions are discussed. Such faults as darkening and softening of the crust, excessive sponginess of the crumb and sour taste are minimised by the use of: water vapour- and aroma-permeable packaging materials; a 1–3 h cooling period in still air (before packing); high proportions of wheat flour in the mix; and the production of bread with floury as against smooth crust. Production recommendations include baking for 5–10 min longer at a temp. 10° lower than usual for unpacked bread, the use of sour dough temp. of 30°, and the use of one-tenth normal amt. of sour dough in combination with souring additives. E. C. Apling.

Aseptic packaging of grade A dairy products. A. L. RIPPEN (*J. Dairy Sci.*, 1970, **53** (1), 111–115. 7 ref.).—Recent developments and possible future trends in ultra-high temp. treatment and aseptic packaging of milk products are reviewed. M. O'Leary.

Guava powder—preparation, packaging and storage studies. M. MURALIKRISHNA, A. M. NANJUNDASWAMY and G. S. SIDDAPPA (*J. Fd Sci. Technol.*, 1969, **6** (2), 93–98. 16 ref.).—Good quality guava powder was prep. from white fleshed Allahabad variety guava. Addn. of sugar to guava pulp improved puffing characteristics, colour and flavour of final product. Of the various methods of drying examined, vac. shelf drying proved the most practical. The effects of drying time on moisture, ascorbic acid, texture and flavour of guava powder were tabulated. In-package desiccation greatly improved retention of ascorbic acid and reduced non-enzymic browning. M. J. Rawlins.

Investigations of the storage stability of manioc flour. K. WEGMANN (*Brot Gebäck*, 1970, **24** (1), 16–18. Ger., 6 ref.).—Studies of the effect of r.h. (65–98%), temp. (20–40°), moisture content and addn. of benzoic, sorbic, lactic or formic acids on the storage life of roots, chips and flour from *Manihot utilissima* are briefly reported. Extension of storage life due to inhibition of the contaminating microflora by acid addn. was significant but insufficient to be important in practice. Extended storage (> 2 yr) is only feasible for manioc flour stored with moisture content < 10% at r.h. < 65%. E. C. Apling.

Storage studies of Indian wheat flour (*Atta*). G. KAMESWARA RAO and M. A. MALATHI (*J. Fd Sci. Technol.*, 1969, **6** (2), 129–130. 3 ref.).—The effect of storage of Indian wheat flour in gunny bags, polyethylene gunny bags and polyethylene lined canvas bags is discussed. The last type of package was found to be the best. M. J. Rawlins.

Effect of ^{60}Co gamma irradiation on the survival of some fungi in single samples of each of three different grades of wheat. M. MOHYUDDIN and W. P. SKOROPAD (*Can. J. Bot.*, 1970, **48** (2), 217–219. 10 ref.).—Grades No. 1, 2 and 3 of wheat grains (*Triticum aestivum*) were treated with 100, 300, 500, 700 and 1000 krad of ^{60}Co γ-irradiation. *Alternaria* and *Fusarium* present in all grades were inactivated by 1000 krad. *Aspergillus*, *Rhizopus* and *Absidia*, less frequently isolated, were less resistant to treatment. The grade of grain did not effect survival of fungi after irradiation. Spore septa of *Alternaria* failed to form with increasing irradiation, and at 700 krad spores failed to form. M. J. Rawlins.

Preparation of segments in syrup from Satsuma and Clemenules (oranges) after refrigerated storage. A. ALBERT B., A. REIG F. and C. PÉREZ-NIEVAS A. (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18** (1), 39–66. Span., 2 ref.).—Studies of the effect of refrigerated storage for up to 53 days at 1–2 or 4–5°, with or without prior treatment with fungicide or fungicide and coating-wax, on yield of whole segments and other processing quality factors are reported in detail. Results were generally satisfactory. Wt. losses increased with time (from 2–11% for Campenules; from 4–30% for Satsuma), but % yield of whole segments was only slightly reduced after refrigerated storage, and the pre-treatments had no detectable effect. E. C. Apling.

Studies on the comparative efficacy of oil coating and lime sealing on the preservation of shell eggs at room temperature. SUSHIL KUMAR, B. PANDA, M. SREENIVASULU REDDY and R. JAGANNATHA RAO (*J. Fd Sci., Technol.*, 1969, **6** (1), 9–11. 13 ref.).—Oil coated eggs maintained a higher Haugh unit score, albumen index, yolk index, % of thick albumen and a lower loss in egg wt. and less variations in pH, % of yolk, % solids of albumen and yolk, than the lime water treated eggs during storage. The control group (untreated) showed considerable deterioration in internal quality and were unfit for consumption after 28 days of storage. I. Dickinson.

Equilibrium humidity as the factor determining the shelf life of confectionery goods. H. H. VÖLKER (*Fd Technol. Aust.*, 1970, **22** (2), 58–61. 12 ref.).—Sorption isotherms illustrate the relationship between humidity in the air and the water in many foodstuffs of differing hygroscopicity. The closely associated equil. relative humidity (*ERH*) value was detd. for foodstuffs by the equation: $\%\ ERH = 100 \div (1 + 0{\cdot}27\,N)$ where N = mol. of dissolved substance in 100 g H_2O. The importance of *ERH* in relation to spoilage by, e.g., micro-organisms, is stressed. M. J. Rawlins.

Preparing foodstuffs and package therefor. DOW CHEMICAL CO. (Inventor: O. R. MCINTIRE) (Br. Pat. 1,177,165, 29.8.67. U.S., 28.9.66).—A polypropylene or irradiated polyethylene resealable pouch used for prepn. of cooked fats, vegetables, meats etc. is described. S. S. Chissick.

Dressing foodstuffs and container and foodstuff package for carrying out the method. WILLI LÖHNERT (Br. Pat. 1,178,290, 29.8.67. Ger., 29.8.66).—The foodstuff (fish, meat, etc.) is heated at the dressing temp. in an airtight, steam permeable plastic container. S. S. Chissick.

Nutrition, Proteins, Amino Acids, Vitamins

Simple, practical method of assessing the nutritional value of grain and certain cereals. Y. COÏC and C. TENDELLE (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (13), 927–931. Fr., 3 ref.).—The technique depends on the removal of non-protein-N and estimation of NH_3 liberated. Finely ground grain (1 g) with known % dry matter, is stirred with 10 ml of 10% trichloroacetic acid for 1 h. The product is filtered on sintered glass and the residue hydrolysed by 10 ml of N-H_2SO_4 under reflux for 30 min. After cooling, the H_2SO_4 is nearly neutralised by 2 N-NaOH. The filtrate is made up to 20 ml and NH_3 detd by Conway's method. M. T. Rawnsley.

Comparative nutritive value of certain vegetable and marine oils in rats. N. AHMED and M. B. SIAL (*W. Pakistan J. agric. Res.*, 1969, **7** (2), 107–113. Engl., 13 ref.).—Rats fed on a basic diet of maize starch 40%, casein 20%, glucose, and vitamin and mineral mixture, had 10% of one of the following oils: coconut, cottonseed, groundnut, cod liver, herring, halibut and liver. The herring diet was insufficient. Lesions occurred in all rats except those fed cottonseed oil. Reasons for this are discussed. M. T. Rawnsley.

Effect of level of protein on protein quality of lysine-deficient foods. E. YANEZ and J. M. MCLAUGHLAN (*Can. J. Physiol. Pharmac.*, 1970, **48** (3), 188–192. 15 ref.).—For 2 weeks male weanling Wistar rats were fed test diets of 5, 10 and 15% protein levels. Groups of controls received non-protein diets. Net protein utilisation (*NPU*) was assayed by the Bender and Doell method. *NPU* for lysine-deficient proteins were markedly higher at 5 than 10% level of protein but *NPU* for methionine, threonine or lysine and threonine yielded similar values at 5 and 10%. It is concluded that protein quality *per se* depends on the level of protein fed even at low and moderate concn. M. J. Rawlins.

Fermentation methods for protein enrichment of cassava. E. J. BROOK, W. R. STANTON and A. WALLBRIDGE (*Biotechnol. Bioengng*, 1969, **11** (6), 1271–1284. Engl., 32 ref.).—Two methods are described for the nutritional upgrading of cassava, (*i*) a direct solid-substrate fermentation and (*ii*) a liquid fermentation; both use starch degrading organisms that produce protein. In (*i*) cassava is converted into a coarse grade flour by conventional methods and is mixed with inorg. salts and urea. An inoculum of *Rhizopus oligosporus* is added and the moist paste is fermented as a spaghetti-like extrusion to form a vegetable cheese. This low-cost method is well adapted to simple apparatus. Method (*ii*) uses a suspension of cassava flour with urea in a nutrient medium under aseptic conditions. The fermentation is effected by *Rhizopus stolonifer* or *Mucor racemosus*. This method requires considerably more elaborate apparatus. J. N. Ashley.

Chemical and physical properties of soyabean proteins. W. J. WOLF (*Baker's Dig.*, 1969, **43** (5), 30–37. 45 ref.).—A variety of soyabean proteins, available in baking, are grits, concentrates and isolates. The proteins were highly extractable with H_2O, indicating that the membranes surrounding the protein bodies are easily broken. Protein concentrate was produced by (*a*) leaching defatted meal with dil. acid at pH 4·5 and (*b*) by washing defatted meal with aq. alcohols. Electrophoresis, ultracentrifugation, chromatog. and immunochemistry indicated great complexity of the protein. Difficulties in attempting to explain the functional properties of soyabean proteins, incorporated in dough, are emphasised. M. J. Rawlins.

Production of microbial protein from carbon sources. Z. FENCL (*Biotechnol. Bioengng Symp.*, 1967, *No. 1, 2nd int. Conf. global Impacts appl. Microbiol.*, 1969, 63–70. 11 ref.).—A review. M. J. Rawlins.

Loss of methionine in casein during storage with autoxidising methyl linoleate. S. R. TANNENBAUM, H. BARTH and J. P. LE ROUX (*J. agric. Fd Chem.*, 1969, **17** (6), 1353–1354. 12 ref.).—A model system of casein:methyl linoleate (9:1) was used to study changes in methionyl residues of proteins as a consequence of lipid oxidn. A modified McCarthy–Sullivan procedure (*J. Biol. Chem.*, 1941, **141**, 871) was developed which could distinguish methionine from its oxidn. products. In each case loss of methionine was proportional to the amt. of protein-bound nonenzymic browning pigment. Methionyl residues may act as peroxide decomposers with concomitant carbonyl compd. formation which in turn leads to nonenzymic browning. I. Dickinson.

Combination of plant and industrial biosynthesis. V. SKOLA (*Allg. prakt. Chemie*, 1970, **21** (2), 35–36. Ger.).—Utilisation of beet sugar for the fermentative production of yeast protein by a new process, patented in Czechoslovakia, is briefly discussed. M. Sulzbacher.

Fermentative production of L-tryptophan. G. TERUI and H. NIIZU (*Biotechnol. Bioengng Symp.*, 1967, *No. 1, 2nd int. Conf. global Impacts appl. Microbiol.*, 1969, 33–52, 10 ref.).—*Hansenula anomala* was used for the fermentative production of L-tryptophan (**I**), which was estimated by the xanthydol method. During fermentation at 27° the O_2 level was controlled. Fermentation was proved to be the growth associated product type. Various substrates were used and corn flour, beet, cane molasses as raw materials gave yields not less than those obtained with glucose. The optimum O_2 concn. was $\sim 10^{-4}$ M and EtOH and glycerol gave increased yields of **I** when added to a glucose-grown culture. M. J. Rawlins.

Developments in the field of vitamins. O. ISLER (*Experientia*, 1970, **26** (3), 225–240. 23 ref.).—A review. C. V.

Model molecules for vitamin B_{12}. N. STAGNI, B. de BERNARD, G. COSTA and G. MESTRONI (*Nature, Lond.*, 1970, **225** (5236), 942-943. 7 ref.).—The org. Co-aquo chelates of bis-(diacetyl-monoxime-imino)-1,3-propane (I) stimulate the growth of a vitamin B_{12}-dependent strain of *Lactobacillus leichmanii*, provided that a small amt. of vitamin B_{12} is present in the medium as catalyst. Each chelate is active only over a narrow range of concn., with max. activity at 0·07 mM (Bz), 0·4 mM (Pr, Ph) and 0·7 mM (Me, Et deriv.). The effect is virtually the same for the corresponding Co-*N*-methylimidazole chelates of I. All chelates are inactive (i.e., no replacement of native cobamide co-enzyme) and all inhibit competitively the propanediol dehydratase reaction. These synthetic complexes should be useful model compd. for studies on the reaction mechanisms of vitamin B_{12}. W. J. Baker.

Determination of thiamine in flour fortification mixtures. H. V. HART and K. H. WILLIS (*Analyst, Lond.*, 1970, **95** (1128), 312–315. 3 ref.).—Recoveries of thiamine (I) from these mixtures (flour, master-mix, chalk, etc.) when analysed by the Ridyard method (*ibid.*, 1955, **80**, 834) are low because of the presence of $KBrO_3$ (II) and $CaCO_3$ (III). II causes destruction of some I and III causes adsorption of I on insol. matter during decomp. of sample in HCl. These two interferences are overcome by addn. of KCl and $FeSO_4$ to the HCl used for dissolution. Recoveries then av. 98–99·5%. W. J. Baker.

Vitamin B_6 components in some meats, fish, dairy products and commercial infant formulas. M. M. POLANSKY and E. W. TOEPFER (*J. agric. Fd Chem.*, 1969, **17** (6) 1394–1397. 15 ref.).—Hydrolysed food extracts were separated by chromatog. on a Dowex 50 resin column prior to microbiol. assay. Vitamin B_6 values ranged for meats from 3 to 8 μg/g, fish from 0·5 to 4 μg/g, dairy products from 0·01 to 4 μg/g and commercial infant foods from 0·4 to 4 μg/g. I. Dickinson.

L-Threonine. KYOWA HAKKO KOGYO CO. LTD. (Br. Pat. 1,176,125, 8.1.68. Jap., 21.1.67).—Used as a nutritive additive to cereal proteins, L-threonine (I) is obtained by aerobically culturing a I-producing micro-organism (*Escherichia, Brevibacterium, Xanthomonas, Vibrio, Aerobacter* spp. for example) in an aq. medium contg. a decomposate of the mycelia of micro-organisms contg. diaminopimelic acid (e.g., *Pseudomonas, Azotobacter, Proteus, Bacillus, Chlorella* spp.). This decomposate is obtained by acid or alk. hydrolysis of the mycelia; its use results in increased production of I. S. D. Huggins.

Producing L-threonine by fermentation. KYOWA HAKKO KOGYO CO. LTD. (Br. Pat. 1,181,592, 9.1.68. Jap., 21.1.67).—A genus of *Escherichia* requiring diaminopimelic acid and optionally methionine, e.g., *E. coli* (ATCC 21148), is cultivated aerobically at 20–40° and at pH 4·5–9 in an aq. nutrient. The main C-source present is fructose, xylose, starch, cane sugar, waste molasses or desalted waste soap liquor. The L-threonine is recovered from the medium. S. S. Chissick.

Unclassified, Tobacco

Advances in food technology. M. PYKE (*Fd Wld*, 1970, **5** (2) 6–8).—A review in which (*i*) effects of food technology on the agriculturalist, (*ii*) effects of vitamin A deficiency and (*iii*) social nutrition are discussed. W. J. G.

Linearisation of empirical rheological data for use in composition control of multicomponent foodstuffs. B. DRAKE and B. NÁDAI (*J. Texture Stud.*, 1970, **1** (2), 223–230. 3 ref.).—A function involving η (viscosity) and n_D (refractive index) which is linear over an extended range of compn. combinations is suggested for the direct, rapid compn. control of 3-component systems, e.g., tomato paste–water–sucrose, by linear vector-vector transformation. S. S. Chissick.

Application of engineering techniques to evaluation of texture of solid food materials. N. N. MOHSENIN (*J. Texture Stud.*, 1970, **1** (2), 133–154. 41 ref.).—Specific mechanical properties (e.g., resistance to shearing, tensile/compressive modulus of elasticity, toughness, stress relaxation, creep, and storage and loss moduli) were measured. Numerical data obtained were used in the fundamental equations of mechanics and rheology. S. S. Chissick.

Determination of trace metals in foods and commodities by atomic absorption spectrophotometry. II. F. ROTH and E. GILBERT (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1969, **19** (6), 430–436. Ger., 2 ref.).—After wet ashing the sample, the metals Cu, Ag, Pb and Zn were extracted with dithizone in CCl_4. Purification of reagents and solvents is described. The first 2 metals were extracted at pH 1·5–1·8 and the last two at pH 9·2–9·6 in the presence of NH_4 citrate. Concn. was detd. by atomic absorption spectrophotometry. Min. levels which could be measured with certainty were: Ag, 0·002; Cu, 0·003; Pb, 0·01 and Zn, 0·002 ppm. Interference by anions is eliminated and the time consuming removal of contaminating metals is not necessary as it is with the visible spectrophotometric procedure. J. B. Woof.

Physical and chemical characteristics of processed arecanuts. S. SHIVASHANKAR, S. DHANARAJ, A. G. MATHEW *et al.* (*J. Fd Sci. Technol.*, 1969, **6** (2), 113–116. 10 ref.).—Six major types of marketed arecanut samples were analysed. Length, dia., nuts/pieces/kg and vol./nut/piece in ml were tabulated. The nuts were analysed for moisture, total H_2O extractives, polyphenols, arecoline, fat, crude fibre, total polysaccharides, ash, and acid insol. ash. The most useful constituents for specification were moisture, total H_2O extractives, polyphenols, crude fibre and ash. M. J. Rawlins.

Enzymic degradation of coconut meal by 'Meicelase-P'. G. RAMA RAO (*J. Fd Sci., Technol.*, 1969, **6** (1), 21–22. 3 ref.).—Coconut meal has a high protein content of good nutritional quality but high fibre content has prevented its use for human consumption. Expt. with the enzyme 'Meicelase-P' (a cellulase prepn. from *Trichoderma viride*) show that this enzyme is capable of degrading the fibre content in coconut meal. I. Dickinson.

Application of cellulase. I. Degradation of vegetable foodstuffs with bacterial enzyme. K. C. GHOSE, A. K. BISWAS and D. P. HALDAR (*J. Fd Sci. Technol.*, 1969, **6** (1), 29–32. 9 ref.).—Extracellular cellulase of the bacteria *Cytophaga hutchinsonii* at different concn. was used in the degradation of slices of beet, carrot, guava and pineapple and sections of sweet potato, potato and radish. All substances were degraded to some degree by partial dissolution of the cell walls. The period of disintegration was shortened with higher enzyme concn. Fibrous materials in the plant tissue were resistant to enzymic attack. Some of the starch was solubilised with enzymic treatment. I. Dickinson.

3.—PEST AND DISEASE CONTROL, SANITATION

Plant Diseases, Pests and Weeds

Some problems of pests and pesticides in Spanish fruit orchards. J. M. DEL RIVERO and SILVERIO PLANES GARCÍA (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18**, 215–239. Span., 38 ref.).—A review. E. C. Apling.

Herbicides

Weed control in Spanish fruit orchards. J. M. DEL RIVERO (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18** (2), 191–213. Span., 17 ref.).—A review of present practice. E. C. Apling.

Rapid esterification of dicamba and chlorophenoxy acids with *N,O*-bis(trimethylsilyl)acetamide for gas chromatographic analysis. T. P. GARBRECHT (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 70–73. 3 ref.).—The pesticides (~10 mg of each) were extracted from fertiliser products into Et_2O, the dried residue from which was treated with the silylating agent in DMF (1 ml, 1:1) and, after 15 min standing and shaking, was analysed on a packed column of DC-200 at 190° with thermal conductivity detection. D. I. Rees.

Fungicides

Influence of sunflower diseases on the seed oil and changes in free fatty acids. E. KURNIK (*Fette Seifen AnstrMittel*, 1970, **72** (1), 10–13. Ger,).—The relationship between low seed oil yield and the presence of diseases such as peronospora and botrytis is examined. Prevention of such diseases by seed dressing, careful selection of fertilisers and sequential planting is shown to improve oil yield and quality. Some developments in the breeding of disease-resistant plants is reported, using an artificial infection procedure. G. R. Whalley.

Methods of determination of the systemic and fungitoxic properties of chemicals applied to plants, with emphasis on control of *Verticillium* wilt with thiabendazole and Benlate [benomyl]. D. C. ERWIN (*Wld*

Rev. Pest Control, 1969, **8** (1), 6–22. 33 ref.).—Methods of testing fungicides in living plants are described including root-dip, stem inoculation and double pot techniques etc. Detn. of the preventive, delaying or therapeutic effects of the compd. are described, together with their detection in treated plants. W. J. G.

Insecticides and Others

Duration of patent protection for new insecticides. J. M. DEL RIVERO (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18** (2), 163–168. Span.).—Arguments in favour of extending the duration of patent protection for new insecticides are briefly expounded. A longer period of protection is advocated in the interests of both manufacturers and agriculturalists. E. C. Apling.

The use of insecticides on the main agricultural crops of the world. III. Cotton. A. D. HANNA (*Wld Rev. Pest Control*, 1969, **8** (1), 23–44. 53 ref.).—A detailed presentation which includes data on the species of cotton pest prevalent in various areas of the world and recommended treatment for their control. W. J. G.

Activity of several insecticides, singly or in admixture, against the cabbage worm *Pieris brassicae* L. J. M. DEL RIVERO, J. J. TUSET, F. J. ROIG and M. LAFUENTE (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18** (2), 145–155. Span., 1 ref.).—Results of preliminary lab. trials for the assessment of 69 formulations are reported. 10 prep. gave 100% mortality within 24 h and a further 26 after 48 h. E. C. Apling.

Experiments in the control of a driller of onion leaves. J. M. DEL RIVERO (*Revta Agroquim. Tecnol. Aliment.*, 1969, **9** (4), 564–568. Span., 3 ref.).—Preliminary trials of various insecticides in the control of caterpillars of *Acropelia assectella* Zell., which cause considerable damage by drilling onion leaves, are reported. Most effective (100% kill) were Ultracid Gusathion, Folithion, Sumithion, N-4543, diazinon, Folimat, Zolone, Mesurol and Sevin. E. C. Apling.

Pesticidal dinitrophenylcyanoalkyl carbonates. FABRIEK VAN CHEMISCHE PRODUCTION VONDELINGENPLAAT N.V. (Br. Pat. 1,181,596, 7.3.68. Neth., 7.3.67).—The carbonates have better fungicidal, acaricidal, and herbicidal properties than CN-free analogues. An example is (2-cyanoisopropyl)-4-6-dinitro-2-s-butylphenyl) carbonate, m.p. 87° (EtOH), prep. by passing phosgene for 1 h at −5° to 0° into a soln. of $CMe_2(OH)\cdot CN$ in toluene; introducing pyridine during 1·5 h at 0–5°; removing excess phosgene with air; then adding toluene contg. $1,6,2,4\text{-}OH\cdot C_6H_2Bu^s\text{-}(NO_2)_2$ followed by pyridine dropwise at 0–5°; and working up the mixture. F. R. Basford.

Production of aromatic monoalkoxyaldehyde acetals. FARBENFABRIKEN BAYER A.-G. (Inventors: E. ROOS and K. WAGNER) (Br. Pat. 1,181,769, 24.9.68. Ger., 17.10.67).—Aromatic compd. having ⩽ 2 $CHCl_2$ groups and otherwise completely substituted by Cl, are condensed with a mono- or polyhydric alcohol in presence of an alkaline agent at 0–200°, to give acetals with herbicidal or fungicidal properties. E.g., $3,4,5,6,1,2\text{-}C_6Cl_4(CHCl_2)_2$ is added slowly at 50–70° to a soln. of NaOH in MeOH. After boiling for 10 h the mixture is worked up to give an acetal mixture of b.p. 186°/0·2 Torr, n_D^{20} 1·6583. F. R. Basford.

Diseases and Pests in Livestock; Veterinary Treatments

Control of Exogenous Pests

No abstracts

Other Treatments

Gas chromatographic determination of ethyl 6,7-di-isobutoxy-4-hydroxyquinoline-3-carboxylate in animal feeding stuffs. R. A. HOODLESS and R. E. WESTON (*Analyst, Lond.*, 1970, **95** (1128), 253–256. 4 ref.).—This compd. (buquinolate) is converted selectively into 1-iodo-2-methyl propane by a modified Zeisel treatment of an aliquot of a $CHCl_3$ extract of the sample. The liberated halide is collected quant. in pentane and detd. by chromatog. on a 60-cm column of Porapak Q at 150° with N_2 as carrier-gas and an electron-capture detector. An aliquot of buquinolate standard soln. is treated similarly; peak-height of sample extract should be comparable with that of the standard. Recoveries from 10 g of each of three poultry feeds were 100%, and there was no interference from acinitrazole, amprolium, decoquinate, dimetridazole, ethopabate, clopidol, furazolidone, Me benzoquate, nitrofurazone, sulphaquinoxaline or zoalene (500 ppm levels). W. J. Baker.

Grazing management in relation to *Trichostrongylid* infestation in lambs. II. Level of infestation associated with increased stocking rate and its effects on the host. N. E. DOWNEY (*Ir. J. agric. Res.*, 1969, **8** (3), 375–395. 16 ref.).—Two stocking rates were used over a 3 yr period (5 ewes + 10 lambs and 7 ewes + 14 lambs per acre). A higher level of infestation on the pasture with larvae of *Nematodirus* spp. was associated with higher stocking rate. More intestinal *Trichostrongylus* spp. were recovered at autopsy (Sept/Oct) from lambs stocked at the higher rate. Total worm burdens did not differ between stocking rates. Untreated parasitic infestation depressed lamb growth more at the higher than the lower stocking rate. M. J. Rawlins.

Effectiveness of chlorhexidine in a postmilking teat dip. W. D. SCHULTZE and J. W. SMITH (*J. Dairy Sci.*, 1970, **53** (1), 38–45. 21 ref.).—0·2% of chlorhexidine in an aq. dip for cows' teats used immediately after each milking reduced the resident microflora on the apical teat skin by ~95%. The chlorhexidine dip also markedly reduced the incidence of infection in teats exposed to *Staphylococcus aureus* contamination. M. O'Leary.

Toxicity of an organic arsenical, 3-nitro-4-hydroxyphenylarsonic acid residues, in chicken tissues. K. B. KERR, J. R. NARVESON and F. A. LUX (*J. agric. Fd Chem.*, 1969, **17** (6), 1400–1402. 5 ref.).—Build-up, plateau and depletion of tissue residues of an org. arsenical administered in the feed of growing chickens are compared with the total As residues in chickens receiving non-medicated feed. Spectrophotometric detn. of residues was based on the procedure of Winkler (*J. Ass. off. agric. Chem.*, 1962, **45**, 80) in which Ag diethylthiocarbamate is used. Highest residues were found in the liver; significant sex differences were not observed. Rapid depletion of residues occurred during the first five days of medication. Continued accumulation of As residues was not observed in any of the tissues. I. Dickinson.

Sanitation, Hygiene and Safety

General Sanitation, Pollution

Water pollution potential estimated from farm nutrient budgets. C. R. FRINK (*Agron. J.*, 1969, **61** (4), 550–553. 15 ref.).—The extent to which runoff contributes to the pollution of waterways by inorg. nutrients is critically reviewed. Comparison of input and output of nutrients on dairy farms indicated that under some conditions the farms could contribute significant amounts of nutrients, particularly NO_3^-, to ground water. Methods of preventing loss of nutrients in this way are discussed. A. H. Cornfield.

Prevention of potato peel pollution. R. P. GRAHAM, C. C. HUXSOLL, M. R. HART *et al.* (*Fd Engng*, 1969, **41** (6), 91–93).—The peeling process results in ~80% of the B.O.D. in the plant effluent. A dry caustic peeling process is described to lower the B.O.D. W. J. G.

Food industry and pollution. P. STAVENGER (*Fd Technol., Champaign*, 1970, **24** (2), 121–126).—In 1969, two thirds solid waste in the U.S. was generated by agriculture. Ratios between food production and waste are tabulated. It is more economic to incinerate waste rather than to use it for fertiliser. The use of reverse osmosis and ultra filtration may salvage from wash streams materials (e.g., carbohydrates and proteins) of potential value to humans. Package materials, selected to resist chem., phys. and biol. attack present a great problem. The future requires a shift away from food products with high wash component costs, a change in packaging strategy, and government regulation of H_2O, air and solid waste pollutants. M. J. Rawlins.

Food Hygiene and Safety

Determination of residual amounts of acrylonitrile in foods. E. KRÖLLER (*Dt. Lebensmitt Rdsch.*, 1970, **66** (1), 11–13. Ger., 9 ref.).—The food sample (~ 100 g) is heated with xylene and the acrylonitrile, (**I**) which is distilled over, is freed from HCN and H_2S by passing through cotton wool impregnated with $CuSO_4$. After removal of solvent, the residue is dissolved in MeOH and treated with Br_2 under a Hg lamp. Cyanogen bromide is released

and, after addn. of ascorbic acid, is reacted with pyridine–benzidine. Absorption of the red product is measured at 525 nm and the concn. of **I** detd. from a calibration curve. J. B. Woof.

Susceptibility of local strains of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) to insecticides. A. Toppozada, F. I. Ismail and M. E. Eldefrawi (*J. stored Prod. Res.*, 1969, **5** (4), 393–397. 8 ref.). Malathion (**I**), lindane (**II**), dichlorvos and diazinon were more toxic than DDT, carbaryl and pyrethrins. *S. oryzae* was very susceptible to **I** and **II**, but *T. castaneum* was tolerant to **I** and **II**. *T. castaneum* was more tolerant than *S. oryzae* to all the insecticides. W. J. G.

Mycotoxins: methods for detection in foods. J. C. Ayres, H. S. Lillard and D. A. Lillard (*Fd Technol., Champaign*, 1970, **24** (2), 161–164. 14 ref.).—Screening tests for aflatoxins and aflatoxin-producing *Aspergilli* are described, using t.l.c. and 'milli-columns'. U.v. light was used to scan large samples, e.g., cultivated strains of *Aspergillus* on agar enriched with an aq. extract of groundnuts. Presumptive tests were time consuming, but gave better evidence than the screening methods. T.l.c. was carried out on conc. extracts. Fluorescent spots on chromatograms were compared with standards. Chem. or biol. confirmatory tests required a fairly pure sample. No single procedure has proved satisfactory for the isolation and purification of toxins in foods. M. J. Rawlins.

Collaborative study of three methods for determination of aflatoxin in peanuts [groundnuts] and peanut [groundnut] products. A. E. Waltking (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 104–113. 14 ref.).—The method of Waltking *et al.* (*J. Am. Oil Chem. Soc.*, 1968, **45**, 880) was found to be more rapid and as accurate as the two AOAC methods (*ibid.*, 1968, **51**, 485 and 488). D. I. Rees.

Aflatoxin in Indian peanut [groundnut] oil. C. T. Dwarkanath, V. Sreenivasamurthy and H. A. B. Parpia (*J. Fd Sci. Technol.*, 1969, **6** (2), 107–109. 6 ref.).—Aflatoxins were extracted from groundnut oil by the Parker and Melnick method. Whilst they were absent in refined and hydrogenated groundnut oils, aflatoxin B_1 and B_2 were present (0·02–0·26 ppm) in unrefined oils. G_1 and G_2 were not found. Addn. of casein or arecanut powder to the groundnut oil and heating brought about considerable reduction in aflatoxins, but imparted an objectionable colour and flavour to the oil. M. J. Rawlins.

Rapid chemical confirmatory method for aflatoxin B_1. I. Development of method. A. E. Pohland, L. Yin and J. G. Dantzman **II. Collaborative study.** L. Stoloff (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 101–102. 6 ref.; 102–104. 4 ref.).—I. By heating aflatoxin B_1 (0·25 μg) on a steam bath for 10 min in a mixture of benzene and CH_3CN (1 ml; 49:1), H_2O (0·1 ml) and conc. HCl (1 drop), a fluorescent spot with an R_F of ~ 10% of that of aflatoxin B_1 is obtained by t.l.c. analysis on SiO_2 gel with Me_2CO–$CHCl_3$ (1:9) as solvent. Similar reaction with Ac_2O (0·25 ml), in place of H_2O, gives two spots slightly behind that of aflatoxin B_1, in the same chromatog. system.

II. The method was found to be more rapid and reliable than the AOAC method (*ibid.*, 1968, **51**, 485). D. I. Rees.

Safety of flavouring substances. R. L. Hall and B. L. Oser (*Flavour Ind.*, 1970, **1** (1), 47–53. 26 ref.).—Problems associated with the use of flavour additives to foodstuffs are considered, and the need for a special criteria of safety is proposed; future work of the Flavour and Extract Manufacturers Association and the U.S. Food and Drug Administration is discussed. G. R. Whalley.

Risk of explosion in air suspensions of sugar dust under the influence of high voltage electrical discharges. A. V. Dan'ko and A. M. Kosten'yuk (*Pishch. Tekhnol.*, 1969, [5 (72)], 88. Russ.).— C. V.

Contamination by Pesticides, Pesticide Toxicity

Residues of 4-amino-3,5,6-trichloropicolinic acid (picloram) in grass from applications of Tordon herbicides. M. E. Getzendaner, J. L. Herman and B. van Giessen (*J. agric. Fd Chem.*, 1969, **17** (6), 1251–1256. 2 ref.).—Three liquid and one granular formulation of the herbicide were used. Liquid formulations deposited residues on grass up to 200 ppm for each lb/acre applied. In all expt., the residual level decreased within one or two weeks after application, then remained near const. for 8 to 16 weeks. Residues from the granular formulation increased through a max. near the 8th week after application and dropped to a lower level within the following 8 weeks. Residue of the herbicide in samples of grass collected the year after application showed a 90 to 100% decrease over the mid-season level. No bound form of picloram was found. I. Dickinson.

Determination of Ciodrin from fortified animal tissues by oscillopolarography of its conversion product acetophenone. A. Westlake, F. E. Hearth, F. A. Gunther and W. E. Westlake (*J. agric. Fd Chem.*, 1969, **17** (6), 1160–1163. 7 ref.).—Polarographic conditions for the detn. of acetophenone are described with the lower limit of detection 0·2 μg of acetophenone in 2 ml. Extraction and clean-up procedures for up to 100-g samples are given for meat, fat, liver, eggs and milk, and recovery data are presented with a lower limit of detection of 0·1 ppm of Ciodrin [*O,O*-Me_2 *O*-(3-hydroxy-α-methylbenzyl crotonate) phosphate] in 14 g samples of all tissues. I. Dickinson.

Rapid gas chromatographic method for analysis of *O,O*-diethyl *O*-3,5-6-trichloro-2-pyridyl phosphorothioate (Dursban) in turkey and chicken tissues. L. M. Hunt, B. N. Gilbert and J. C. Schlinke (*J. agric. Fd Chem.*, 1969, **17** (6), 1166–1167. 7 ref.).—The residues are extracted with petroleum ether and aliquots analysed, without prior clean-up, by g.c. A non-polar column and a high temp. electron-capture detector is used. Residues as low as 0·05 ppm can be detected. Recoveries of Dursban from tissues ranged from 72–99%. Samples of body tissues from birds dusted or fed various levels of Dursban were analysed. Residues were found only in the skin and fat tissues. I. Dickinson.

Depletion of DDT residues from laying hens. R. L. Wesley, A. R. Stemp, R. B. Harrington *et al.* (*Poult. Sci.*, 1969, **48** (4), 1269–1275. 9 ref.).—Effects of several treatments on rate of depletion of DDT residues in abdominal fat and egg yolk of laying hens during 20 weeks after capsule administration of DDT into the oesophagus were studied. Residues decreased with time after DDT treatment, but dietary protein level (20–75%), androgen treatment (0·015 g per bird), and starvation for 48–96h before analysis had little effect on final residues in fat or yolk. Addn. of 5% fat to the diet increased DDT residues in body fat. A. H. Cornfield.

Contamination by chlorinated pesticides. III. Insecticidal residues and their metabolites in the eggs of chickens fed with a mixture of insecticides. G. Baluja, J. N. Francos, M. A. Murado and M. E. Pereiro (*Revta Agroquím. Tecnol. Aliment.*, 1969, **9** (4), 578–585. Span., 17 ref.).—Chickens were fed for one month on a diet prep. by the Institute of Pharmacology and Veterinary Toxicology of the University of Utrecht (OECD collaborative study), and eggs collected in the following week were analysed. Residues found (as ppm referred to fresh egg wt.) were: lindane, 0·04; dieldrin, 0·13; *o,p'*-DDT, 0·21; *p,p'*-DDE, 0·10; *p,p'*-TDE (*p,p'*-DDD), 0·23; *p,p'*-DDT, 0·50. E. C. Apling.

Organochlorine pesticides and the dairy industry. G. F. Fries (*J. Dairy Sci.*, 1970, **53** (3), 367–371. 20 ref.).—Some aspects of organochlorine residue problems of particular significance to the dairy industry are reviewed. M. O'Leary.

Fate of organochlorine pesticides during processing of milk into dairy products. C. F. Li, R. L. Bradley, jun. and L. H. Schultz (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 127–139. 31 ref.).—In general, the pesticides were found to be very stable to ordinary dairy processing operations and subsequent storage. However, dieldrin, lindane and chlordane showed a 27, 34 and 11 % decrease resp., for spray-dried products. In manufacturing Cheddar cheese, most of the pesticides showed some bacteriostatic or bactericidal action against starter micro-organisms. D. I. Rees.

Separation and determination of trace amounts of tin present as organotin residues on fruits. H. B. Corbin (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 140–146. 6 ref.).—The sample (≡ < 1 ppm of Sn) is wet ashed and the Sn is separated from other elements by either distillation of $SnBr_4$ with a mixture of HBr and HCl with 98% recovery or by extraction of SnI_4 from aq. H_2SO_4–KI soln. with 100% recovery. The isolated Sn is detd. by the dithiol colorimetric method. By slight modifications of the separation techniques, interference from As (> 1·5 ppm) and Sb (> 20 ppm) is removed. D. I. Rees.

Ultra-violet determination of naphthaleneacetic acid in apples and potatoes. R. C. Randall (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 149–151. 6 ref.).—The chopped product [≡ 0·1 to 10 ppm of naphthaleneacetic acid (**I**)] is extracted with $CHCl_3$. The extract (after treatment with aq. $KMnO_4$ soln. in the case of potatoes) is cleaned up on a Florisil column and **I** is detd. spectrophotometrically at 283 nm. An av. recovery of 93% was obtained. D. I. Rees.

Insecticide residues in agricultural products in California. E. NAVELLIER (*Fruits d'outre mer*, 1970, **25** (1), 61. Fr.)—A review of a book by E. A. Gunther which includes legal aspects, tolerance values, testing and sampling methods etc. in five countries. The California section covers 25 yr use of 35 pesticides, esp. on Valencia and Navel oranges, and Eureka lemons.
M. T. Rawnsley.

Contamination of agricultural produce with pesticides. III. Changes in residual hydrocyanic acid in oranges following fumigation. J. CARRASCO, R. M. MARTINEZ and P. CUÑAT (*Revta Agroquím. Tecnol. Aliment.*, 1969, **9** (4), 574–577. Span. 3 ref.).—Orange trees of varieties Murtera and Washington Navel were fumigated with 90 g of HCN per tree in March and July resp.; residues were detd. on fruit harvested just after treatment. Initial residues were 1·0–1·6 ppm on skin and < 0·1 ppm in the pulp, decreasing after 4 days to < 0·1 ppm on skin and undetectable in the pulp.
E. C. Apling.

Toxicity of pesticides to the crustacean *Gammarus lacustris* H. O. SANDERS (*Tech. Pap. Bur. Sport Fish. Wildlife*, 1969, No. 25, 18 pp. 10 ref.).—Relative acute toxicities of some pesticides were detd. by static bioassays. LC_{50} was detd. for 24, 48 and 96 h exposures at 70°F. Organophosphorus insecticides were generally more toxic than chlorinated hydrocarbon insecticides.
W. J. G.

Impotence in farm workers using toxic chemicals. M. L. E. ESPIR, J. W. HALL, J. G. SHIRREFFS and D. L. STEVENS (*Br. med. J.*, 1970, **i** (5693), 423–425. 21 ref.).—Four out of five members of a team of farm workers using various herbicides and pesticides in intensive agriculture became impotent. When contact with the chemicals ceased and hormone therapy was given, sexual function recovered although in one case recovery took ~ 1 yr. Of the nine groups of compd. (18 compd.) concerned, no one particular substance appeared to be involved; it is probable that impotence was due to the toxic effects of more than one chemical. C. V.

Preparations containing urea or thiourea derivatives for use as molluscicides. CIBA LTD. (Br. Pat. 1,178,563, 10.2.67. Switz., 8.3.66).—Molluscs, esp. watersnails, are controlled by means of a compn. contg. $NR^{II}R^{III}\cdot CX\cdot NRR^{I}$ wherein R is aryl or aralkyl; R^{I}–R^{III} are H or alkyl or R^{II} is, e.g., alkenyl, alkoxyalkyl, cyanoalkyl, aryl or R^{III} is alkoxy; and X is O or S. A typical agent is 1-(*p*-chloro-*m*-trifluoromethylphenyl)-3-(*p*-chlorobenzyl) urea, m.p. 159–160·5°; prep. by adding a soln. of 1,4,3-NCO-$C_6H_3Cl(CF_3)$ in dioxan slowly to dioxan contg. *p*-$Cl.C_6H_4\cdot CH_2NH_2$, then diluting with water after 30 min. F. R. Basford.

[Pesticidal] phosphorus amidates. CIBA LTD. (Br. Pat. 1,178,889, 23.5.67. Switz., 26.5.66).—Esters of formula $OR^{I}(NR^{II}R^{III})PX\cdot O\cdot C_6H_2Cl_2Y$-1,2,5,4 wherein X is O or S; Y is Br or I; R^{I}–R^{III} are lower-alkyl or alkenyl or R^{II}–R^{III} are H or R^{III} is lower-alkoxyalkyl or $NR^{II}R^{III}$ is heterocyclyl, are insecticides of low toxicity and residual activity, esp. effective against stored product pests such as cockroach and larder beetle. In an example, 1,2,5,4-$OH\cdot C_6H_2Cl_2I$ is reacted at 25–28° with $POCl_3$ in pyridine to give 2,5-dichloro-4-iodophenyldichlorophosphate; this is then reacted with MeOH in CCl_4 soln. and the HCl formed is swept out with N_2. A cold soln. of NH_2Me in ether is added, the product is washed and worked up to give 2,5-dichloro-4-iodophenyl Me (methylamido) phosphate, m.p. 110° (hexane). F. R. Basford.

4.—MISCELLANEOUS

Glasgow University radiocarbon measurements. M. S. BAXTER, M. ERGIN and A. WALTON (*Radiocarbon*, 1969, **11** (1), 43–52. 19 ref.).—Samples (14) of malt whisky (1933–1966) were studied. ^{14}C findings agree closely with a series of Danish cereals. C. V.

Rapid determination of strontium-90 in tissue, food, biota and other environmental media by tributyl phosphate. E. J. BARATTA and T. C. REAVEY (*J. agric. Fd Chem.*, 1969, **17** (6), 1337–1339. 8 ref.).—The sample is prep. by either dry- or wet-ashing techniques. It is solubilised in HNO_3 and the ^{90}Y is extracted into equilibrated tributyl phosphate. The separated ^{90}Y is further purified by selective stripping and fluoride pptn. to remove the remaining contaminants which may have been carried over. ^{89}Sr can also be detd. in the same sample by pptn. of Sr prior to the ^{90}Y extraction. The method is sensitive to < 1 pCi per sample of tissue or food ash. I. Dickinson.

Determination of plutonium in biological materials by extraction and liquid scintillation counting. R. F. KEOUGH and G. J. POWERS (*Analyt. Chem.*, 1970, **42** (3), 419–421. 9 ref.).—The sample is reduced to C-free ash by heating at 450° and digestion with HNO_3 and HNO_3/HF. The residue is dissolved in HNO_3/H_3BO_3 and aliquots are counted in a vial in presence of urea and a toluene soln. of di(2-ethylhexyl) hydrogen phosphate (D2-EHPA). 0·05 disintegration min^{-1} g^{-1} can be detected if preliminary concn. by copptn. with, e.g., $Bi_3(PO_4)_2$, is used. S. S. Chissick.

Estimation of diacetyl in presence of other carbonyl compounds. G. J. LEES and G. R. JAGO (*J. Dairy Res.*, 1970, **37** (1), 129–132. 4 ref.).—An examination of the spectra of some volatile carbonyl semicarbazones indicated that only compd. contg. vicinal carbonyl groups formed semicarbazones which absorbed at 270 nm. This observation was used for the quant. estimation of diacetyl. The presence of both AcH and acetoin did not interfere.
M. O'Leary.

A technique for studying the build-up and prevention of milk film on hard surfaces. L. F. L. CLEGG and C. M. COUSINS (*J. Dairy Res.*, 1970, **37** (1), 61–76. 7 ref.).—Glass and stainless steel slides are used, suitable for studying the build-up of milk film on hard surfaces and its removal with detergent–disinfectant mixtures. Results indicate that removal of milk film was more efficient when NaOCl was added to certain detergent soln. than when the materials were used separately. A final rinse with 0·1% H_3PO_4 virtually eliminated mineral matter from the film residue. Use of warm rather than cold water in pre-rinses and final rinses caused a substantive reduction in residues. M. O'Leary.

Polyurethane foams from dried whey. G. O. HUSTAD, T. RICHARDSON and C. H. ADMUNDSON (*J. Dairy Sci.*, 1970, **53** (1), 18–24. 19 ref.).—A description is given of the prepn. of rigid and semi-rigid polyurethane foams using lactose, sweet whey powder, lactose-sweet whey powder mixtures, or lactose–acid whey powder mixtures as the principal polyhydroxy compd. The phys. properties of whey based foams were found to compare favourably with those of conventional polyether type foam. M. O'Leary.

A critical review of the utilisation of methane. V. F. COTY (*Biotechnol. Bioengng Symp.*, 1967, *No. 1, 2nd int. Conf. global Impacts appl. Microbiol.*, 1969, 105–117. 41 ref.).—Microbes which grow on CH_4 were studied, together with techniques employed in their isolation, apparatus, media and gas atm. used for their culture. Heterotrophic assimilation of CH_4 through MeOH, HCHO and formate is discussed. M. J. Rawlins.

High resolution gas chromatography in aroma research. R. TERANISHI (*Flavour Ind.*, 1970, **1** (1), 35–40. 11 ref.).—Application of g.c. to the detailed analysis of odour materials is described, considering specifically a comparison between human and instrumental detection limits, the effect of sample load on both open and packed tubular columns and the effect of different columns on the relative retention times of a 5 component model mixture.
G. R. Whalley.

α-Amino-ω-acylamino carboxylic acids. VEB DEUTSCHES HYDRIERWERK RODLEBEN (Inventor: G. LAUERMANN) (Br. Pat. 1,176,549, 21.12.67).—Used in the synthesis of ω-diamino carboxylic acids (additives for animal and human food), the compd. are obtained by reacting 1 mole α-halogen-ω-aromatic acylamino carboxylic acid, 8–500 (10–100) mole water and 5–150 mole NH_3 at < 120° for < 4 h. S. D. Huggins.

Organofluorosilanes as fumigants. DOW CORNING CORP. (Inventor: W. K. WHITNEY) (Br. Pat. 1,178,220, 29.5.68).—Matter such as grain, cloth, nursery stock, infected with nematodes, insects, arthropods, bacteria, yeasts or fungi, is exposed in a confined space to a compd. of formula $R_nSiF_{(4-n)}$ where R is a lower alkyl, alkenyl or alkynyl group and *n* is 1, 2 or 3, e.g., Me_3SiF, in the liquid or vapour state. S. S. Chissick.

5.—RECENT BOOKS AND JOURNALS

South African Journal of Dairy Technology. 1969, **1** (1), quarterly (Pretoria: South African Society of Dairy Technology).
W. J. G.

Wood Science. 1968, **1** (1), quarterly (Madison, Wis: Forest Products Research Society). W. J. G.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

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Printed in England by Staples Printers Limited at their Kettering, Northants, establishment

16 MAR. 2513