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CEREAL PRODUCT FORTIFICATION: THE B VITAMINS, WITH SPECIAL REFERENCE TO THIAMINE LOSSES IN BAKED PRODUCTS

By J. B. M. COPPOCK, (MISS) B. R. CARPENTER and R. A. KNIGHT*

Certain aspects of cereal product enrichment with vitamins of the B group, especially thiamine, are discussed with particular reference to wheaten flour and rice.

Losses of thiamine during the baking of bread and certain cakes and biscuits were found by the thiochrome method to be 17·3–23·3%, 22·7–33·1% and 26·0–29·7%, respectively. During the toasting of bread, thiamine losses ranged from 13·4 to 31·0% depending on the thickness of the slice. There was no indication that thiamine is destroyed during fermentation of bread dough.

It is suggested that some degree of enrichment of existing National flour, in addition to that of low extraction flours, may be desirable in Great Britain.

This paper is divided into three sections: (1) a discussion of the enrichment of wheaten flour and rice with vitamins of the B group; (2) a review of the available data on the thiamine losses and certain causative factors thereof in the baking process, discussing these in the light of new evidence, and (3) an examination of the significance of these losses in relation to levels of flour enrichment.

Mention should be made that maize is enriched in the Southern States of the U.S.A. to an extent of about 40% of the total production. In America the enrichment of macaroni, spaghetti and noodles with B vitamins is officially permitted but only about half of the output is treated in this way.[1]

Wheaten flour enrichment

It is now well known that as the extraction rate of flour falls there is a progressive decrease in its vitamin content, and Table I shows the nutrients in the B complex for flours of various extraction rates[2] derived from (1) a Manitoban wheat, and (2) an English wheat.

Table I

The effect of extraction rate on the quantities of thiamine, riboflavin and nicotinic acid in flour (mg./100 g.)

Wheat	Extraction rate	Thiamine	Riboflavin	Nicotinic acid
Manitoban	41	0·03	0·05	0·7
	70	0·07	0·07	0·8
	80	0·22	0·08	1·1
	100	0·40	0·17	5·5
English	46	0·05	0·05	0·5
	70	0·09	0·06	0·75
	80	0·19	0·08	0·9
	100	0·32	0·17	4·8

Before the war, apart from bread made from brown flour of 90–100% extraction, white flour of about 70% extraction or even lower was used for making white bread. This comprised about 95% of the public demand and there is now clear statistical evidence[3] that 'white' bread is generally preferred. During the last war National flour was introduced which, in the main, was of 80–85% extraction, a typical average composition[4] for 80% flour in respect of the B vitamin group being thiamine 0·26 mg./100 g., riboflavin 0·05 mg./100 g. and of nicotinic acid 1·20 mg./100 g. Prior to the war, and due, no doubt, to persistent medical and scientific statements that white bread was deficient in vitamins of the B group, it was suggested[5] to the British Milling Industry that it should consider enriching its white flour with synthetic thiamine at the rate of about 0·2 g./280 lb. of flour, which would raise its vitamin-B_1 content by 0·15 mg./100 g. It will be observed from the preceding figures that this results in a thiamine content approximately

* Read before the Nutrition Panel of the Food Group on 14 December, 1955

equal to that of an 80% extraction flour. Thus, we see emerging the basis of the policy of restoring certain token nutrients to the levels of those naturally found in 80% extraction flour, as occurred when white flour of 70% extraction was re-introduced for breadmaking in August, 1953.

Indeed, this policy would appear to be no more than a logical extension of the practice of flour enrichment, resulting from Dodds's suggestion,[5] followed by the British Flour Millers early in the war. In March, 1942, about 38%[5] of the total flour consumed was enriched, the then 73% extraction flour possessing an average natural thiamine content of 0·09 mg./g., being increased by the addition of synthetic vitamin B_1 to approximately 0·24 mg./g. The increase in extraction rate, however, due to war-time conditions, caused the cessation of this practice. However, in 1945, the Conference on the Post-War loaf[6] recommended that a National flour should contain the three token nutrients, thiamine, nicotinic acid and iron, in minimum quantities of 0·24, 1·60 and 1·65 mg./100 g., respectively. It will be noted that the thiamine content is identical with that realized by the millers in 1942, and the minimum levels of this and the other two token nutrients were made obligatory on the introduction of 70% extraction flour in 1953 as mentioned above. The achievement of these levels requires the addition of a concentrate in the form of a dry powder or 'master mix' of composition thiamine 0·21, nicotinic acid 1·00, Ferrum redactum 0·89 and flour (10% moisture content) 26·25 g./oz.; 1 oz. being added to 280 lb. of 70% extraction flour and 1·1 oz. to 280 lb. of a patent flour of somewhat lower extraction rate. Whether or not these levels are adequate, or the policy of enrichment in this country shall be continued, is now the subject of an enquiry by an independent panel convened by the Government under the chairmanship of Professor Sir Henry Cohen.*

The compulsory enrichment of flour and bread was commenced in the United States in 1941 and the voluntary (and widely carried out) enrichment of white bread in Canada in 1953. The levels of enrichment in both countries are closely similar, are about three times those enforced in this country (see Table II), and include the addition of riboflavin. In the United States, most of the flour milled is patent flour and about one-third is still sold direct by the grocer to the consumer. This family flour is enriched by the miller, the minimum and maximum levels permitted being thiamine 2·0–2·5, riboflavin 1·2–1·5, nicotinic acid 16·0–20·0, iron 13·0–16·5 mg./lb. Enrichment of bread, however, is usually made by the bakers, utilizing wafers or tablets, who claim that this method is both cheaper and permits of variations in additives (mainly riboflavin) to take into account differences in bread formulae (particularly in the contribution of riboflavin from skimmed milk powder content). The minimum and maximum levels of enrichment are thiamine 1·1–1·8, riboflavin 0·7–1·6, nicotinic acid 10·0–15·0 and iron 8·0–12·5 mg./lb. of bread.[7] The following table cited by Horder, Dodds & Moran[5] compares the mandatory enrichment levels in Great Britain and the United States of America for comparable flours and apparently assuming an average thiamine content of 0·05 mg./g. for patent flours.

Table II

Enrichment in mg./100 g. flour

Addition	Great Britain	U.S.		Approximate enrichment necessary to bring to whole wheat levels
		Min.	Max.	
Thiamine	0·19	0·44	0·55	0·3
Riboflavin	—	0·26	0·32	0·1
Nicotinic acid	0·90	3·56	4·45	4·7
Iron	0·89	2·89	3·69	2·9

Thus, summarizing, it is seen in general terms that in this country a non-enriched National 80% extraction flour contains about 2·0–2·5 μg./g. of thiamine, an 'enriched' flour of 70% extraction or a patent flour about 2·5 μg./g. of thiamine. It is, however, more correct to describe British practice as restoration of nutrients to the 80% extraction level, rather than enrichment.

* *Note added in proof.* This report is now issued (Cmd. 9757, 1956). It concludes that the available nutritional evidences does not reveal any measurable difference between National 80% extraction flour and flours of lower extraction rate, provided that vitamin B, nicotinic acid and iron have been restored in the amounts specified in the Flour Order, 1953 (see Table II).

In America, however, true enrichment is carried out and a fortified patent flour contains about 5·0–6·0 μg./g. of thiamine and about 3–3·5 μg./g. of riboflavin.

Enrichment of rice

As in the case of white flour, white polished rice is regarded as deficient in the vitamins of the B complex, the average quantities being about 0·06 mg./100 g. compared with brown rice 0·25 mg./100 g. The enrichment of rice in the Philippines is compulsory.[1] Although the nutritional problem is, therefore, the same, namely to effect an increase in the vitamin content, the methods of attainment would appear to be not quite so easy to achieve as in the case of fortifying wheaten flour by a simply prepared and added ' master mix ' or by adding the enriching agents, as is done in America, at the doughmaking stage of bread production. The different ways in which wheat and rice are consumed, the latter being in the form of boiled or steamed grains, clearly complicates the picture. The origin of the natural vitamins is, however, the same, i.e., they are concentrated in the outer coats of the grain and in the germ (embryo and attached scutellum). It has been found[8] that milled parboiled rice is much richer in thiamine, because during parboiling the scutellum is not detached as it is in milled boiled rice. Furthermore, it appears that the greater part of the thiamine in the rice grain can be made to penetrate the endosperm by parboiling, and before polishing, in the presence of various dilute aqueous acid solutions, such as hydrochloric and acetic acid (1%). Thus, it has been reported that soaking brown rice for 24 hours at 38° causes the thiamine content of the finally produced white rice to increase to 2 μg./g., approximately equal to the thiamine content of wheaten flour in this country.

Sakurai[10] stated that, when brown rice was soaked in water, about 50% of the thiamine of the bran and embryo was transferred to the endosperm and about 10% lost in the supernatant liquid. The vitamin loss could be reduced by soaking at 55° for 1·5 hours which was preferred to 37° for 5 hours or 25° for 19 hours. He concluded the transfer was greatest in 2% aqueous sodium chloride followed in decreasing effectiveness by tap water, 1% acetic acid and 8% ethyl alcohol in that order. It would appear, however, that these methods have not yet reached a commercial scale, but that in these later experiments enrichment levels of 2·5 to 2·9 μg./g. have been achieved. It also seems that riboflavin-enriched rice at the level of 17·5 μg./g. develops yellowness,[11] rendering the product less acceptable to the consumer, a point which must always be borne in mind in human nutrition.

Commercial methods of rice enrichment are nevertheless based in part on these observations. Thus, Kondo *et al.*[12] describe the preparation of an enriched rice by soaking washed white rice in 1% acetic acid containing 100–400 mg.-% of thiamine hydrochloride at room temperature for 6 to 24 hours, draining, steaming for 2–3 minutes and drying with hot air (70–80°) for 30 minutes. It appears that, for daily use 1% of enriched rice containing 400 μg./g., thiamine is mixed with ordinary white rice, resulting in a higher thiamine content than ordinary brown rice. Thus, we see the enrichment level is here about 4 μg./g. to give a product with thiamine content approaching that of enriched patent flour in the U.S.A. and that the tendency is to incorporate a pre-mix of rice of high thiamine concentration similar to United Kingdom practice in relation to flour.

Szazs[13] has described the Roche process of enrichment with soluble vitamins and iron. In this a portion of the rice (0·5%) is treated with a solution of vitamins (the quantities vary according to the destination of the rice) which is then dried and treated with a film-forming substance, added to a mixture of talc and iron salts, and then again treated with a film-forming substance. Matsumuro *et al.*[14] outline an alternative method of spraying on to an aliquot of the main bulk an aqueous solution of thiamine hydrochloride containing a small amount of dextrin, the rice being contained in a ventilated pan. These authors then describe an investigation into the use of various film coatings to prevent thiamine losses during washing of rice. A coating produced by an alcoholic solution of polyvinyl acetate alone proved successful, reducing the thiamine losses on washing from 17·3% for uncoated rice to 6·5% for coated rice. The cooking loss was also examined and said to be 20%. Certain other workers state cooking losses of thiamine from unenriched rice as 78% and from enriched rice 28%,[9] but very much higher figures ranging from about 15 to 90% have also been reported,[15] depending on the method of cooking. For example,[16] rice heated in hot fat for 5 minutes, then baked in a small amount of water lost 46–72% thiamine and when toasted in an oven and then baked in a little water 50–91%. It is clear, therefore, that

more data are required on both the influence of washing and cooking on the thiamine retention of cooked rice. In view of such wide variations it is difficult to say whether an enrichment level of 2·5–4 μg./g. of thiamine, as would appear to be the case, is adequate or not.

Thiamine losses in baked products

It is clear from the foregoing that an enrichment policy of a primary food or ingredient, indeed any nutritional policy as is well known, must take into account the nutrients available in the food as finally consumed. Thus, it is important to have data on vitamin losses in baking. These would appear mainly concerned with the destruction of the thermolabile vitamin B_1, riboflavin and nicotinic acid having been found thermostable.[17, 18] Most of the available data relates to bread and toasted bread, data on the losses in cakemaking and biscuit manufacture being scanty. The earliest work[19] suggested there was practically no loss of thiamine in the baking of whole wheat, rye and white bread. Subsequent feeding tests indicated[20] a loss of 5–9% depending on the type of bread and toasting losses of 0–17%, and in Melba toast 26%. On the other hand Horder *et al.*[5] state that in toast the destruction is frequently complete. With the advent of a chemical analytical procedure for determining thiamine, the data might be expected to become more accurate. The first experiments using the thiochrome method indicated losses of about 11% in white bread[21] and also that when a yeast high in thiamine content was utilized in baking, the quantity present in the yeast cell was destroyed only to the extent of 8%. In an extension of this work[22] the same authors found that in white bread enriched with thiamine the baking loss was 22%, and in breads from (*a*) 85% National and (*b*) 100% extraction stone-ground wholemeal flours, 27 and 35%, respectively. These results were criticized by Schultz *et al.*[23] who, using a typical American bread formula containing 5% sugar and 6% milk solids, found no difference in the rate of destruction of naturally occurring and synthetic thiamine. Using the yeast fermentation method, they concluded that under normal baking conditions the overall loss in enriched white bread was 20%, the major destruction occurring at the crust, and this was not significantly greater in whole wheat bread. By the same method Meckel & Anderson[24] obtained thiamine losses of 14–24% in various types of U.S. Army bread. Goldberg & Thorpe[25] in 1949 confirmed the loss of 20% under good baking conditions.

Since 1950 many conflicting statements have been made. Thus, the treatments to which flour is subjected before baking have been said by Menger[26] to cause thiamine losses and the same author states that the loss in cakes is 20% and in Melba toasts 40–50%. Chemical leavening agents are also said to favour thiamine destruction: the retentions in loaves, muffins and sticks have been given as 85, 79 and 66%, respectively, in self-rising maize bread made with sour milk, 80%, and made with sweet milk, 46–69%.[27] Certain Russian workers have found retentions of 86–88% in bread made from wheaten flour and 70% in bread made from rye flour, but state that synthetic thiamine is less well retained in wheaten bread (75–80%).[28]

Thiamine losses to the extent of 10% on baking have also been attributed to the presence of calcium irrespective of the source.[29] A novel view of thiamine losses has been put forward by Van der Mijll Dekker,[30] who states that the greatest losses occur in doughmaking and not in baking. He claimes losses of 0–60% dependent on the yeast used. The loss is only observed when thiamine is determined by the diazotization reaction but not by the thiochrome reaction, indicating that in the dough vitamin B_1 is converted into a substance that can be oxidized to thiochrome, but which is not determined by the diazo reaction. Baking is said to destroy this compound, but it is nevertheless difficult to reconcile these high losses with an approximately 80% retention of thiamine in bread when determined by the thiochrome method.

Finally, reference should be made to a paper by Farrer[31] in which two factors said to be significant in the destruction of thiamine in bread baking were criticized. It has been mentioned earlier[21, 22] that flours of different extractions showed differing retention rates. These were attributed to (1) changes in pH, although little differences were detected in the breads, and (2) absorption of free thiamine from the dough by the yeast during its fermentation and protection of it in the subsequent baking. This phenomenon, being more complete in the lower-extraction flours of lower thiamine content, would give rise to higher destruction rates in the higher extraction flours if the vitamin inside the yeast cell was less vulnerable to heat, and especially if the thiamine in these flours is bound to the bran and less available to the yeast. However, Schultz

et al.[23] could find no difference between the losses in bread baked from high and low extraction flours. Goldberg & Thorpe[25] on the other hand reported differences, attributing them to the longer baking time of wholemeal bread. Farrer criticizes the yeast-cell retention theory on the grounds that the greater part of the thiamine in yeast is in the form of cocarboxylase which has been shown to be more thermolabile than thiamine itself. He noted, however, that if the ash figures for the flours examined by Dawson & Martin[22] were plotted against thiamine destruction, a linear relationship occurred. Farrer, therefore, repeated this work and found for flours of 75, 85, 98 and 100% extraction, with ash figures of 0·35, 0·49, 0·97 and 1·06%, respectively, that the thiamine losses increased in the same order, viz. 18·0, 21·7, 30·0 and 31·0% determined by the thiochrome method. Thus, he concluded that the effect was due to an increase in the inorganic constituents. Bearing in mind that present-day British National flour contains creta preparata at the rate of 14 oz./sack with an average ash content of about 0·70% (mean of 10 samples), on Farrer's linear relationship the thiamine loss should be about 25%. It will be shown later that this does not appear greatly to exceed 20%, and thus it may be concluded that the presence of creta has little or no effect on thiamine destruction. Similar baking losses found in wholemeal bread (100% extraction flour) also support the view that ash is of little significance in determining thiamine destruction.

It was because of this and other conflicting evidence on the thiamine destruction in bread and in toast that we examined a number of typically British breads prepared and toasted under varying conditions and included in our studies certain cakes and biscuits to make the survey more complete.

The method used was the thiochrome procedure developed by the Sub-Committee on Vitamin Estimations of the (then) Society of Public Analysts and Other Analytical Chemists.[32] The extraction was carried out with diluted hydrochloric acid followed by ' Taka-diastase ' and using base-exchange silicate for removal of interfering substances. All samples were finely ground before examination and triplicate determinations were carried out on each flour and the baked product derived from it at the same time and the mean value obtained taken for the calculation of the ' processing loss '. No difficulty was experienced with the extraction procedure, low ' blanks ' being obtained even when fatty products, e.g., cake and biscuits, were examined. In the case of bread, toasts, cake and biscuits, the air-dried product was assayed. Apart from the bread doughs examined for fermentation losses and toasts, each ingredient was separately analysed before use for its thiamine content, and thus the precise initial concentration in the final mix was calculated.

Thiamine losses in breadmaking

The breads examined were mainly of the simplest formula as used in our standard test baking procedure. The flour and yeast were initially assayed for thiamine content. White and National bread was prepared from flour 1000 g., yeast 18 g., salt 14 g. and water 553 g., the dough was fermented at 80° F for 3 hours, final proof 55 minutes, baking temperature 475° F for 30 minutes. Wholemeal bread was prepared from flour 1000 g., yeast 27 g., salt 14 g. and water 607 g., fermentation of the dough was at 80° F for 1 hour, final proof 50 minutes, baking temperature 475° F for 35 minutes. The thiamine content of the yeast used was frequently checked and contributed 4·2 μg./g. (representing approximately 3·7% of the thiamine) to the white bread doughs examined for fermentation losses. Breads were prepared from the following flours:

(*a*) white patent flour containing 1·06 oz. of master mix per sack with an initial thiamine content of 2·12 μg./g., (*b*) a National ' simulated ' 80%-extraction flour unbleached and untreated containing 1·71 μg./g. thiamine, (*c*) a National ' simulated ' 80%-extraction flour containing 1·37 μg./g. thiamine, to which a typical commercial bread improver was added during dough-making; the flour contained 8·4 p.p.m. of potassium bromate, (*d*) a National ' simulated ' 80%-extraction flour treated wth 2·15 oz. of chlorine dioxide per sack (normal commercial treatment) and (*e*) a 100%-extraction wholemeal flour containing no additives. All other flours contained creta preparata at the rate of 14 oz./sack.

The mean baking losses (average of three separate determinations each performed in triplicate) were (*a*) 21·0%, (*b*) 17·3%, (*c*) 20·7%, (*d*) 19·1% and (*e*) 23·3%. Baking losses in American Army type canned bread were also determined and were 19·6%. These results are

in close agreement with those of Goldberg & Thorpe[25] and Schultz *et al.*[23] The variation in individual results may, however, be as much as 4% and we presume that other workers must also have observed this, although controls carried out on the corresponding flour samples always gave excellent replication. Thus, a reasonable conclusion is that baking losses of thiamine in bread are of the order of 20%; there is little evidence to show that synthetic thiamine added by the miller in the master mix differs from natural thiamine, or that treatment by the miller or baker has any significant effect, nor does the thiamine content of wholemeal bread exhibit more than a slightly greater destruction which may well solely be due to the greater baking time. The pH of the doughs when made were all within the range 5·2–5·5 and on entering the oven 5·0–5·5, except in the case of canned bread which was 4·75.

Two methods of doughmaking were studied for fermentation losses, (i) involving normal dough mixing and fermentation for 3 hours at 80° F plus 55 minutes proof and (ii) an overmixing technique using unbleached and untreated flour[33] to which enzyme-active unprocessed soya flour had been added at the rate of 13 oz./280 lb. The thiamine present in the dough was assayed before and after fermentation, a gain of 1·5% in (i) and of 0·2% in (ii) being obtained (duplicate determinations differing in each case by 0·5%). This apparent gain is attributed to experimental error, but it is clear that the thiochrome method gives no indication of fermentation loss.

Thiamine losses in toasting

Bread in the form of slices of varying thickness was toasted to a golden brown colour using a gas-heated grill. The thinnest toast had a longer toasting time to prevent curling. The thiamine contents shown are expressed on the air-dried sample:

(*a*) Slices 12 mm. thick from a National bread prepared by us containing 1·16 μg./g. thiamine; toasting loss, 13·4%.
(*b*) Slices 9 mm. thick from a commercial sliced National bread containing 1·68 μg./g. thiamine; toasting loss, 14·7%.
(*c*) Slices 5 mm. thick from a National bread prepared by us containing 1·51 μg./g. thiamine; toasting loss, 31%.

Assuming a 20% baking loss in breadmaking, these results indicate total losses of 30·7, 31·8 and 44·8%, respectively, and suggest that the thermolabile character of thiamine is the principal cause of its destruction in processes involving heating, the thinner the product the greater the heat penetration and the greater the destruction.

Thiamine losses in cake making

Madeira cakes were examined. The flour used was a cake flour of approximately 70% extraction containing creta preparata and 1·2 oz. of master mix/sack; the thiamine content was 2·98 μg./g. Cakes were prepared (*a*) containing no self-raising ingredients, from flour 284 g., cake margarine 228 g., sugar 228 g. and reconstituted egg containing 0·36 μg./g. of thiamine (dried egg 1·43 μg./g.) 284 g.; the sugar batter method was used and the cake was baked at 380° F for 70 minutes; the pH of the batter was 6·1; and (*b*) with self-raising agents present using the same formula, process and baking time but with 1·8 g. of baking powder present (sodium pyrophosphate cream powder 2 parts, sodium bicarbonate 1 part). The pH of the batter was 6·4.

The mean baking loss in (*a*) was 22·7% and in (*b*) 33·1%. The influence on baking loss of self-raising agents is clearly marked, and may be due to the difference in pH of (*a*) and (*b*) and to possible local concentrations of raising agent.

Thiamine losses in biscuit baking

Soft dough sweet biscuits were examined. The flour used was 'M' flour and contained in (*a*) 2·6 μg./g. of thiamine and in (*b*) 1·82 μg./g. Two types of self-raising agent were used; (*a*) contained pyrophosphate–bicarbonate baking powder (2 : 1) 1·8 g., flour 228 g., margarine 85 g., sugar 85 g. and water 21 g., whilst (*b*) contained ammonium carbonate 14·2 g., flour 680 g., margarine 453 g., reconstituted milk 113 g., sugar 453 g. and dusting flour 41 g. Biscuits (*a*) were baked at 380° F for 18 minutes and (*b*) at 330° F for 23 minutes. The pH of dough

(*a*) was 6·3 and of dough (*b*) 8·4. The mean baking loss in (*a*) was 26·0% and in (*b*) 29·7%. Bearing in mind the slightly longer baking time of (*b*) and the variations that can occur between individual results, it would not appear that the influence of pH or nature of the raising agent is very significant. The results suggest an average thiamine loss in biscuit baking of about 28%.

A consideration of these results in bread, biscuits and cake, and remembering the deduction already suggested on the basis of the thiamine destruction in toast, it would appear that by far the most important factors involved in baking losses are the final temperature, the time of heating and bulk of the product undergoing baking. In another connexion,[34] data have been obtained on the internal temperatures attained during baking of various breads and related products. Applying this data to 1-lb. loaves such as were used in this present work, it is found that the number of minutes the loaf is heated above 80° C is 13 and the final temperature exceeds 80° C by 19° C. These values already take into account the thickness or bulk of the product. Similar data for cakes are 20 minutes and 22° C and for biscuits 15 minutes and 36° C. Thus, it might reasonably be assumed that the product of these times and temperatures, viz. 198, 247 and 840, would at least be in the same order as the baking losses, which they clearly are, and further if the average values for bread (20%), cake without raising agents (23%) and biscuits (28%) are plotted against this product they exhibit an approximately linear relation. The high loss in the cake containing raising agents is probably explained by the longer baking time in relation to biscuits and the greater localized distribution of the aerating agents in the former due in part to the differences in liquor content. Thus, it is concluded that thiamine destruction in baked products is mainly thermal and only to a relatively less extent affected by other factors.

Significance of thiamine losses in relation to flour enrichment

In this paper it is not proposed to discuss the merits or otherwise of enrichment beyond commenting that the results of Widdowson & McCance,[35] obtained by feeding enriched 70%-extraction flour to undernourished German school children, suggested from an examination of growth- and weight-changes that there was no significant difference between this and non-enriched 70%-extraction flour when a mixed diet was available. Thus, in considering any enrichment policy the diet as a whole must be taken into account. If bread is considered to which certain nutrients (and in particular thiamine) have been restored to the same level as non-enriched 80%-extraction flour (i.e., 2·40 μg./g. of thiamine), then as 280 lb. of flour yields approximately 384 lb. of bread and assuming a baking loss of 20%, it is calculated that 1 lb. of bread contains 0·65 mg. of thiamine.

The thiamine requirements for carbohydrate metabolism are 0·6 mg./1000 non-fat kg.-cal. and as 1 lb. of bread contains approximately 1100 kg.-cal.,[5] it is seen that the present restoration level is just about adequate to deal with the bread carbohydrate ingested, even when baking losses are taken into account. Nevertheless, in Great Britain the margin on this basis would appear somewhat slender, whereas in the U.S.A. and Canada a true enrichment policy gives a greater allowance, and permits greater flexibility in dealing with varying individual consumptions of bread depending on financial circumstances, number in a household and type of work performed.

In this country, bread supplies from 30–40% of our total thiamine intake,[5] in America it is probably somewhat less owing to the influence of other competitive foods. Thus, although a more plentiful mixed diet is available, this might in part explain the greater additions of the vitamins of the B group to bread in the U.S.A. and Canada, so that some excess of them is available, and it would be reasonable to claim that with the present falling bread consumption a slight upward trend in our own enrichment policy might be desirable. Indeed, the variability in thiamine content of the flours used in this work and drawn from a number of sources, suggest that some degree of enrichment in both National and 70% extraction flour would be a reasonable insurance against thiamine deficiency.

British Baking Industries Research Association
Chorleywood
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STUDIES ON THE EFFECTS OF TREATMENT WITH CHLORINE DIOXIDE ON THE PROPERTIES OF WHEAT FLOUR. IV.*—The Biological Properties of Untreated, Normally Treated and Overtreated Flours

By A. C. FRAZER, J. R. HICKMAN, H. G. SAMMONS and M. SHARRATT

Previous studies indicate that no significant nutritional damage to flour protein was incurred by the use of chlorine dioxide, even at ten times the normal level of treatment, and that no abnormal substances were formed.[1–3] In confirmation of this previous work, further studies on animals have been made which form the subject of this report.

The object of the experiment described in this paper was to apply classical group feeding experimental methods to the comparison of normally and ten times normally chlorine dioxide-treated flour. Since it was already known[1–3] that no significant damage to known nutrients had occurred, the main emphasis was on the possible occurrence of any consistent differences indicative of toxic action. As a further check on this study, a group received the same flour untreated in Generations 0 and 1. All the observations were also compared with standard figures obtained from a much larger group of animals not receiving chlorine dioxide-treated flour, but otherwise living under similar conditions.

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Experimental

Materials

Flours.—The same flour was used for the preparation of all groups of test materials. *Flour G* was treated at normal level with chlorine dioxide; *Flour H* at ten times this level; *Flour I* was left untreated. Several batches of flour were necessarily used in the course of these and other experiments, but each batch was divided similarly into three parts. An independent analysis and physical test of each batch at each treatment level was made. Throughout the experiments each flour showed the normal characteristics of material treated in the way described.

Rats.—These were Wistar strain albino rats, taken from our own stock immediately after weaning at 30–40 g. body weight.

Diets.—The basal diet consisted of:

	%
Fish meal	7·2
Flour under test	56·8
Full cream dried milk	25·6
Yeast extract	3·5
Cod liver oil	2·8
Dried yeast	3·5
Steenbock salt mixture	0·6

Diets G, H or I contained flours G, H or I, respectively

Methods

Groups of ten male and thirty female rats were taken and each group was fed on one of the diets G, H or I. These groups constituted Generation O (Gen. O). Their general health and wellbeing was assessed by frequent observation. The weight gain of each rat was measured weekly. Special attention was paid to any illness of the animals, and a post-mortem examination was carried out on those that died. The animals were kept in groups, three in each cage (females), or five in each cage (males), the temperature being maintained at 65–70° F throughout the experiment. The cages were moved into different relative positions in the animal house at monthly intervals, to even out positional differences in light and temperature.

After 15–20 weeks, the animals were mated by putting one male into each cage of three females. The females were separated from the males after 21 days and were placed in individual cages for parturition. The fertility was assessed, the number of offspring in each litter counted, and the young rats were weighed daily from birth until they were 21 days old.

At weaning, ten males and thirty females were selected at random from the available offspring to form the next generation. Groups G and H were carried through for four generations and Group I for two.

At about one year to fifteen months of age, several animals from each group were sacrificed to determine the relative organ weights. A histopathological examination was carried out on the principal organs, including the testes, liver, kidneys, heart, adrenals, small intestine, caecum and spleen. If any consistent significant differences were observed, suitable function tests were carried out on the remaining animals of the group. The survival rate at the time of determination of the relative organ weight was calculated.

Throughout these experiments, the diet and water were fed *ad libitum.*

Determination of relative organ weights.—The animals were lightly anaesthetized with an ether–air mixture and weighed. They were bled by making incisions into the femoral veins. The principal organs were quickly removed, all adhering connective tissue was trimmed away, and the wet weight was determined.

$$\text{Relative organ weight in mg./100 g. body wt.} = \frac{\text{wet weight of organ in mg.}}{\text{wet weight of intact animal in g.}} \times 100$$

Results

Percentage survival

Percentage survival at 55 weeks in groups of rats on Diets G and H, and their comparison with normal survival of this colony of rats, is shown in Table I.

Table I

Survival rate of rats at 55 weeks on control and experimental diets

Generation	Percentage survival	
	Diet G Normal treatment	Diet H 10 × normal treatment
0	95·0	97·5
1	97·5	97·5
2	86·5	92·5
3	89·7	97·5

Mean percentage survival 94·2 ± 4·24
Mean percentage survival in normal rats not receiving chlorine dioxide-treated flour in groups of this size at 55 weeks 94·6 ± 6·5

There is no significant difference between any of the groups examined.

Body weight gains at 100 days

Body weight gain was measured weekly. The situation at 100 days is shown in Table II.

Table II

Body weight gains at 100 days in four generations of rats

Generation	Diet G Normal treatment g.	Diet H 10 × normal treatment g.	Diet I Untreated g.	Statistical significance (P value) G/H	G/I	H/I
Males						
0	274·4 ± 22·1	259·9 ± 21·3	286·3 ± 35·4	N.S.	N.S.	N.S.
1	276·6 ± 14·4	251·0 ± 31·6	280·1 ± 24·0	> 0·02	N.S.	> 0·02
2	302·7 ± 19·5	292·5 ± 21·5	—	N.S.	—	—
3	276·8 ± 20·8	213·1 ± 54·2	—	0·005	—	—
Females						
0	210·0 ± 19·7	189·4 ± 13·4	194·2 ± 17·8	< 0·001	< 0·001*	N.S.
1	200·4 ± 24·5	181·3 ± 23·5	190·6 ± 25·2	> 0·001	N.S.	N.S.
2	211·3 ± 16·9	201·1 ± 19·7	—	N.S.	—	—
3	193·2 ± 22·1	187·9 ± 18·3	—	N.S.	—	—

N.S. = not significant (P value > 0·05)
* = greater weight gain in animals receiving treated material

No consistent significant difference was found between groups receiving flours treated at different levels with chlorine dioxide or between those receiving untreated flour.

Fertility and number of offspring

The fertility in each group was assessed 21 days after mating and the number of offspring in each litter was recorded. The results are shown in Tables III and IV.

Birth weights

Birth weights were measured in each group and are shown in Table V.

These birth weight figures are not significantly different from those found in rats not receiving material treated with chlorine dioxide. Rather wide variations occur in individual figures, due to the different number of offspring in litters.

Table III

Percentage apparently non-fertile at 21 days

Generation		Diet G Material normally treated with chlorine dioxide	Diet H Material treated at ten times normal level with chlorine dioxide	Diet I Untreated material
0	Male	0	0	0
	Female	11	15	17
1	Male	20·0	22·2	—
	Female	12·5	0	—
2	Male	0	10	—
	Female	14·3	17·2	—

Table IV

Number of offspring per litter

Generation	Diet G	Diet H
0/1	9·7	10·1
1/2	8·5	9·2
2/3	8·4	9·3

Mean for all groups = 9·2
Average for normal rats in this colony not receiving chlorine dioxide = 9·0 ± 0·69

Table V

Birth weights

Generation	Diet G Normal treatment g.	Diet H 10 × normal treatment g.	G/H Significance
0/1	5·80 ± 0·89	5·98 ± 1·20	N.S.
1/2	6·10 ± 0·98	5·93 ± 0·88	N.S.
2/3	6·5 ± 1·83	6·4 ± 1·72	N.S.

Weight gains during lactation

The average weights at 14 days and survival at weaning are shown in Tables VI and VII. Body weight of offspring was measured daily for 14 days.

Table VI

Body weight gains of offspring for first fourteen days

Generation	Body weight at 14 days: Diet G Normal treatment g.	Diet H 10 × normal treatment g.	G/H Significance
1	23·57 ± 3·18	22·22 ± 2·54	N.S.
2	24·6 ± 3·5	20·9 ± 4·9	> 0·02
3	27·3 ± 3·42	23·8 ± 3·02	> 0·01

The mean body weight at 14 days for rats on untreated material was 25·4 ± 5·1 g. There is no significant difference between this weight and those recorded in the table, except for Generation 2 in Group H.

The average number per litter at weaning for the normal rats in the colony not receiving chlorine dioxide-treated material was 8·2 ± 0·92. No differences were observed between groups receiving treated or untreated material.

Table VII

Number surviving at weaning

Average number per litter at weaning

Generation	Diet G Normal treatment	Diet H 10 × normal treatment
0/1	8·5	9·4
1/2	7·9	6·9
2/3	7·8	9·2

Average for all groups = 8·3
Average for the normal rats in the colony not receiving chlorine dioxide-treated material = 8·2 ± 0·92

Different relative organ weights

Organ weights were measured in groups receiving bread made from flour treated with chlorine dioxide at normal level or ten times normal level, after fifteen months on these diets. The results over three generations are shown in Table VIII.

Table VIII

Differential relative organ weights, mg./100 g.

Organ	Diet G Normal treatment	Diet H 10 × normal treatment	G/H P value	Diet G Normal treatment	Diet H 10 × normal treatment	G/H P value
Generation 0				Generation 1		
Liver	3440±230	3060±370	0·05>P>0·01	3321±190	3299±300	0·9 >P>0·8
Kidneys	703±48	704±103	1·0 >P>0·9	674±68	658±51	0·6 >P>0·5
Spleen	316±33	294±35	0·2 >P>0·1	286±31	276±35	0·6 >P>0·5
Heart	292±24	296±41	0·8 >P>0·7	278±19	300±30	0·1 >P>0·05
Adrenals	25±6·1	27·5±5·1	0·5 >P>0·4	24·8±3·5	23·9±3·3	0·4 >P>0·3
Small intestine	3250±430	3120±370	0·5 >P>0·4	3447±340	3190±420	0·2 >P>0·1
Large intestine	407±63	498±62	0·01>P>0·001	392±64	445±94	0·2 >P>0·1
Testes	804±130	750±119	0·4 >P>0·3	817±48	735±72	0·01>P>0·001
Uterus	211±81	233±59	0·5 >P>0·4	212±60	183±29	0·2 >P>0·1
Generation 2				Generation 3		
Liver	3234±399	3511±417	0·2 >P>0·1	3174±340	3363±291	0·3 >P>0·2
Kidneys	664±83	705±61	0·3 >P>0·2	608±101	723±77	0·05>P>0·02
Spleen	277±39	289±80	0·7 >P>0·6	260±44	226±38	0·2 >P>0·1
Heart	290±83	291±72	1·0 >P>0·9	252±20	283±10	0·01>P>0·001
Adrenals	25·1±6·1	29·0±4·4	0·2 >P>0·1	23·2±3·4	27·0±2·8	0·4 >P>0·3
Small intestine	3242±363	3545±382	0·1 >P>0·05	2926±360	3388±378	0·05>P>0·02
Large intestine	359±38	411±80	0·1 >P>0·05	300±33	372±34	P<0·001
Uterus	164±67	232±75	0·1 >P>0·05	—	—	—
Testes	786±60	744±215	0·6 >P>0·5	741±83	861±211	0·2 >P>0·1

Except for certain individual differences discussed later, no significant changes were observed either in differential organ weights or histopathology as between the two groups studied. The relative organ weights, except for those commented upon later, fall within the normal range for rats receiving untreated materials.

Discussion

The further evidence presented in this paper lends support to the general conclusion that chlorine dioxide, from a nutritional point of view, has no deleterious effect on wheat flour, even at ten times the normal level of treatment. In Table II the body weight at 100 days was occasionally just significantly less in some groups of animals having material treated with ten times

the usual amount of chlorine dioxide than in those having normally treated flour. This occurred in the males in Generation 1 and in the females in Generations 0 and 1. We already know,[1–3] however, that the nutritional value of the flour protein is not impaired by chlorine dioxide treatment. These occasional differences in weight gain may well be due to decreased food intake, resulting from reduced palatability of over-treated flour, which has been shown to occur in some animals.[3] This effect in the flour varies from batch to batch and might, therefore, be expected to cause this type of inconsistent effect.

In Generation 0, the rats on material treated at normal level had a significantly lower mean relative liver weight and significantly higher mean relative large intestine weight than the animals receiving the material treated at ten times normal level. The mean figure was, in each case, just outside the normal range so far recorded. However, no such differences were observed in either case in Generations 1 and 2, and the findings were reversed in Generation 3. No abnormalities were seen on thorough histopathological investigation. It is not considered that these differences had any biological significance.

There was a significant difference in relative testes weights in Generation 1. The males of this generation showed reproductive difficulties. The difference in testes weights was not seen in subsequent generations and reproduction was normal. There is, however, rather a wide spread of values observed in the animals receiving material treated at ten times normal level in Generations 2 and 3, which is not observed in the animals receiving material treated at the normal level.

Summary

General health and survival, body weight gain, fertility and number of offspring, birth weight and weight gain during lactation, histopathological appearances and relative weight of the main organs were studied over three generations in groups of rats receiving 56·8% of the diet in the form of flour. The flour supplied to the different groups was either untreated, or treated at normal level, or ten times normal level, with chlorine dioxide. There was no indication in any of these experiments that the treatment or over-treatment of the flour with chlorine dioxide caused any deleterious effects in these animals. From this and previously published evidence, it is concluded that chlorine dioxide treatment does not significantly affect the nutritional value of flour for human subjects.

Final conclusion

It may be concluded from these and previous studies[1–3] as well as from the work of many other investigators[4–8] that the chlorine dioxide treatment of flour at normal level does not have any significant deleterious effect on its nutritional value, as shown by chemical analysis of the flour proteins, or assessment of their biological value. The changes in vitamin E content that occur on normal storage and with aeration treatment, as well as with chlorine dioxide treatment, are not considered to be significant in terms of human nutrition. There is no evidence of the formation of abnormal or toxic products. At ten times normal level of treatment with chlorine dioxide, the flour is almost unusable for bread-making, the bread crumb goes rancid more readily and this may result in some loss of palatability. However, there is still no evidence of significant nutritional damage, or formation of toxic products.

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THE ANALYSIS OF FORMULATED SCHRADAN INSECTICIDES

By K. GARDNER and B. D. OWEN

A tentative method, based on differential hydrolysis and solvent extraction, followed by phosphorus determination, is suggested for the analysis of formulated schradan insecticides.

Introduction

From time to time joint Committees of the Ministry of Agriculture, Fisheries and Food and the Association of British Insecticide Manufacturers have been formed to draw up specifications and methods of analysis for insecticides, fungicides and weedkillers. An account of the history of the development of these Committees has already been given,[1] and the specifications and methods prepared have been published in the Ministry's Technical Bulletin No. I.[2]

In May 1953, a Committee was set up to investigate methods of analysis for schradan (bisdimethylaminophosphonous anhydride) and other organo-phosphorus insecticides. A considerable amount of collaborative work has been carried out and, although results so far obtained are not entirely satisfactory, some measure of agreement has been obtained among different laboratories. Schradan formulations are now widely used as systemic insecticides and in view of the urgent need for a method of analysis, the Committee feels justified in putting forward its analytical procedure as a tentative method in the hope that further experience will lead to a generally acceptable standard method.

This report is published with the approval of the Ministry and the Association.

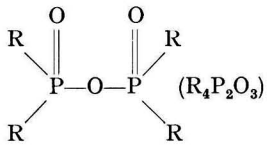
Experimental

Principle of method

The method of analysis described by Hartley *et al.*[3] is not easily applicable to certain formulated products owing to the formation of emulsions during extractions, whilst distillation techniques are not easily applicable to the analysis of formulated materials. A modification of Hartley's method was therefore proposed in which the analysis was carried out on a semi-micro scale and the separate ingredients determined by photometric determination of phosphorus instead of volumetric determination of amine. Despite its inherent lower precision, the photometric phosphorus method was preferred in the interests of simplicity and speed to a semi-micro volumetric method.

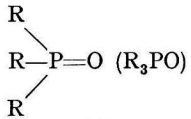
Schradan formulations normally contain schradan (I) as active ingredient, and tris(dimethylamino)phosphine oxide (II). They may also contain triphosphoric pentadimethylamide (III) and small amounts of other phosphorus compounds such as $R_3P_3O_6$ (IV), R_2POCl, R_2PO' amine salts and esters, and dimethylamine hydrochloride. Small amounts of solvents and water may also be present. Wetting agents and dyestuffs are usually added during formulation.

The method proposed is based on differential hydrolysis and extraction of the major components. Physical data are given in a paper by Heath & Casapieri.[4] Initial extraction with chloroform from aqueous solution isolated I, II and III from the other phosphorus compounds. As IV has not been detected in any recent commercial formulations, its presence is ignored in the proposed method.

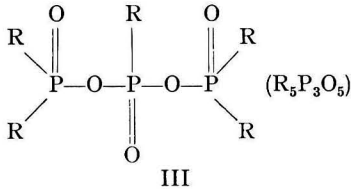


Schradan

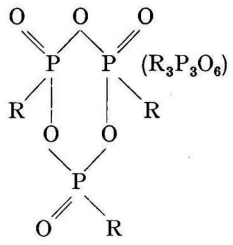
I



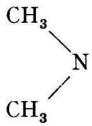
II



III



IV

where R = 

An aliquot portion of the chloroform solution is evaporated in the presence of water and portions of the resulting aqueous solution are used for determination of the three ingredients. Mild alkaline treatment of a portion of the aqueous solution hydrolyses more than 99% of III and about 7% of I. The hydrolysed products remain in the aqueous layer after extraction with chloroform which extracts all of II and the remaining 93% of I. Strong alkaline treatment of a separate portion of the aqueous solution hydrolyses all I and III and the products from these remain in the aqueous layer, II being estimated in the chloroform solution.

The latest method used in collaborative work is as follows:

Method

Apparatus

Class 'A' volumetric glassware should be used throughout (Note *d*)

Copper tube (preferably silver-plated internally) 6″ × 1″ diam.

Reagents

All reagents used should be of analytical reagent quality

Sodium hydroxide 200 g./litre (kept in polythene bottles), and diluted to 1·00 N and 2N as required

Ammonium molybdate 50 g./litre, prepared by dissolving the salt $(NH_4)_6Mo_7O_{24},4H_2O$ in warm water

Ammonium vanadate: dissolve 2·5 g. of ammonium vanadate NH_4VO_3 in 500 ml. of boiling water, cool, add 20 ml. of conc. nitric acid and dilute to 1 litre.

Procedure

Weigh a representative sample containing 1–1·3 g. of schradan (W g.) into a 250-ml. separating funnel containing 80 ml. of chloroform. Add 100 ml. of 2N-sodium hydroxide, shake and allow to separate. Transfer the lower phase to a 250-ml. volumetric flask and repeat the extraction with two further portions of 80 ml. of chloroform, combining the chloroform layers in the 250-ml. flask. Make up to the mark with chloroform.

Transfer a 25-ml. aliquot (note *b*) to a 250-ml. B.24 round-bottom flask, add 20 ml. water and 5 ml. of N-NaOH. Fit the flask with a distillation head and coarse air leak and remove

the chloroform by aspirating a slow stream of air through the chloroform layer while the flask is immersed in a water-bath at 55–60°. (The time taken must not exceed 1 hour.) Transfer the residue quantitatively to a 100-ml. volumetric flask washing in with water and dilute to 70 ml. Add 20 ml. of N-NaOH slowly and with shaking and make up to 100 ml. with water. This solution will be referred to as Solution A.

(i) Pipette a 10-ml. (Note *a*) aliquot portion of Solution A (Note *b*) into a B-19 glass boiling tube immersed in a boiling water-bath. Fit a reflux condenser to the boiling tube and place the latter in the water-bath for 60 ± 1 minutes, beginning the timing when the pipette is half empty. Cool immediately and quickly under the tap. Transfer to a separating funnel, washing well with small amounts of water. Extract immediately with 3 × 10-ml. portions of chloroform (Note *c*). Discard the chloroform layers, transfer the aqueous layer to a 50-ml. volumetric flask and dilute to the mark. Pipette a 20-ml. aliquot portion into a small Kjeldahl flask and proceed as in (iv).

(ii) Pipette a further 10-ml. aliquot portion of Solution A into a silvered copper tube and add 10 ml. of 5N-NaOH. Heat in a pressure cooker at 120–122° for 2 hours (15 lb./sq. in. in a domestic-type pressure cooker). Allow to cool, transfer to a separating funnel and extract with 3 × 10-ml. portions of chloroform using 3 ml. of chloroform for washing after each extraction. Combine the chloroform extracts in a 250-ml. squat beaker. Transfer the aqueous phase to a 100-ml. volumetric flask and dilute to the mark. Pipette a 10-ml. aliquot portion into a small Kjeldahl flask and proceed as in (iv).

(iii) To the combined chloroform layers from (ii) add 20 ml. of water and carefully evaporate the chloroform. Cool and make the aqueous phase up to 50 ml. in a volumetric flask. Pipette a 20-ml. aliquot portion into a small Kjeldahl flask and proceed as in (iv).

(iv) To each of the Kjeldahl flasks obtained in (i), (ii) and (iii) add 4·5 ml. of 60% perchloric acid (sp. gr. 1·54) and 1 ml. of nitric acid, sp. gr. 1·42. Add 2–3 glass beads and evaporate carefully to fumes of perchloric acid. Reflux gently for 5–10 minutes avoiding excessive loss of perchloric acid, allow to cool and add 10 ml. of water. Boil for 2 minutes, cool and transfer to a 50-ml. volumetric flask. Dilute with water to about 35 ml. and add 5 ml. of ammonium vanadate solution and 5 ml. of ammonium molybdate solution in that order. Make up to the mark and shake well (Note *b*). Set aside for 30–45 minutes and measure the colour on a 'Spekker' absorptiometer using 4-cm. cells and Ilford-601 filters or a spectrophotometer at 470 mμ (4 cm. cell) against a reagent blank prepared by fuming 4·5 ml. of perchloric acid and 1 ml. of nitric acid and proceeding as above.

Calculate the weight in mg. of phosphorus present in the aliquot portions from (i), (ii) and (iii) by comparisons with a standard graph.

The standard graph should be checked at the same time by preparing a solution of potassium dihydrogen phosphate (A.R. material, dried for 4 hours at 110°) and taking suitable aliquot portions for treatment as above.

Blanks.—A complete set of blanks covering the entire procedure should be carried out. These blanks are usually very low and constant enough to enable several complete estimations to be carried out using one set of blank determinations.

Calculation

Let P_1, P_2, P_3, be the wt. in mg. of phosphorus in the aliquot portions from (i), (ii), and (iii), respectively.

Then

$$\% \ R_5P_3O_5 = \frac{4{\cdot}537(25P_1 - 7P_2)}{W}$$

$$\% \ R_3PO = \frac{144{\cdot}5P_3}{W}$$

$$\% \ R_4P_2O_3 = \frac{495{\cdot}6(P_2 - 0{\cdot}25P_1)}{W}$$

Notes

(*a*) If the formulation has a high $R_5P_3O_5$ content, a smaller aliquot portion may be required and an adjustment made to 'P_1'.

(*b*) The compounds $R_4P_2O_3$ and $R_5P_3O_5$ are toxic and their chloroform or alkaline solution should be pipetted with the aid of a rubber bulb or a pump.

(*c*) The operation up to and including the extraction with chloroform should be carried out as quickly as possible after Solution A is made up.

(*d*) Since only small amounts of phosphorus are determined in each case, all apparatus should be scrupulously clean (especially separating funnels) and care taken in all transferences.

(*e*) A check on recovery of phosphorus may be carried out by estimating phosphorus on an aliquot portion of Solution A by converting all the 'R–P' compounds to PO_4''' as in (iv).

Investigation of method

The main points investigated in the method were:

(A) The precise determination of $R_5P_3O_5$
(B) The conversion of 'R–P' compounds to PO_4'''
(C) The determination of phosphate.

(A) The main error in this determination is due to the fact that only 7% of the $R_4P_2O_3$ is hydrolysed. First experiments were done in N-NaOH for 15 min. and theoretically an error of $\pm$ 1½ min. in the time taken or of $\pm$ 0·1 in the normality of the sodium hydroxide will give an error of only 1% in the amount of $R_4P_2O_3$ hydrolysed. Thus in a formulation containing 50% schradan and assuming a 7% hydrolysis, a plus error of either 1½ min. or 0·1N should give a result of about 0·5% $R_5P_3O_5$ and 49·5% $R_4P_2O_3$.

In practice, however, the results showed far greater inter-laboratory discrepancies than expected from theory. In order to try to increase the precision, the time was increased to 60 min. and the sodium hydroxide strength reduced to 0·25N. Although intra-laboratory precision was found to be $\pm$ 3% (95% confidence limits), Table II shows that far greater discrepancies occurred in collaborative work.

(B) The published method[3] for unformulated materials converts 'R–P' compounds to phosphate by mineral acid hydrolysis. The discovery that ceric sulphate rapidly oxidizes the compounds to phosphate led to its employment in this method. It was subsequently found that fuming perchloric acid was equally successful and rather more convenient to use. Nitric acid was added as a precaution against explosions.

(C) Phosphate was first determined by the molybdenum blue method using ferrous sulphate reduction.[5] Again, intra-laboratory tests gave a satisfactory precision, but collaborative work gave indifferent results. The vanadophosphomolybdate method[6] was then tried in a slightly modified form, but again certain workers obtained irregular results on synthetic samples and commercial formulation.

Discussion of results obtained

Tables I and II give the results of analysis of synthetic and commercial samples. The results are arranged to show the phosphorus recovered per g. of sample on each section of the estimation. P_a/W, P_b/W, and P_c/W represent the total phosphorus in μg. per g. of sample as determined in portions (i), (ii) and (iii), respectively. The column I and II gives (on synthetic samples) the total recovery of $R_4P_2O_3$ and eliminates errors in calculation indicated by the $R_5P_3O_5$ determination. Comparison of the last two columns gives an indication of recovery of phosphorus compounds from extraction of 'Solution A'.

Considering Table I first, both samples H and I contained pure $R_4P_2O_3$ and R_3PO only. The figure P_b/W for the samples gives the recovery of $R_4P_2O_3$ and P_c/W the recovery of R_3PO. Statistical treatment of the results is shown in the Tables.

Table I

Analysis of synthetic samples

	% recovery				P_a/W, μg./g.	P_b/W, μg./g.	P_c/W, μg./g.	$\frac{P_b + P_c}{W}$ mg./g.	P total, W mg./g.
	I $R_5P_3O_5$	II $R_4P_2O_3$	I + II as $R_4P_2O_3$	III R_3PO					
Sample H									
Theoretical	Nil	60·24	60·24	10·0	950	13,060	1732	14·80	14·78
Laboratory 1	2·41	58·4	61·0	10·0	1458	13,230	1720	14·95	—
	1·45	59·2	60·8	10·0	1246	13,188	1726	14·9	—
Laboratory 2	—	—	56·9	9·9	—	12,320	1715	14·0	—
	—	—	58·9	9·4	—	12,750	1620	14·4	—
	—	—	56·8	9·5	—	12,300	1640	13·9	—
Laboratory 3	2·8	57·7	60·5	9·8	1500	13,100	1690	14·8	—
	1·3	59·4	60·9	9·9	1210	13,200	1710	14·9	14·8
Laboratory 4	2·00	57·7	60·1	10·2	1352	13,020	1763	14·8	14·9
	2·40	57·2	60·2	11·3	1481	13,058	1958	15·0	15·06
	2·16	57·6	60·2	10·5	1387	13,039	1813	14·85	15·22
Laboratory 5	2·11	58·8	61·1	10·8	1395	13,265	1859	15·1	15·5
	1·34	62·9	61·2	10·8	1276	13,480	1863	15·3	15·65
	1·41	60·0	61·6	13·0	1247	13,370	2238	15·6	15·3
Sample I									
Theoretical	Nil	31·14	31·14	30·42		6751	5268	12·02	12·02
Laboratory 1	2·08	29·3	31·4	28·8	941	6815	4973	11·91	—
	1·21	30·3	31·5	28·7	747	6838	4958	11·87	—
Laboratory 2	—	—	30·4	28·8	1410	6600	4980	11·87	—
	—	—	31·9	27·8	1460	6920	4810	11·85	—
	—	—	31·3	28·5	940	6780	4930	11·58	—
Laboratory 3	1·4	30·3	31·8	29·6	804	6910	5120	12·0	—
	1·5	30·3	31·8	29·3	812	6910	5070	12·0	12·3
Laboratory 4	1·0	30·0	33·7	31·3	732	7301	5416 ?	12·7	12·5
	0·86	28·2	31·4	29·7	668	6827	5145 ?	12·0	11·7
Laboratory 5	1·69	30·5	31·5	30·7	868	7061	5320	12·2	13·1
	1·45	31·0	32·8	31·6	818	7116	5479	12·6	12·9
	0·73	31·7	33·4	31·0	649	7249	5369	12·5	13·1

$R_4P_2O_3$ *determination*

Sample H

	μg./g.
Theoretical	13,060
Found average	13,025
Standard deviation	361
95% fiducial limits	13,025 ± 226 (± 1·74%)

Sample I

Theoretical	6751
Found average	6944*
Standard deviation	203
95% fiducial limits	6944* ± 133 (± 1·91%)

* 2·8% high

Table II

Analysis of commercial formulations

	I $R_5P_3O_5$	II $R_4P_2O_3$	I + II calc. as $R_4P_2O_3$	III R_3PO	P_a/W, μg./g.	P_b/W, μg./g.	P_c/W, μg./g.	$\frac{P_b + P_c}{W}$ mg./g.	$\frac{P\ total}{W}$ mg./g.
Sample J									
Laboratory 1	10·2	48·1	59·3	9·25	3170	12,860	1600	14·5	14·4
	10·1	48·0	59·1	9·25	3130	12,820	1600	14·4	
Laboratory 2	9·9	48·3	60·0	10·1	3150	13,000	1734	14·7	14·76
	10·9	45·4	57·2	10·8	3240	12,410	1882	14·3	14·3
Laboratory 3	12·7	47·2	61·1	12·15	3719	13,242	2171	15·4	16·9
Laboratory 4	10·3	46·7	58·6	12·8	3144	12,580	2225	14·8	14·4
	10·3	45·0	56·4	12·4	3144	12,220	2169	14·4	14·1
	9·7	47·6	58·2	13·7	3008	12,610	2369	15·0	14·7
	9·7	47·5	58·2	13·7	3019	12,610	2369	15·0	14·6
Sample K									
Laboratory 1	24·5	50·3	76·9	9·15	6565	16,680	1580	18·3	18·4
	24·4	50·6	77·3	9·1	6540	16,750	1570	18·3	
Laboratory 2	26·4	49·5	78·2	9·9	7170	16,960	1708	18·7	18·7
	26·6	47·6	76·0	9·9	6850	16,460	1717	18·2	18·1
Laboratory 3	25·4	46·5	74·3	10·8	6721	16,110	1860	18·0	20·7
	26·3	47·7	76·5	11·1	6962	16,593	1926	18·5	21·2
	25·2	49·5	77·0	11·3	6720	16,700	1938	18·6	19·9
Laboratory 4	22·0	52·9	77·0	11·3	6027	16,695	1952	18·6	18·2
	21·8	53·4	77·6	11·4	5987	16,815	1969	18·8	18·2
	21·2	50·4	73·8	11·5	5672	16,000	1995	18·0	18·4
	21·4	50·6	74·0	11·5	5837	16,040	1991	18·0	18·4

R_3PO determination

Sample H

	μg./g.
Theoretical	1732
Ignoring the third result of laboratory 5,	
Average	1774*
Standard deviation	99·7
95% fiducial limits	1774* ± 65 (± 3·6%)

* 2·4% high

Sample I

Theoretical	5268
Found average	5131*
Standard deviation	218
95% fiducial limits	5131* ± 152 (± 2·9%)

* 2·6% low

The results for '$R_4P_2O_3$ plus $R_5P_3O_5$' (P_b/W) show a variation which was considered to be marginally satisfactory. Those for R_3PO (P_a/W) are less satisfactory, but since the compound is insecticidally and toxicologically insignificant, it was decided to proceed with the analysis of commercial samples. The '$R_5P_3O_5$' figures (which should be nil) were variable and unsatisfactory. The results for $R_5P_3O_5$ were obtained by the 15-minute hydrolysis procedure in Table I and by the 60-minute hydrolysis method in Table II.

The results in Table II show that the deviations in '$R_4P_2O_3$ plus $R_5P_3O_5$' are of the same order but those for $R_5P_3O_5$ (P_a/W) and particularly R_3PO ($P/_cW$) are worse than those previously obtained. Disturbing discrepancies are noted in total phosphorus determinations (particularly in the case of laboratory 3).

The chemical basis of the method, however, is considered to be satisfactory, and the discrepancies may be ascribed to lack of precision in the hydrolysis of $R_5P_3O_5$ and in the determination of phosphorus.

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EFFECT OF DRYING CONDITIONS ON THE NUTRITIVE VALUE OF A PROCESSED STOCK DIET FOR ANIMALS

By A. J. HARMS and PATRICIA P. SCOTT

Tests carried out with weanling rats showed that a diet containing fish, whalemeat, meat and liver meal, biscuit waste and propyl gallate, dried in air at temperatures below 140°, supported good growth, whereas the same diet dried at 140–160° produced successively inferior growth. Rats given the diet dried at 160° were thin, lethargic and incapable of reproduction, resembling rats maintained on a diet deficient in protein or in amino-acids rather than in vitamins. Chromatographic analysis did not indicate any destruction of essential amino-acids, but the diet dried at 160° was more resistant to peptic digestion. It is suggested, and is supported by the findings of other workers, that the poor nutritional value of the diet dried at 140° and above was due to failure to digest the protein which had been ' damaged ' at this relatively low temperature by the formation of *N*-glycosides in the presence of reducing sugars.

Introduction

Variations in the nutritive value of different batches of dried compressed animal diets have frequently been suspected, even where the constituents of the diet are known to be adequate and carefully controlled in amount and quality from one batch to another.

These variations may have been due to the high temperatures used in some commercial processes, which appear to have a deleterious effect on the nutritive value of foodstuffs, particularly those containing protein, although the precise effects and temperatures required to produce them have seldom been clearly defined. Mecham & Olcott[1] found by experiments *in vitro* that wheat gluten and cattle hoof were no longer hydrolysed by pancreatin after being heated above 153°. Using the rat repletion method, Frazier, Cannon & Hughes[2] were able to show that, whereas purified fibrin had to be heated at 210° for 30 minutes before it became nutritionally valueless, when reducing carbohydrates were mixed with the fibrin, or with casein, heat injury occurred at much lower temperatures. This was due to the reaction occurring between reducing aldoses and the reactive groups of proteins, described by Maillard[3, 4] and by Gottschalk & Partridge,[5] leading to the formation of *N*-glycosides, which proceeds more rapidly as the temperature rises.

Although Bruce[6] found that autoclaving did not have any effect on a stock diet for breeding mice, it seemed worth while investigating the effects of varying the temperature of the current of air used for drying a diet designed primarily for dogs.

Experimental

Preparation of diets

Whalemeat and white fish were cooked for 1 h. in a steam-jacketed cooker, then drained until the water content was approximately 55–60%. Two batches of the diet were mixed in the proportions shown in Table I and water was added to give a total moisture content of 30–33%. The diet was extruded in strips, through a plate having holes $\frac{1}{8} \times \frac{1}{2}$ in., some heat being generated in the process, so that the temperature rose to 55–60°. The extruded diet was divided into eight portions and dried on wire trays in a current of steam-heated air in a special cabinet. The time and temperature for which each sample was exposed are shown in Table II. Since it was not

Table I

Composition of diet

Constituent	Parts by weight
Cooked white fish	67
Cooked whalemeat	32
Liver meal	10
Meat meal	16
Biscuit waste	100
Sugar (reducing and non-reducing)	9
Propyl gallate	trace

Table II

Treatment of diet during processing

Sample No.	Batch 1				Batch 2			
	Dried in steam cabinet		Dried in oven		Dried in steam cabinet		Dried in oven	
	Time, hours	Temperature °C	Time, hours	Temperature °C	Time, hours	Temperature °C	Time, hours	Temperature °C
VI	2	90	—	—	2	90	—	—
V	1½	100	—	—	1½	100	—	—
VIII	1½	110	—	—	1½	110	—	—
II	1½	120	—	—	1½	120	—	—
IV	1	90	1	130	1½	130	—	—
I	1	90	1	140	1	90	1	140
III	1	100	1	150	1	100	1	150
VII	1	100	1	160	1	100	1	160

possible to attain the highest temperature required in the cabinet, some of the samples were dried in an oven for a further period, as indicated in the Table. All the samples appeared thoroughly dried, their moisture contents ranging from 0·81% to 4·39%. Some browning was observed in the samples dried at, or above, 140°. The amounts of the first batch of samples (2–3½ lb. each) were insufficient for completing the animal experiments, and a second batch of diet was made up and dried as before, except that only three of these samples were subjected to further heat treatment in the oven (Table II).

Bulk analyses for the batches of dry diet were protein 29·6%, fat 8·8% and ash 5·60%. The calcium content was 1·69% and the phosphorus 0·97% (as P) of the dry weight of the diet. The samples were numbered at random and the drying conditions were not disclosed during the animal experiments.

Design of animal experiments

Experiment 1.—Sixty-four Wistar strain weanling rats, approximately 28 days old, were used. They were derived from eight litters, each of which had been reduced shortly after birth to eight individuals. The growth rates of these animals were normal at the beginning of the experiment, the mothers having received Diet 41 (Bruce & Parkes[7]) supplemented by greens, carrots and oats. The individual rats were distributed between eight groups in such a way that the average weights of the groups were similar (Table III) and each group contained a representative from each litter, the sexes being distributed as evenly as possible. The members of each group were placed together in wire-gridded cages and fed *ad lib.* on one of the eight diet samples, which were distributed at random in relation to the groups. No supplements were given, but water was provided in drinking bottles. The rats were weighed three times weekly and their condition was noted. The experiment was continued for 4 weeks.

Experiment 2.—The rats that had been fed the diet sample giving the smallest increase in weight (dried at 160°) were sub-divided into two groups, balanced for weight and sex, the members of the sub-group being caged together. One group was fed on a diet sample that had given satisfactory weight increases (dried at 110°), the other group continuing to receive the diet sample that had given the smallest weight increases (dried at 160°). Weighings and observations were continued on these animals for 4 more weeks. At the end of this period the rats were killed with nembutal and examined by *post-mortem* dissection. Portions of liver, intestines and reproductive organs were fixed for routine histological examination, and sections were cut in paraffin for staining with haematoxylin and Biebrich scarlet.

Chemical estimations

Samples of diet dried at 100°, 120° or 160° were extracted twice with boiling dilute acetic acid, and the filtrates, after cooling, adjusted to pH 4·4 and incubated with Takadiastase at 37°. Thiamine was estimated in these samples as thiochrome.

Determination of the vitamin-A content of the diets dried at 110° and 160°, and of the weighed livers from two rats fed on these diets in Experiment 2, was kindly carried out for us by Dr. T. Moore of the Dunn Nutritional Laboratory, Cambridge, using a spectrophotometric method.

To ascertain whether any essential amino-acids had been destroyed during drying, samples of diets dried at 100°, 120°, 140° and 160° were hydrolysed with 5N-baryta under reflux for 24 hours. Neutralization was effected with H_2SO_4, the mixture was filtered, and the filtrate was concentrated to a small volume and then run on two-dimensional paper chromatograms. Tryptophan was overshadowed by phenylalanine and valine, so that this amino-acid had to be separated by precipitation with mercuric sulphate. The regenerated tryptophan was run on one-dimensional paper. Lysine was similarly run after precipitation with phosphotungstic acid. In all tests the solvent was butanol–acetic acid.

Digestibility tests were carried out on the diets dried at the same temperatures, 2 g. of each sample being extracted with light petroleum B.P.C. (40-60°) and the extracted material was dried and transferred to a flask, with 100 ml. of distilled water: 3 ml. of N-HCl and 20 ml. of pepsin solution (B.P. 1948) were added and digestion was carried out at pH 2·2 for 18 hours at 37°. The solution was made up to 200 ml. and centrifuged, and the soluble nitrogenous products were estimated in 50-ml. aliquot portions. A blank estimation was carried out on the HCl and pepsin solutions and the result of this subtracted from the total pepsin-soluble nitrogen. The water-soluble nitrogenous constituents were found to be less than the acid-soluble nitrogenous constituents and were estimated in a similar manner to the nitrogenous constituents of the pepsin-soluble fraction, but without the addition of HCl and pepsin.

Results

Experiment 1

The average gains in weight made by the groups of rats for the whole experimental period are shown in Table III. An analysis of variance showed that over the whole period there was no significant difference in the rate of weight increase of rats receiving the diets heated at 90°, 110°, 120° and 130°. The diet dried at 100° gave no significantly different increase from the diets mentioned above until the fourth week when, with one exception, all the rats receiving it lost weight. It is probable that this terminal loss was not due to the nature of the diet, but to some other factor, such as infection. The diet dried at 160° gave a significantly smaller increase in weight in every week of the experiment ($P < 0.01$ for the whole 4-week period). The smaller gains in weight on the diets dried at 140° and 150° were significantly less than those made on the diets dried at lower temperatures in some but not all weeks. Growth curves for the groups of rats are shown in Fig. 1.

The rats receiving the diet dried at 160° showed little interest in their food and were much less active than the other animals. Rats receiving the diet dried at 150° or 160° showed delayed sexual maturity (undescended testes, closed vaginae) compared with animals in the other groups.

Table III

Effect of drying temperature on the nutritive value of a diet, measured by weight gains in weanling rats

Diet dried at °C	No. rats in group		Initial mean weight, g.	Mean weight gain in 28 days with its standard error, g.
	Male	Female		
90	3	5	48	76·4 ± 8·2
100	4	4	47	50·7 ± 5·2
110	5	3	45	75·6 ± 5·0
120	4	4	48	81·0 ± 8·0
130	5	3	47	78·5 ± 8·7
140	2	6	46	59·9 ± 4·5
150	5	3	48	50·1 ± 3·7
160	4	4	49	19·3 ± 2·9

* Low value due to failure to gain in last week of experimental period, up to which time growth had been normal.

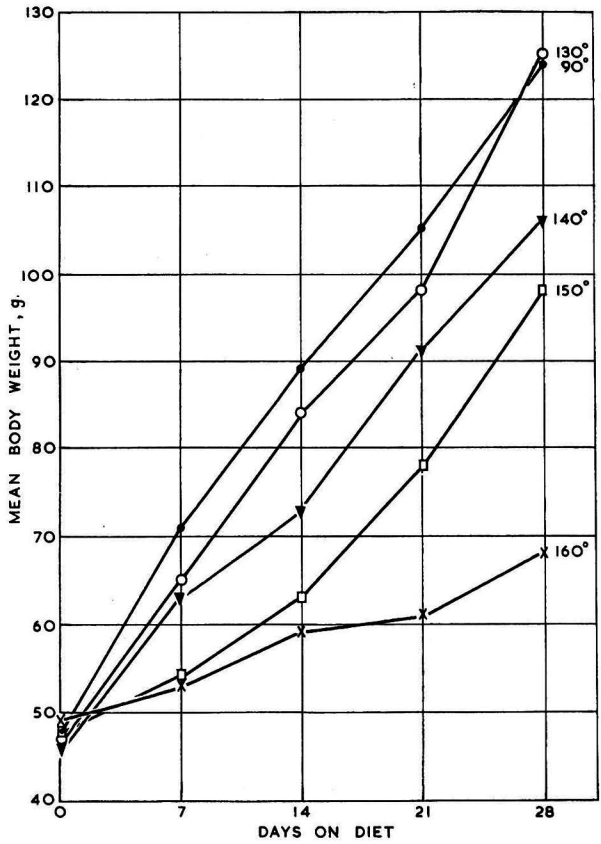

FIG. 1.—*Graph to show the mean growth rate of groups of* 8 *rats receiving the diet dried at temperatures ranging from* 90 *to* 160° *for* 4 *weeks*

The curves for the diets dried at 100°, 110° and 120° have been omitted for clarity, since they do not differ significantly from those of the rats receiving the diet dried at 90° and 130°

Experiment 2

Sub-group A maintained on diet heated to 160°.—The four rats in this group made gains in weight averaging 51 g. (range 33 to 52) during this experimental period (4 weeks). At the end of this time they were lean and lethargic, with poor coats, two showing loss of hair at the back of the neck. At *post-mortem* examination the uteri were pale and atrophic, the ovaries in the two females lacking any mature follicles. In one male the testes were still undescended, and this rat showed an almost complete absence of mesenteric and perirenal fat. The testes had descended in the other male, in which moderate amounts of fat were present round the abdominal viscera. Histological examination of the testes showed atrophic degeneration of the spermatogenic tissue, ranging from absence of spermatozoa to complete disappearance of spermatogonia (Fig. 2). The seminiferous tubules were reduced to about one-third of their normal cross-sectional diameter (compare Fig. 3). Giant cells were present in some parts of the seminiferous tubules, and the interstitial tissue was deficient and atrophic. The livers were small, averaging 5·9% of body-weight, but did not show any histological abnormality; the intestines were also histologically normal.

Sub-group B transferred to diet heated to 110°.—These four rats made significantly greater gains than the rats in sub-group A, averaging 80 g. (range 76 to 91), during the experimental period. They became active and sleek, with dense, healthy coats. The testes were descended

in both the males, having been undescended in one animal at the beginning of the period, and had a normal histological appearance (Fig. 3). Both the females were pregnant, one having 4 conceptuses, the other 11. Normal amounts of fat were found associated with the abdominal viscera in all animals in this group. The livers were on the average 6·6% of the body-weight and appeared normal, as did the intestines also.

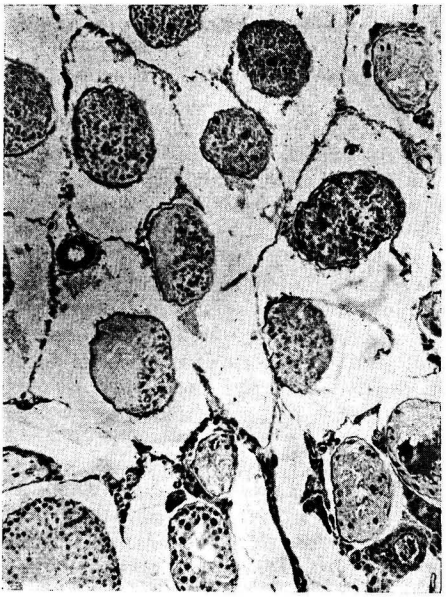

FIG. 2.—*Section of testis, from rat which had received the diet dried at 160° for 8 weeks, showing atrophy of the seminiferous tubules*
× 120. Stained haematoxylin and Biebrich Scarlet

FIG. 3.—*Section of testis from rat which had received the diet dried at 160° for 4 weeks, followed by the diet dried at 110° for a further 4 weeks*
× 120. Stained haematoxylin and Biebrich Scarlet

Chemical tests

Table IV shows that some destruction of thiamine and vitamin A had occurred in the samples dried at the higher temperatures, but that it was by no means complete. However, combined with a decrease in food intake, this destruction was sufficient to reduce the vitamin-A content of the liver of a male rat in sub-group A (diet dried at 160°) to 720 i.u., compared with 2780 i.u. in the liver of a male from sub-group B (diet dried at 110°). Although the values for total nitrogen were substantially the same for all drying temperatures, the proportion of

Table IV

Results of chemical tests

Drying temp. °C	Thiamine i.u./g.	Vitamin A i.u./g.	Digestibility		
			N per 100 g. of diet, g.	Water-soluble N %	Pepsin-soluble N %
100	0·5	—	4·25	26·4	72·2
110	—	12·6	—	—	—
120	0·4	—	4·35	27·7	77·0
140	0·2	—	4·32	27·5	62·8
160	0·25	9·2	4·35	18·1	49·7

nitrogenous material susceptible to solution by water or by peptic digestion was much reduced in the diet dried at 160°.

When the amino-acid composition was determined by the chromatographic method described, no difference in concentration of individual amino-acids was apparent between the diets dried at low and at high temperatures.

Discussion

In considering the effect of dry heat on a mixed diet, it has been observed that the addition of maize starch caused no greater deterioration of fibrin at 190° than its absence,[2] but the addition of glucose partly inactivated fibrin at 150° and completely at 165°. Fructose partly inactivated fibrin at 130° and completely at 160°.

These temperatures appear significant in relation to the results described in this paper, since only the weanling rats receiving the diet dried at 130°, or below, made satisfactory weight gains. Those rats having the diets dried at 140° and 150° showed fluctuating gains, whereas those on the diet dried at 160° showed anorexia, stunting, poor coats and failure of reproductive function. These symptoms resemble those shown by rats fed diets deficient in protein or in amino-acids, such as tryptophan.[8] Overby & Frost[9] found that destruction of tryptophan exceeded by far that of any other amino-acid when a solution containing 5% fibrin hydrolysate and glucose (pH 5·5) was autoclaved at 115° for 100 min. Rat repletion studies confirmed that shortage of tryptophan was the most important limiting deficiency produced, the rats suffering from anorexia, which reduced their total nitrogen intake to less than half that of rats fed the same mixture heated for 1 hour or less. Even so, these authors also noted that prolonged heating produced, in the hydrolysate–glucose solution, changes that could not be counteracted by the addition of tryptophan alone.

Although the symptoms do not resemble those of any specific vitamin deficiency, it can be seen from Table IV that some reduction had occurred at 160° in the contents of thiamine and vitamin A, and loss of other heat-labile constituents, such as folic acid and possibly tocopherols, may be postulated. Biely, March & Tarr[10] showed that, for the chick, the nutritive value of commercially flame-dried herring meal was less than that of the same material dried in air at relatively low temperatures (approximately 40°). Further investigations[11] by means of microbiological assay, showed a marked loss of folic acid from the flame-dried meal. When folic acid was added to test diets containing this meal, the growth of cockerels was equal to that of those receiving a diet containing the herring meal dried at the low temperature.

Since chromatographic analysis of the diet dried at 160° showed that the essential amino-acids were still present, it seems that the loss of nutritive value was mainly due to the inability of the proteolytic enzymes of the alimentary canal to digest the heat-treated protein. This is supported by the results of the digestibility test with pepsin and by the nitrogen balances carried out by Frazier *et al.*,[2] which showed a fall in nitrogenous intake, a marked fall in urinary nitrogenous output and a rise in faecal nitrogen whenever heat-injured protein was fed to their rats.

It is therefore clear that mixed diets containing fish, meat and reducing carbohydrates should not be subjected to temperatures exceeding 120° during drying processes.

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STUDIES ON EGG SHELLS. VII.*—Some Aspects of Structure as Shown by Plastic Models

By C. TYLER

When egg shells are embedded in plastic it would appear that the plastic can form a clean mould of the inner surface of the shell with its knob-like projections, it can also penetrate into the pore channels and even into the body of the shell itself. The shell can then be dissolved away leaving the plastic moulds behind. It is thus possible to obtain a clear picture of the shape and size of pores and their relationship to the air spaces in the mammillary layer. The egg shells of various species of birds have been examined and differences in pore size and shape clearly demonstrated. The plastic residue from the shell itself also seems to indicate that the shell consists of layers of material, the layers being arranged differently in different species.

Introduction

The information which exists about the finer details of shell structure, including pore shape and size, is not very great. Romanoff & Romanoff[1] give values for the long and short axis of pores in shells from a number of species, values which imply that the cross-section of a pore is oval. However, it is interesting to note that these data were obtained by von Nathusius[2] in 1868, and further that he states in the original paper that the measurements are not of great significance because the position of the horizontal cross-section could not be standardized. Haines & Moran[3] have measured a pore at the top (mouth) and bottom and give values of 13 μ and 6 μ respectively. In addition, a number of diagrams and photographs of vertical cross-sections through pore channels have been published (von Nathusius,[2, 4] Stewart,[5] Romanoff & Romanoff,[1] and Marshall & Cruikshank[6]).

With regard to general structure, it is well known that the inner or mammillary layer consists of knob-like projections and that above it there is a layer, the wrongly named spongy layer, which is very compact, and which, according to von Nathusius,[7] shows, on staining, sub-layers having different amounts of protein in them.

It was felt that further information on these matters might be forthcoming if a different approach were used and therefore an attempt has been made to prepare true plastic models of pores.

At the same time it was hoped that other aspects of shell structure might be revealed.

* Part VI: *J. Sci. Fd Agric.*, 1955, **6**, 170

Methods

Embedding the shell

Pieces of shell were embedded in plastic as described by Tyler,[8] except that, immediately after adding the liquid monomer and the plasticizer to the shell, the liquid was boiled for a few seconds to expel air from the shell.

Developing the specimen

General.—The block of plastic with a piece of shell embedded in it must be treated in such a way that part of the shell is exposed. This can be achieved in a number of ways, but after considerable preliminary work three methods were ultimately decided upon.

Whichever method is used, the plastic has to be removed to expose the shell. This is done first with a coarse file, then with a finer file and the surface of the specimen carefully finished off with the finest of files or some suitable abrasive.

The exposed surface or surfaces are then treated with a solvent which removes the shell material leaving some, but not all, of the plastic.

Ethylenediaminetetra-acetic acid (EDTA), made up as a saturated solution and adjusted with sodium hydroxide to pH 7, removes shell materials slowly and gently, but often a jelly-like mass of plastic remains which ruins the specimen. Dilute acids tend to do the same, but concentrated hydrochloric acid removes shell material rapidly and vigorously, its vigour serving to get rid of most of the jelly-like material as well. In this way clean specimens are obtained.

The jelly-like material is certainly best removed, because on drying it may form strands and sheets and powder, which, as artefacts, proved at first to be very misleading.

Method 1.—The plastic is ground away to expose a smooth radial section with the face at right angles to the surface of the shell. The specimen is then immersed in concentrated hydrochloric acid for about 10 seconds, washed, stained with acid fuchsin, which shows up the specimen better, washed again and dried. The acid attacks the exposed surface but etches different layers of the shell at different rates. Thus in 10 seconds some layers are scarcely touched, whilst others are removed to a considerable depth. By repeated and careful grinding it is possible to obtain ultimately a pore near to, or in the face to be etched, and subsequent etching and staining not only makes it possible to see the different layers but also the pore in relation to them. Etching for a few more seconds sometimes improves the section further. These times of etching are suitable for hen-egg shells, but longer or shorter times may be required for other shells.

Sometimes the sections are found to have a crack or cracks running along the shell more or less parallel to the surface and probably caused by stresses set up in the plastic. These specimens are very unsatisfactory because the clearness of the etched model is disturbed owing to acid getting into the cracks and attacking at the wrong points. These specimens should be rejected.

Method 2.—The plastic is removed only from the outer surface of the shell leaving the edges (radial faces) still firmly in contact with the plastic. This is best done by filing off the plastic very carefully until the convex shell surface is exposed. It is fairly easy, with a little practice, to tell when this point has been reached, but as a useful check the surface can be touched with acid and the patches, if any, where plastic still remains will effervesce much less than the exposed shell, or not at all.

Treatment with concentrated hydrochloric acid ultimately removes all the shell leaving a depression with base and sides of plastic. On the floor of this depression is the mould of the mammillary layer and projecting like stalagmites from it are a number of pore moulds (Figs. 1 and 2).

The base has an irregular honeycomb pattern, each mammilla of the original shell giving rise to a single cell of this honeycomb. Where a number of cells, usually three or more, come together there is nearly always a spike of varying length, whilst in some cases there is a pore. Thus it appears that each pore channel is a continuation of the space between a group of mammillae. The pores themselves widen gradually towards the top and often spread out suddenly to give a mushroom shape.

It is fairly easy to dissect off these pore moulds whilst viewing the specimen under a low-power microscope and to mount them singly or in groups on a slide. The pores may be mounted

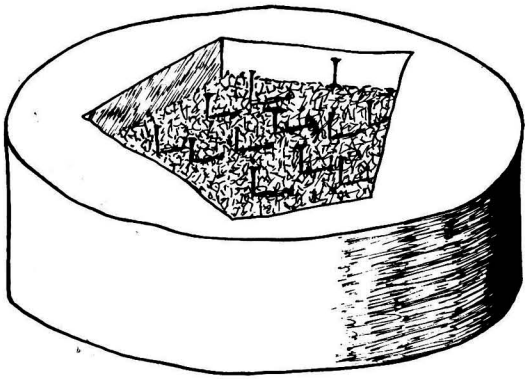

FIG. 1.—*Diagram showing cylinder of plastic with depression which previously held the shell*

The base of this depression shows the honeycomb structure and projecting from it are a number of pore moulds

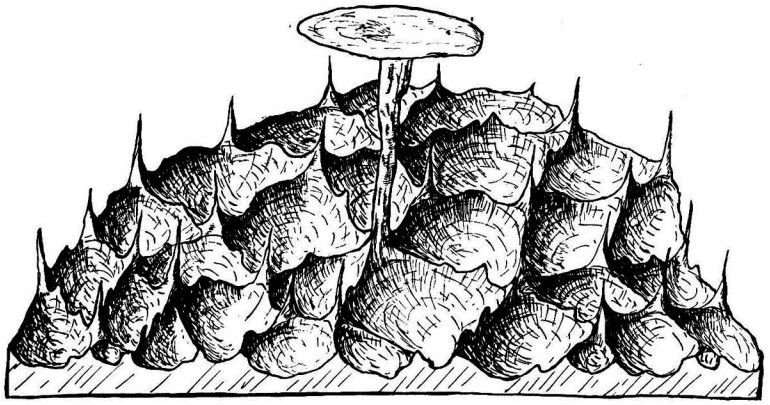

FIG. 2.—*Portion of Fig. 1 enlarged and somewhat conventionalized*

The depressions are the individual cells of the honeycomb formed by the mammillary knobs. The spikes of plastic have been formed where three or more of these knobs meet, but they are not pore moulds. However, pore moulds start from similar points and one is shown

in any of the usual mounting media or sealed dry in a cavity slide. In either form they can be measured and photographed using a high-power (× 200) microscope.

Method 3.—The plastic is ground away to expose the edges of the piece of shell all the way round. The shell is then dissolved away completely, using concentrated hydrochloric acid, and finally washed with water. This results in two pieces of plastic separated by a gap previously occupied by shell. Across this gap, and holding the two pieces of plastic together, are plastic pillars which are the pore moulds (Fig. 3).

The two pieces of plastic may then be carefully pulled apart causing the pillars to break, usually at the narrowest point. The layer of plastic which was originally in contact with the outer surface of the shell thus comes away still having attached to it the pore models. The bottom layer of plastic shows the typical honeycomb pattern, as shown by Method 2, but with no pores attached. This method is useful in special cases as will be seen later.

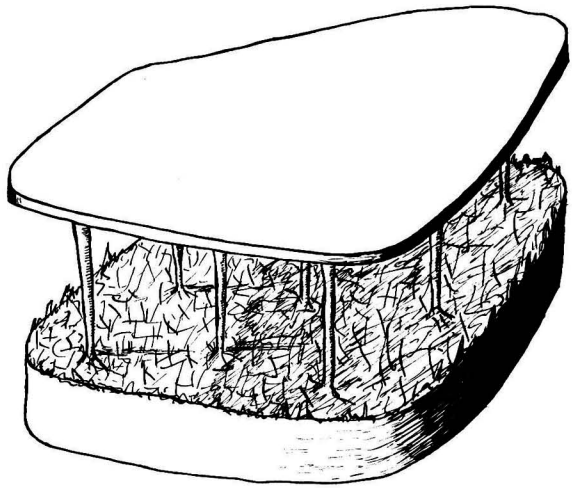

FIG. 3.—*Top and bottom layers of plastic, with plastic pillars, i.e. pore moulds, between*

The bottom layer has, on its upper surface, the honeycomb structure from which the pores arise

Examining the models

The best instrument for general inspection of the models is a stereoscopic microscope giving magnification of about × 12·5, × 50 and × 100. Excellent views of all structures are obtained, but unfortunately, such views cannot readily be reproduced in a journal. The best that can be done is to make drawings and supplement these with suitable photographs.

Results

General

The photographs in Plate 1*a–g* show the general effect of etching by Method 1. The clearly recognizable layers have been designated L1, L2 and L3 to represent the three layers usually found in the normal hen-egg shell and modifications, where necessary, of this standard nomenclature have been applied to the other shells.

For comparative purposes sections of representative shells have been drawn in a stylized form and are shown in Fig. 4*a–f*. Using a micrometer eye-piece, the thickness of each layer of a shell was measured at a number of points and the mean taken. However, in the diagram all the shells have been given the same total scale thickness so that the relative thicknesses of the different layers may be compared between species. Pores have also been drawn in these figures, again to the appropriate scale, so that their relative size in relation to shell thickness may be seen. Differently etched layers are shown by different types of shading, but of course, these rates of etching are only truly comparable within, and not between, individual specimens. Measurements in microns are also given on the diagram.

The pores seen in the specimens run from between the mammillae, through the various layers and finally reach the surface, usually in a depression. The emu shell is an exception which will be discussed later.

The layer most resistant to acid appears to have a fibrous structure with the fibres running parallel to the surface of the shell, but, in some cases, these fibres tend to show a streamlined effect where a pore passes through, as if the pore had been pushed through this layer from the outside.

There also appear to be minute nodules of resistant material in many of the honeycomb cells. It is possible that these nodules represent the cores of protein known to occur in each mammillary knob.

Size of pores

As already stated, Method 2 makes possible the separation and mounting of pores. From Method 1 it is also possible to get good measurements of pores because it is usual to obtain sections in which the pores are only exposed after etching and hence have not been rubbed away so much as to give erroneous results.

However, there are difficulties in measuring pores. Towards the outer surface a pore widens gradually, but then, in some cases, there is a final rapid widening to a mushroom head. Since this mushroom head may merely represent a depression in the surface of the shell, is it to be regarded as part of the pore ? All that can be done is to try to judge where the neck of the pore widens into its mushroom head and measure at that point. Similarly, at the inner end of the pore, the pore channel is continuous with the space between a group of mammillae and it is therefore again difficult to decide where the pore truly ends. An attempt has been made to break off pores at their narrowest point, from moulds made by Method 2, but, by doing this their length will be less than the thickness of the shell by about the depth of the mammillary layer.

The measurement of pore moulds may be made using a microscope with micrometer eyepiece, and measurements were taken at the top (excluding the mushroom head), middle and bottom (narrowest point) of the pore. Such measurements are purely arbitrary in the sense that they do not necessarily indicate the exact shape of the pore. Method 1 with a drawing of a pore to scale is in some ways more satisfactory, but, of course, fewer pores can be measured in this way. A further advantage of Method 1 is that the junctions of the differently etched layers form very useful points at which to take measurements of the pores.

Notes on different species

Hen

A number of shells have been examined by Method 1 and it would appear that there is a thin outer layer (L1) which dissolves very rapidly, then an acid-resistant layer (L2) and finally, an inner layer, including the mammillae which dissolves fairly rapidly (L3) (Plate 1*e* and Fig. 4*e*). In some eggs the outer layer is very difficult to see, whilst in others it is very pronounced. The other two layers are generally easily visible. The pores run from the mammillae to the surface passing clearly through all three layers.

Using Method 2, mean values for pore moulds from two different shells were :

top, 63 μ and 45 μ,
middle, 35 μ and 25 μ,
bottom, 23 μ and 17 μ.

These should be compared with the respective values of 42 μ, 14 μ and 14 μ given in Fig. 4*e*. If von Nathusius[2] took his measurements towards the middle of the shell then the present values of 35, 25 and 14 μ are of the same magnitude as the diameters which he gave, namely 29 $\times$ 22 μ for the largest pore and 11 $\times$ 9 μ for the smallest. But the values differ considerably from those of Haines & Moran[3] who gave a value of 13 μ for the top and 6 μ for the bottom. However, Haines & Moran only measured one pore. On the other hand, it must be admitted that there is no evidence yet to show that our method gives models of all pores. In fact, from the number seen on a specimen it is most unlikely. It may be that the liquid plastic does not penetrate into the finer pores, but it is more likely that the finer ones are broken off during the acid treatment, for if plastic can penetrate into the body of the shell itself it should have no difficulty in penetrating all pore channels. However, the measurements obtained give values for some, probably the larger, pores in shells and such measurements, made on actual moulds, should be more accurate than those made on stained protein sections.

Plate 1*g* shows a pore from a fully incubated hen-egg shell. This pore is much cleaner than the other specimens (Plate 1*e* and *f*) and apparently of greater diameter, but it is the only specimen we have yet made and therefore it is too early to comment further.

The specimen shown in Plate 1*f* is that of a rough-shelled hen egg. Such eggs are often referred to by practical poultry men as ' porous eggs ' and it is said that they do not hatch well. Unpublished work in this department has shown that such eggs have very low porosity

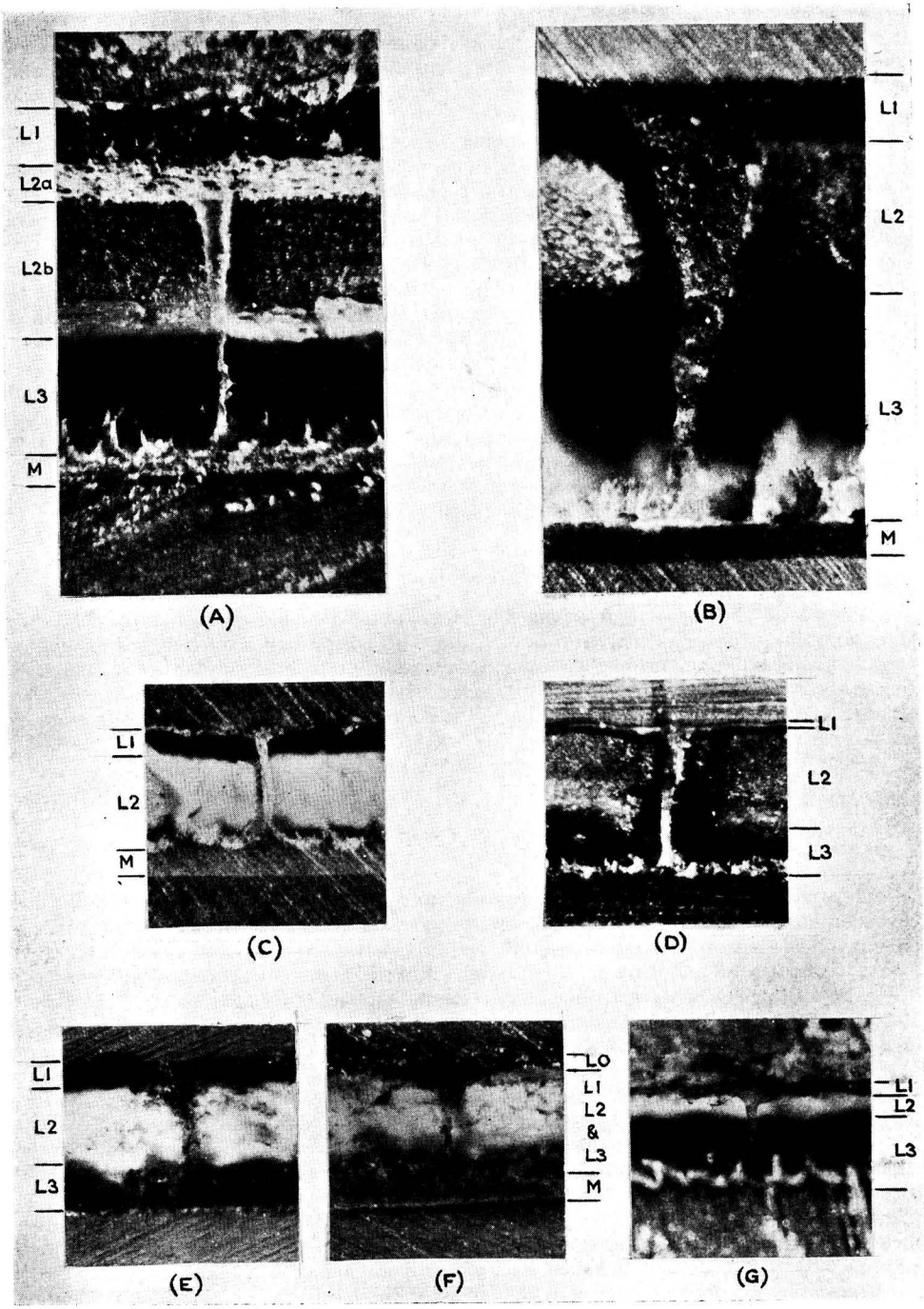
L1
L2a
L2b
L3
M
(A)
L1
L2
L3
M
(B)
L1
L2
M
(C)
L1
L2
L3
(D)
L1
L2
L3
(E)
LO
L1
L2
&
L3
M
(F)
L1
L2
L3
(G)

PLATE 1A–G

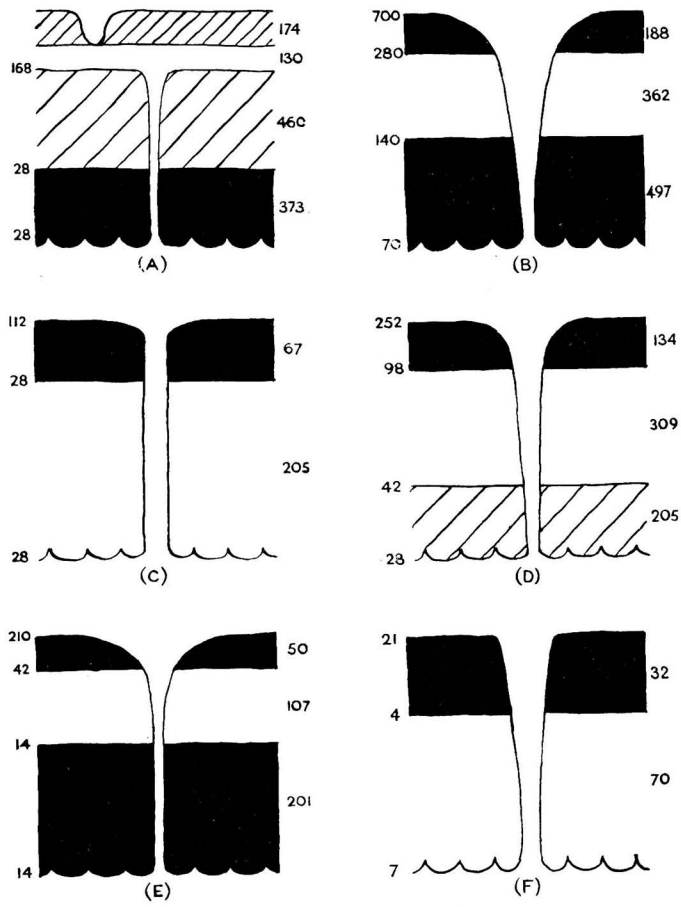

FIG. 4A–F.—*Conventional diagrams of different types of shell*

Black indicates areas rapidly etched, white indicates slowly etched and shading indicates intermediate values

A. Emu C. Skua E. Hen
B. Rhea D. Swan F. Song Thrush

Numbers on the right-hand side of each figure represent the thickness of each layer, those on the left the diameter of the pore at that level. All measurements are absolute and are given in microns

PLATE 1A–G (*opposite*).—*Photographs of radial sections of shells : embedded in plastic and etched with hydrochloric acid*

Layers—Lo : extra L2 : weakly etched
L1 & L3 : strongly etched M : membrane
A. Emu C. Skua E. Hen, normal G. Hen, incubated
B. Rhea D. Guinea Fowl F. Hen, rough-shell

coefficients and there are many measurements to support this. The photograph shows a pore channel and over the whole surface including the pore mouth is an extra layer of readily etched material (Lo) which presumably blocks the pore mouths and also accounts for the roughness of the shell. The three layers do not show up clearly in this specimen.

Other birds of the Galliformes whose egg shells have been examined are the turkey, pheasant, pea hen and guinea fowl. All of them show the same structure as the hen-egg shell, in so far as there are three typical layers and pores of the same general shape. Plate 1*d* shows the guinea fowl shell with a very thin outer layer of etched material (L1) and a very thick second layer of unetched material (L2) as well as the inner etched layer (L3).

Ostrich

Method 1 gives a structure similar to that of the hen egg with an acid-resistant layer L2 sandwiched between two more soluble layers, L1 and L3, and is therefore not shown in the plate or figure. On the other hand, the pore system is quite different. The pore channel arises, as usual, from between a group of mammillae but immediately begins to branch until at the surface there are a number of pore channels opening into one depression in the shell. Fig. 5 shows a drawing of a plastic mould of the multi-branched ostrich pore. The outer ends of the many branches were broken off short in order to show them clearly separated.

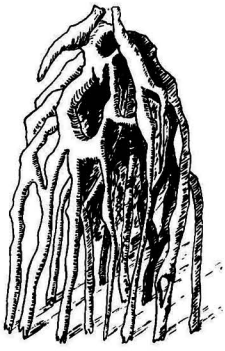

FIG. 5.—*Drawing of a single ostrich pore*

The top represents the inner end of the pore and the bottom the multiple channels leading to the outer surface of the shell

Emu

Method 1 gives the result shown in Plate 1*a* and Fig. 4*a*. The outer layer (L1) has been dissolved away fairly rapidly. This layer is of variable thickness and represents the corrugations or ridges present on the surface of an emu shell. The variation in thickness is not shown in the photograph which cuts right across a ridge but it is shown diagrammatically in the figure. The next layer, very faintly green in colour, is fibrous in form and is very acid-resistant : it has been designated L2a. Below this is a much thicker pale green layer L2b, dissolved away more rapidly than the layer above, but fairly resistant. Finally the innermost layer is very rapidly dissolved (L3). The pore mould in this specimen starts as usual from the mammillary space and passes through two layers but it ends in the very resistant layer. It does not go through to the outer surface as do the pores in other types of shell.

Method 3 strongly confirms this structure, for when such a specimen is prepared the top layer of plastic is quite loose and comes away easily. On its inner surface it carries the impression of the shell corrugations. There still remain two other layers of plastic. The upper one when removed carries with it the pore moulds and the bottom one shows the typical honeycomb pattern. The pores are therefore firmly attached to this second layer (L2a), again suggesting that they do not pass right through the shell. The presence of this second layer may have some influence on the mechanism of evaporation from the eggs of this species.

Method 2 does not give good pore models with this species because of the special layer mentioned above, but the pores may be dissected from this layer as given by Method 3. They are shaped rather like spear heads, that is to say, they are narrow at the base and gradually widen towards the top in one plane but in the plane at right angles to this they are of equal thickness throughout.

Rhea

Method 1 as shown in Plate 1*b* and Fig. 4*b* again gives a typically resistant layer (L2) between two very soluble layers (L1 and L3), and running through this are pores which start from the mammillary spaces and run to the outer surface. These pores are narrowly oval in cross-section, and show a very pronounced spear-head shape.

Swan

The structure as shown by Method 1 and depicted in Fig. 4*d* is rather different from that of the hen-egg shell. There is the readily soluble outer layer (L1) and the second acid-resistant layer (L2). The lower half of this second layer is, however, slightly etched, and it is therefore reasonable to refer to layer L2 as consisting of L2a and L2b as was the case with the emu. Layer L3, however, is missing in the swan. The pores seen in these sections appear to be of the usual type, and this is confirmed by the use of Method 2.

It is of interest to note that egg shells of the duck and the grey lag goose, both also Anseriformes, show very much the same type of structure.

Other species

Examination by Method 1 of egg shells of the skua and the razorbill, both Charadriiformes, shows that they are different from the other species examined but like each other. The skua shell shown in Plate 1*c* and Fig. 4*c* clearly shows the readily soluble outer layer (L1), but the acid-resistant layer (L2) extends throughout the rest of the shell, so that the layer L3 is missing: neither is there any sign of some etching of the lower half of this layer as seen in the swan in layer L2b.

Egg shells from three Passeriformes, the song thrush, chaffinch and house sparrow, show a similar structure to the skua, but the inner resistant layer (L2) is relatively thinner. On the other hand in Fig. 4*f* representing the egg of the song thrush, the pore is seen to be relatively thick.

As a matter of great interest the opportunity was taken to examine a piece of egg shell of the extinct Aepyornis. Method 1 gives a section which appears to be uniformly resistant to acid except for a slightly greater attack at the outer and inner surfaces, but there are no clear cut layers as in other shells. There was no sign of any honeycomb pattern from the inner surface. The pores pass right through the shell, and near the outer surface they become flattened or even two-pronged, finally opening into a deep groove.

Discussion

The method employed in this study of egg shell structure appears capable of distinguishing between different arrangements of layers in the egg shells of different species and also of showing pores of different shapes. It is hoped that in the future it may be possible to establish other species differences and maybe to differentiate between shells from individual hens.

The first point for discussion is the significance of the various layers. At the outset it must be stressed that, with Methods 2 and 3, most or all of the space occupied originally by the shell is cleaned out if the acid is allowed to act long enough. The layers produced by Method 1 are therefore merely representative of the speed with which the acid can attack, hence the differential etching. It is well known that a shell without membrane consists of about 98·5% mineral matter with 1·5% protein, therefore there cannot be a great deal of difference in the mineral content of the various layers. For example, if the three layers of an egg are taken as 20 : 40 : 40 units thick and it is assumed that the two outer ones are 100% mineral matter, then it can easily be shown that to give an overall percentage of 98·5% mineral matter the middle layer would have to contain 96·25%. On the other hand, this means that the two outer layers would have no protein, whilst the middle layer would have 3·75%. It may, therefore, be the case that there are differences in protein content between the variously etched layers of shell, and support for this comes from von Nathusius[7] who states that protein stains show different amounts of protein in different layers quite clearly. Now, if the liquid plastic can find its way along the protein fibres of the shell and there polymerize, it follows that this effect might in turn influence the rate of etching, and protein does in fact become embedded in plastic as may be seen from the colouring given to some plastic layers by coloured shells. However, it is not possible to state that the percentage protein in a layer will be quantitatively related to the rate of etching. One layer may be composed of large crystal aggregates of mineral matter, chiefly calcium carbonate, with protein running in channels between them, but not necessarily completely enclosing the individual aggregates; such a layer should be readily attacked by acid. Another layer of the

same general structure but with small crystal aggregates might be attacked at the same rate but the proportion of protein in it would be much larger. Still other layers might have crystal aggregates each completely surrounded and cut off from its neighbours by protein. Such layers would be only very slowly attacked or perhaps not at all and yet the percentage of protein might vary a great deal, depending upon the amount of protein separating the crystal aggregates. Until further work is done we must, therefore, be content to refer to different structures giving rise to a greater or lesser penetration of plastic with a resultant effect on the rate of etching.

Of the shells so far examined there are certainly very marked variations in the arrangement of the layers, with some indication of similarities in species belonging to the same family, but so far this is only a hint. Of great interest is the almost completely resistant layer which lies immediately beneath the surface corrugations in the emu-egg shell. This is the only layer yet encountered which leaves a structural residue after prolonged acid attack. It can be separated off and handled easily. Under the microscope it looks like a loosely woven mass of fibres, all running parallel to the shell surface. The rhea-egg shell shows a thick, slowly etched layer and although this does not, on prolonged acid treatment, retain its identity, yet it does give rise to a wet, jelly-like layer, which on drying produces a very thin sheet of brittle plastic. Some shells leave a residue of jelly, which, as mentioned above, must be removed if artefacts are to be avoided, but it is of importance to notice that, on drying, this jelly gives a fine white material, which, under the microscope, appears to consist of very short thin fibres running in all directions. These three different kinds of residue, the layer from the emu-egg shell which maintains its structure throughout, that from the rhea which dries to a fine sheet and the completely different one from some other birds, which dries to a mass of fine fibres may represent the residue from plastic which was related in turn to three different ways of depositing the mineral matter–protein complex in the shell. Material rapidly etched would represent yet another mode of combination.

The other important point for discussion is the question of pore structure. There is no doubt that pores have different shapes and dimensions and further that they arise from the channel formed by groups of mammillary knobs lying side by side. Except in the emu-egg shell, these pores go right through all the layers to the surface of the shell, where they usually open out into a depression in the shell. This may take the form of a shallow depression or a deep narrow grove, or some shape between these two extremes. It has not yet been possible to decide whether there is a direct contact between the protein in the various layers, especially in the slowly etched layers, and the pore walls. The fact that the pore is not etched and ultimately stands out clearly from the rest of the specimen proves nothing in this connexion.

Examination of many specimens shows that spikes of plastic arise from every channel between groups of mammillary knobs and that a proportion of these spikes are replaced by complete pores. It is possible that each of these spikes represents a potential pore, and, in this sense, it is correct to say that they represent aborted pores, but it must be realized that the percentage of abortions will always be very high. This introduces the question as to what it is that decides whether a channel between the groups of mammillae becomes closed up by succeeding deposits of mineral matter and protein, or whether it is kept open despite the added deposits. In other words, how are the pore channels formed? There appear to be three possibilities. Firstly, bundles of threads of some substance, such as protein, might arise from the membrane and stand up more or less vertically from it. The mammillae would then have to be deposited in such a way that the bundles of threads passed between them and deposition of the shell layers above would have to form around them so that their integrity was maintained right through to the surface. Secondly, some liquid or some gas might pass through the forming egg shell into the egg. It is well known that this happens, but in order to form pore channels it would have to commence by passing through all channels between the mammillae, then gradually more and more of these would become blocked until the final shell was formed and only a relatively small proportion remained which were the pores. Liquid flowing in in this way might also explain the directional stream-lining round the pores of the fibres in the unetched layer of some egg shells. However, it must be pointed out that after the membranes are formed, material is only supposed to enter the egg for about four hours. Thirdly, there is the exact opposite of this, namely the escape of some liquid or gas from the egg, which would first find its way out through all channels

between the mammillae, but more and more of these would ultimately be blocked until finally only those remained which were true pores.

Of these possibilities, the second one is perhaps the most promising, but all are speculative and the correct answer may have to be sought in other directions. Furthermore, whatever the answer, it must be remembered that Tyler[9] has shown that the distribution of pores over the surface of a shell is not at random, hence the true answer to this problem must fit that particular fact.

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THE CHEMICAL ESTIMATION OF VITAMIN-E ACTIVITY IN CEREAL PRODUCTS. IV.*—ε-Tocopherol

By P. W. RUSSELL EGGITT† and F. W. NORRIS

ε-Tocopherol has been isolated from wheat bran and some of its properties and its reactions have been studied. Its absorption spectrum, and that of nitroso-ε-tocopherol, are almost identical with the corresponding spectra for β-tocopherol. ε-Tocopherol, when oxidized with the Emmerie–Engel reagents, yields a tocopheroxide, which may be converted into the isomeric ε-tocopherylquinone. The latter shares a characteristically shaped absorption spectrum with β-tocopherylquinone. With nitric acid in ethanol ε-tocopherol forms a red *o*-quinone and other products with a reaction spectrum again very like that for β-tocopherol. Only the β- and ε-homologues produce a brilliant violet unstable colour (not the quinone) with nitric acid in chloroform. In these reactions the new tocopherol behaves as if it is 5-methyltocol and this structure is confirmed by the fact that ε-tocopherol yields 5 : 7-dimethyltocol (ζ-tocopherol) under conditions that convert β-tocopherol to α-tocopherol. Difficulties encountered in applying the Quaife nitrosation technique to the tocopherols are discussed. The factor converting depth of colour to concentration when ε-tocopherol is assayed by the modified Emmerie–Engel method described previously[1, 2] is 96.

The use of paper chromatography in assaying the individual tocopherols of millers' offals led to the suggestion in Part I[1] that a previously unknown monomethylated tocopherol (5-methyltocol) occurs in wheat. Subsequent work confirmed the nature of the substance (ε-tocopherol) and in Part II[2] it was shown to be concentrated mainly in the bran, whereas the

* Part III: *J. Sci. Fd Agric.*, 1955, **6**, 689
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α- and β-homologues reside mainly in the germ. ε-Tocopherol is likely to prove of considerable significance in the nutrition of farm animals, since it accounts for a high proportion of the total tocopherols in such feeding-stuffs as bran (67%), middlings (43%) and compound rations (typical chick mash 30%). It has also been found in barley, rye and oats and is probably widely distributed in cereals.[3]

The isolation of milligramme quantities of ε-tocopherol, by use of reversed-phase partition chromatography, was described in Part III[4] and certain similarities with β-tocopherol were discussed. It is now intended to present some of the properties and reactions of ε-tocopherol which confirm its identity as 5-methyltocol and to compare its behaviour with that of its homologues in some of the colorimetric methods used in the past for tocopherol assay.

Experimental

Isolation of ε-tocopherol

The ε-tocopherol required was isolated in several separate batches of 5 mg., each batch from 180 g. of freshly milled bran, so as to avoid using large-scale extraction and large chromatograph columns. The bran oil was extracted with *cyclo*hexane and saponified in the presence of pyrogallol, and the sterols and carotenoids were separated from the tocopherols in the unsaponifiable residue by adsorption on Floridin earth.[1, 2] The ε-tocopherol was then separated from its homologues by partition chromatography using a stationary phase of liquid paraffin, supported on a column of kieselguhr made hydrophobic with dimethyldichlorosilane, using 75% aqueous ethanol as mobile phase.[4]

Fig. 1 illustrates the ε-tocopherol peak forming part of the elution curve of one of the preparative chromatograms. This was plotted by withdrawing 0·25-ml. aliquots from alternate 3-ml. elution fractions, diluting them to 3 ml. and measuring the red colour produced by 0·5 ml. of each of the Emmerie–Engel reagents in 2 minutes using the standardised procedure described in the earlier papers.[2, 4] The eight fractions falling between 45 and 66 ml. were combined and evaporated to a reduced volume on the water-bath under a stream of nitrogen and finally to dryness under reduced pressure. Losses of ε-tocopherol during this evaporation were small and yields of well over 90% of the theoretical maximum calculated from the elution curve were obtained.

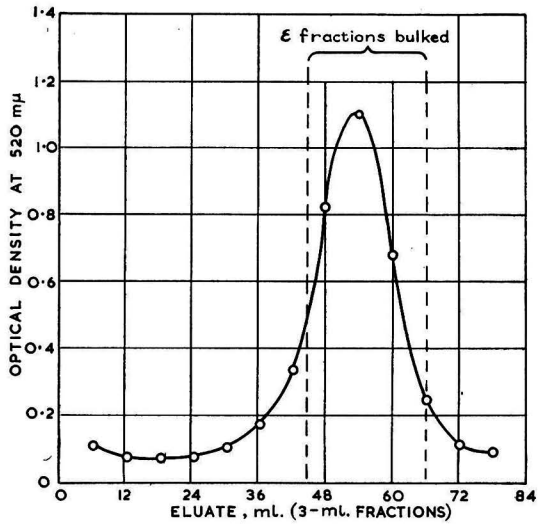

FIG. 1.—*The isolation of ε-tocopherol*

The ε-tocopherol elution peak from a preparative chromatogram of the tocopherols from 180 g. of wheat bran. Emmerie–Engel determinations were made on 0·25-ml. aliquots drawn from alternate 3-ml. fractions of eluate.

The ε-tocopherol may be treated more gently by diluting the aqueous ethanol eluate with water and extracting the tocopherol into light petroleum or peroxide-free ether before evaporation.

In selecting the fractions to be combined, the leading edge of the elution peak was avoided, as previous chromatograms, from which more points were plotted, revealed an inflexion between 30 and 40 ml. due to a possible impurity.[4] The ε-tocopherol isolated proved to be free from other tocopherols or reducing substances, when checked chromatographically by the two-dimensional adsorption–partition method of Green *et al.*,[3] producing a single spot in the unambiguous ε position (Fig. 9A). As quickly as possible after evaporating the eluate, the ε-tocopherol was dissolved in absolute ethanol and stored in the refrigerator.

Owing to the slight solubility of the liquid-paraffin stationary phase in the 75% aqueous ethanol, the ε-tocopherol was obtained mixed with a little of the hydrocarbon. To overcome any possible distortion of the absorption spectra presented in this paper, the reference blank was always prepared from a matching aliquot of an ethanolic solution of the trace of paraffin obtained by evaporating a volume of the mobile phase equal to the combined eluate.

The use of a more volatile paraffin such as octane as the stationary phase has already been suggested[4] should it be necessary to obtain ε-tocopherol free from traces of hydrocarbon. Following this suggestion, R. J. Ward[5] has found that octane or *iso*-octane may replace liquid paraffin with no other alteration in the procedure.

The ultra-violet absorption spectrum of ε-tocopherol

Fig. 2 illustrates the absorption curve of ε-tocopherol (0·055 mg./ml.) in ethanol, showing maximum absorption at 295·5 mμ and a minimum at 257·5 mμ. From several determinations a mean value of $E^{1\%}_{1\,cm.}$ at 295·5 mμ of 87·5 was obtained. The concentration of ε-tocopherol was determined by the modified Emmerie–Engel determination[2] using the same conversion factor as for α- and β-tocopherols, a procedure justified by the work outlined below.

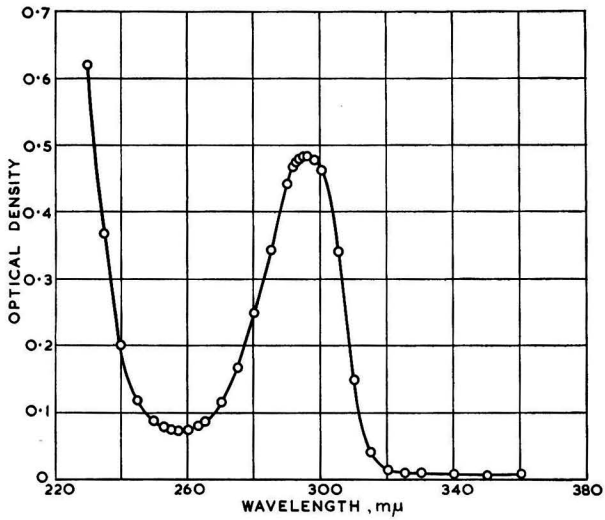

FIG. 2.—*The ultra-violet absorption spectrum of ε-tocopherol in ethanol* (0·055 mg./ml.)

It was noted previously[4] that, although the absorption spectra of the tocopherols are all very similar, they may be divided into like pairs on the basis of the small differences in the positions of the maxima. γ- and δ-tocopherols form a pair with maxima at 298 mμ, β- and ε-tocopherols a second pair with maxima at 296 and 295·5 mμ, respectively, whereas α-tocopherol stands alone with a maximum at 292 mμ. Recently Green *et al.*[3] have detected 5 : 7-dimethyltocol in bran, naming it ζ-tocopherol, and they succeeded in obtaining sufficient of it by paper

chromatography for spectroscopic examination. Their preparation gave a maximum at 292·5 mμ but the shape of the published absorption curve suggests the presence of impurities, particularly as synthetic 5 : 7-dimethyltocol gave a maximum at 294 mμ. Their figures show, however, that, even if ζ-tocopherol does not form a third pair with α-tocopherol, its ultra-violet absorption maximum occurs at a wavelength at least 2 mμ lower than that of the β- and ε-tocopherol pair, as would be expected from its structure.

The absorption spectrum of nitroso-ε-tocopherol

Quaife[6] found that β-, γ- and δ-tocopherols, but not α-tocopherol, form yellow nitroso-derivatives on treatment with nitrous acid and that the absorption spectra of these compounds appeared to obey Cronheim's[7] rules for the positions of the maxima and minima of the spectra of *o*-nitrosophenols. Cronheim[7] stated that: (1) the wavelength of minimum absorption increases with the number of substituents in the benzene ring and (2) the wavelength of maximum absorption depends upon the position relative to the hydroxyl group of the nearest substituent, except the nitroso-group itself.

As other evidence suggested that ε-tocopherol is 5-methyltocol, we decided to examine the absorption spectrum of its nitroso-derivative, in the expectation of finding a minimum at 340 mμ (only one methyl group in the benzene ring) and a maximum at 410 mμ (*ortho*-substituted, like β-tocopherol).

Method

Reagents: Glacial acetic acid, A.R.
Sodium nitrite (2 g. per 100 ml. of distilled water—prepared fresh daily).
Potassium hydroxide (20 g. per 100 ml. of distilled water).
n-Hexane (special for spectroscopy).

All operations were performed in subdued artificial light. Exactly 5 ml. of an absolute ethanol solution containing 1 mg. of the tocopherol were pipetted into a stoppered 50-ml. conical flask, 0·2 ml. glacial acetic acid was added and the flask was swirled to mix. Then 3 ml. of sodium nitrite solution were blown in from a fast pipette, the flask was swirled vigorously for 5 seconds and then allowed to stand exactly 60 seconds from the time the nitrite pipette was blown out. Next 2 ml. of potassium hydroxide were added rapidly and mixed, followed by 10 ml. of distilled water, a pinch of anhydrous sodium sulphate and exactly 10 ml. of *n*-hexane. The stoppered flask was shaken vigorously for 1 minute and its contents were poured into a stoppered 50-ml. separating funnel. A blank was then prepared without delay from 5 ml. of ethanol in exactly the same way, with particular care to time the nitrosation reaction with the same accuracy as for the test sample. The absorption spectrum of the hexane phase was then examined with the Unicam spectrophotometer using 1-cm. cells and with the blank solution in the reference cell.

The above method, used to compare the nitrosotocopherols, differs in a few details only from that devised by Quaife.[6] Under our conditions, vigorous shaking for 30 seconds was insufficient to extract all the nitrosotocopherol into the hexane phase and the aqueous phase remained pink, particularly in the case of γ-tocopherol. This colour must be completely discharged. Increasing the extraction time to 1 minute achieved this and gave reproducible results.

In our earlier experiments a small kink often occurred near the minimum at 360 mμ of an otherwise smooth nitrosotocopherol absorption curve. When a freshly prepared nitrosotocopherol test solution was examined, using a reference blank prepared some time previously and which may have evaporated slightly, a curve modulated between 320 and 400 mμ like that in Fig. 4(*a*) was obtained. The backwards and forwards adjustments necessary to set the spectrophotometer to 100% transmission on the blank solution as the wavelength was steadily decreased suggested that the blank itself absorbed strongly and irregularly over the spectrum range used. The absorption spectrum of a 1-minute blank (5 ml. ethanol allowed to react for 1 minute with nitrous acid before neutralizing and extracting with hexane) was measured against pure hexane. The spiky spectrum of ethyl nitrite was obtained with maxima at 314, 323, 333, 345, 357, 370 mμ and an inflexion at 383 mμ as shown in Fig. 3 (*a*). The concentration of ethyl nitrite in a blank was found to increase steadily the longer the nitrous acid was allowed to remain in contact with

the ethanol before adding the alkali. In Fig. 3 the absorption spectra of a 1-minute blank, a 10-minute blank and a 0·1% ethanolic solution of amyl nitrite are compared, each being measured against the appropriate pure solvent. Accurate timing of both the test and blank reactions therefore proved essential in order to prevent a mismatch in the background absorption.

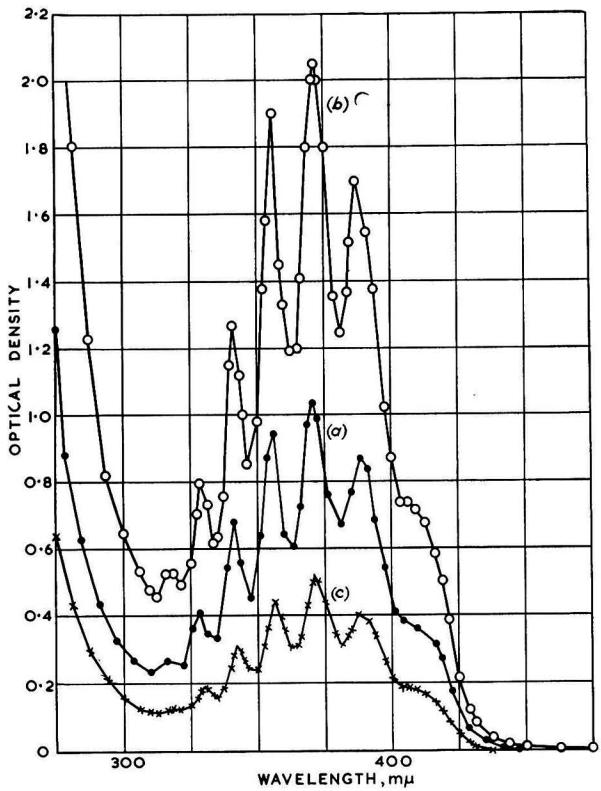

FIG. 3.—*The absorption of the reference blank in Quaife's method of determining tocopherols by nitrosation*

(*a*) The spectrum of a 1-min. blank (5 ml. ethanol allowed to react for 1 min. with nitrous acid before adding alkali and extracting with hexane) measured against pure hexane.
(*b*) The spectrum of a 10-min. blank measured against pure hexane.
(*c*) The spectrum of a 0·1% ethanolic solution of amyl nitrite measured against pure ethanol.

The curves in Fig. 4 were obtained by nitrosating δ-tocopherol. Curve (*a*) shows the effect of a mismatch of 30 seconds, the δ-tocopherol solution being allowed to react for 1½ minutes with nitrous acid before adding alkali and extracting, whereas the blank for the spectrophotometer reference cell was allowed to react for only 1 minute. This is compared with a normal matched curve (*c*) from a 1-minute nitrosation. Fig. 4 (*b*) illustrates the effect of nitrosating δ-tocopherol for 10 minutes with the absorption spectrum measured against a 10-minute blank prepared almost simultaneously. This demonstrates that the ethyl nitrite concentration in the test and blank reactions remained in step for a much longer period than normally employed when the method is used for assay purposes, as even after 10 minutes the mismatch produces only a slight ripple on the absorption curve between 330 and 410 mμ. After this long contact time the test solution was a deep orange colour compared with the usual bright yellow. The bathochromic effect is reflected in the extension of the curve and shows that the reaction went beyond the formation of the simple nitrosotocopherol. The curve published by Quaife[6] for a 10-minute nitrosation of δ-tocopherol is distorted by the ethyl nitrite peaks at 357 and 370 mμ.

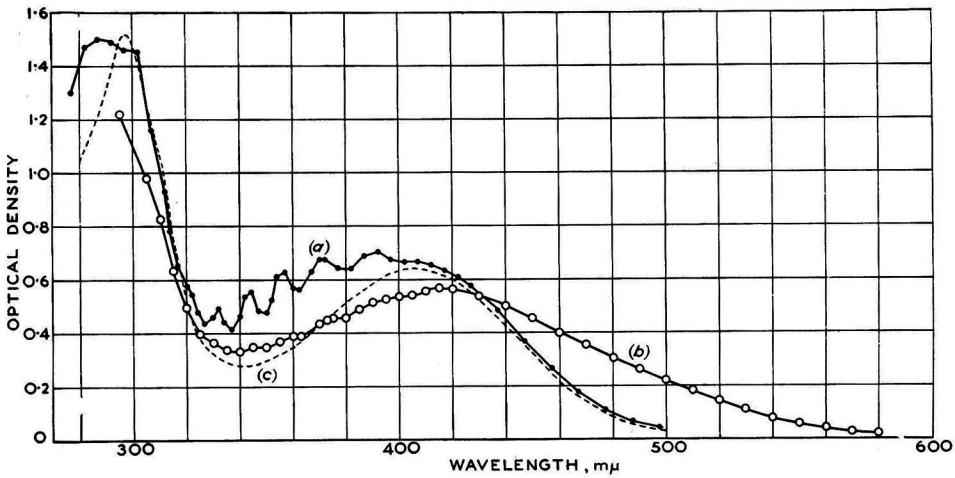

FIG. 4.—*The nitrosation of δ-tocopherol*

(*a*) 1 mg. of δ-tocopherol nitrosated 1½ min. before adding alkali, extracting into 10 ml. of hexane and measuring against a 1-min. blank (30 sec. mismatch).
(*b*) 1 mg. of δ-tocopherol nitrosated 10 min. before adding alkali, etc., and measuring against a 10-min. blank, prepared simultaneously.
(*c*) 1 mg. of δ-tocopherol nitrosated 1 min. before adding alkali, etc., and measuring against a matching 1-min. blank (normal nitroso-δ-tocopherol curve).

It has since been observed that Polister[8] has reported previously on difficulties encountered in the use of Quaife's method for tocopherol assay caused by the formation of ethyl nitrite. She found the interference so great that the method was only workable if the ethanol used as the solvent during the nitrosation was replaced by peroxide-free dimethoxyethane. Under our conditions, however, accurate timing of both blank and test reactions enabled undistorted absorption curves to be obtained using the original method with only minor modifications. Quaife[6] recommends measuring the yellow colour at 410 mμ when using the method for tocopherol assay. At this wavelength a mismatch of some seconds in the blank has very little effect on the result, as ethyl nitrite has little absorption at this point in the spectrum (Fig. 3).

Results

The absorption spectra of the nitroso-compounds prepared from β-, γ- and δ-tocopherols are shown in Fig. 5 (*a*). In each case 1 mg. of the tocopherol was nitrosated and the nitroso-compound extracted into 10 ml. of *n*-hexane. The curves are closely similar to those obtained by Quaife,[6] although they have been extended to include the maxima near 300 mμ. The spectrum of nitroso-ε-tocopherol proved to be virtually identical with that of nitroso-β-tocopherol so that it was necessary to illustrate it separately in Fig. 5 (*b*). The standard solutions of β-, γ- and δ-tocopherols for these experiments were prepared by weighing, whereas the concentration of the ε-tocopherol solution was determined accurately by the Emmerie–Engel reaction using the factor 96 in the method described previously.[2]

The spectrum constants given in Table I emphasize the similarity between nitroso-ε-tocopherol and nitroso-β-tocopherol and support other evidence that ε-tocopherol is 5-methyltocol. In the β- and ε-compounds the nitroso group enters the chroman nucleus at position 7 whereas the more reactive 5-position is available in the γ- and δ-homologues. Thus only β- and ε-nitrosotocopherols share the sequence 5-methyl, 6-hydroxy, 7-nitroso for the positions in the benzene ring, differing only in the unreactive 8-position.

Nitroso-ε-tocopherol, with a maximum at 410 mμ, complies with Cronheim's rule (2) for the spectra of simple *o*-nitrosophenols. Rule (1) for the position of the minimum is not followed, the discrepancy being nearly 20 mμ. It seems that the presence of the dihydropyran ring makes the rule inapplicable to the nitrosotocopherols.

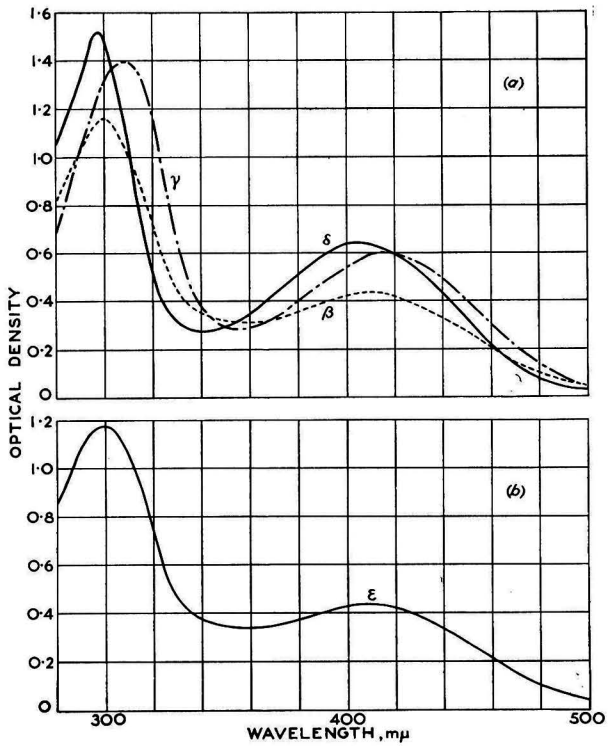

FIG. 5.—*Absorption spectra of the nitrosotocopherols in* n-*hexane*

(*a*) Nitroso-β-, nitroso-γ- and nitroso-δ-tocopherols
(*b*) Nitroso-ε-tocopherol
(1 mg. of each tocopherol nitrosated in ethanol for 1 min. before adding alkali, extracting into 10 ml. of *n*-hexane and examining with the Unicam spectrophotometer against a matching blank)

Although β- and ε-tocopherols are easily separated by partition chromatography,[4] they form a common band in adsorption chromatograms where the R_F values depend on the position of methyl substitution relative to the hydroxyl group.[3] The work of Quaife[6] suggests that the nitroso-derivatives of β- and ε-tocopherols may also run together on columns of zinc carbonate as she found only one non-α-tocopherol in wheat germ oil, although it is now known that ε-tocopherol forms up to 10% of the total tocopherols of commercial wheat germ oil depending on the quantity of branny material in the wheat germ meal before extraction.[2, 4]

The oxidation of ε-tocopherol with ferric chloride in the presence of αα'-dipyridyl

The oxidation of α-tocopherol with ferric chloride has been shown to produce α-tocopherylquinone.[9, 10, 11] The reaction is incomplete at room temperature[11, 12] unless the reduced iron

Table I

Spectrum constants of the nitrosotocopherols

Nitroso-tocopherol	Wavelength of minimum absorption mμ.	Wavelength of maximum absorption mμ.	$E^{1\%}_{1\,cm.}$ at maximum
β	358	410	43·3
γ	355	415	59·4
δ	340	405	63·5
ε	358	410	43·6

is complexed with αα′-dipyridyl[13] or potassium ferricyanide[14] as it is formed. For more than a decade the quinone was regarded as the first oxidation product stable enough to be isolated. More recently Boyer[15] has succeeded in isolating an intermediate biologically active oxidation product, α-tocopheroxide, after oxidizing α-tocopherol with ferric chloride and αα′-dipyridyl in a bath of ice and salt. He has presented evidence that the tocopheroxide possesses an epoxy group and an intact chroman oxygen bridge, and that it is a structural isomer of α-tocopheryl-quinone into which it is readily converted under mildly acidic conditions.

The reaction products given by α-, β-, γ-, δ- and ε-tocopherols, when oxidized with the Emmerie–Engel reagents under the same conditions as described previously in this series for assay purposes,[2] have been compared spectroscopically. This was done in the hope of obtaining further evidence on the identity of ε-tocopherol and on the value of the factor relating its concentration to the depth of colour produced in the assay procedure. The presence of a little paraffin in the otherwise pure samples of ε-tocopherol obtained from the partition chromatograms in the present work prevented the direct determination of the factor by weighing.

In a preliminary experiment, 6 ml. of an ethanolic solution of α-tocopherol (240 μg.) were oxidized for 1 minute at room temperature with 1 ml. of each of the Emmerie–Engel reagents, diluted with water and the reaction products extracted into *n*-hexane. It proved necessary to remove αα′-dipyridyl from the hexane phase, by washing with a dilute aqueous solution of ferrous sulphate, before it was possible to examine the oxidation products in the spectrophotometer against a reference blank prepared in the same way. The spectrum then obtained was that of α-tocopheroxide and not that of α-tocopherylquinone. The latter was produced, however, by transferring the tocopheroxide into acidified 95% ethanol, keeping for half an hour before diluting and re-extracting into hexane.[15] The following procedure was used to compare the behaviour of ε-tocopherol with that of the previously known tocopherols.

Method

Reagents: Ferric chloride (hydrated), A.R., 0·2% in absolute ethanol.
αα′-Dipyridyl, 0·5% in absolute ethanol.
Ethanol, absolute, redistilled from potassium hydroxide and potassium permanganate.
Ferrous sulphate, A.R., 0·5% in distilled water.
n-Hexane (special for spectroscopy B.D.H.).

In subdued artificial light, 1 ml. of αα′-dipyridyl solution was pipetted into 5 ml. of an ethanolic solution of tocopherol (200 μg.) in a stoppered 50-ml. conical flask, followed by 1 ml. of ferric chloride solution, the flask was swirled to mix and allowed to stand 1 minute from the time the ferric chloride pipette was blown out. As quickly as possible 10 ml. of water and then exactly 10 ml. of *n*-hexane were added, the flask shaken vigorously for 1 minute and the contents poured into a 50-ml. stoppered separating funnel. The timing of these additions was standardized to make the tests with the different tocopherols exactly comparable. A blank was next prepared from 5 ml. of ethanol in exactly the same way. The blank and test hexane phases were then washed successively with 10 ml. of 50% ethanol, 10 ml. of 0·5% ferrous sulphate solution, 10 ml. of 50% ethanol and finally 10 ml. of distilled water with 30 seconds' vigorous shaking of the separating funnel for each washing. After shaking the hexane solutions with a little anhydrous sodium sulphate, the absorption spectrum of the primary oxidation products was determined using the blank in the spectrophotometer reference cell.

The hexane solution containing the oxidized tocopherol was then poured from the spectrophotometer cell into a 150-ml. stoppered flask, together with that remaining in the separating funnel, and both cell and funnel rinsed with a little hexane. The combined hexane solution was evaporated to dryness under reduced pressure and 10 ml. of absolute ethanol, 0·5 ml. of water and 0·1 ml. of concentrated hydrochloric acid A.R. run into the flask, which was then stoppered, shaken and placed in an incubator at 45° for 30 minutes. The acid-ethanol was next diluted with 10 ml. of water and extracted with 10 ml. of hexane. After shaking the flask for 1 minute, the contents were poured into a clean 50-ml. separating funnel. The hexane phase was washed once with 10 ml. of water, dried with a little anhydrous sodium sulphate and examined in the spectrophotometer against the blank which had been taken through the same procedure.

Results

The absorption spectra obtained by applying these tests to ε-tocopherol and to the four homologues previously known are compared in Fig. 6.

Fig. 6A, curve (*a*), shows that α-tocopherol gave α-tocopheroxide almost quantitatively when oxidized with the Emmerie–Engel reagents under the conditions specified. $E^{1\%}_{1\,cm.}$ at 238 mμ was 286 when calculated from the weight of α-tocopherol oxidized or 276 when corrected for the change in molecular weight. The corresponding figure for purified α-tocopheroxide, as judged from the absorption spectrum published by Boyer,[15] is 267. The inflexion in curve (*a*) at 265 mμ reveals, however, that a little α-tocopherylquinone was produced either during the oxidation itself or during the washing procedure which was necessary before spectroscopy. After isomerization in acid-ethanol and re-extraction into hexane, curve (*b*) was obtained, showing the well-known bicuspid absorption peak of α-tocopherylquinone[16] with maxima at 259·5 and 268·5 mμ. From this curve $E^{1\%}_{1\,cm.}$ at 259·5 mμ was 414, based on the weight of α-tocopherol taken, or 400, if allowance was made for the uptake of one atom of oxygen per molecule of tocopherol. $E^{1\%}_{1\,cm.}$ for pure α-tocopherylquinone, calculated from the molecular extinction given by Tishler & Wendler,[16] is 419 which also agrees well with the results of Frampton *et al.*[17] Thus at least 95% of the theoretical yield of α-tocopherylquinone was obtained in our test, in spite of possible losses involved in measuring the tocopheroxide spectrum immediately following the oxidation.

β-Tocopherol also gave its tocopheroxide initially, as shown by Fig. 6B, curve (*c*), together with enough of the tocopherylquinone to cause the inflexion at 265 mμ in the otherwise typical tocopheroxide absorption spectrum. ($E^{1\%}_{1\,cm.}$ at 232 mμ = 271 based on the weight of tocopherol oxidized.) A high yield of the quinone was obtained on isomerization, over 90% of that obtained in the case of α-tocopherol assuming equal molecular extinction values. Curve (*d*) illustrates that β-tocopherylquinone has a characteristic absorption spectrum with a single peaked plateau extending from the maximum at 255 mμ to 263 mμ suggesting that the second peak in the spectrum of the α-compound is connected with the methyl group in position 7 of the chroman structure. The loss of this group in β-tocopherol also appears to involve a shift of the absorption spectra of both its oxide and quinone of 5 to 6 mμ towards the shorter wavelengths.

The instability of γ-tocopheroxide, which prevented Boyer[15] from preparing a pure sample of the compound, is reflected in the behaviour of γ-tocopherol recorded in Fig. 6C. The tocopheroxide curve (*e*) was normal and undistorted by the presence of quinone. ($E^{1\%}_{1\,cm.}$ at 231 mμ = 275 based on the weight of tocopherol oxidized.) The oxide was thus the primary product of the oxidation, but on isomerization only about 67% of the theoretical yield of γ-tocopherylquinone was obtained. The work of Boyer[15] suggests that the inflexion and higher absorption shown in the quinone curve (*f*) below 230 mμ, compared with that for α-tocopherylquinone, were due to the formation of stable extraneous products rather than to incomplete conversion of the tocopheroxide. Boyer separated a colourless fraction from a crude preparation of γ-tocopheroxide resembling in properties the non-reducing dimer he obtained by heating α-tocopheroxide which had a weak absorption maximum at 293–295 mμ. The formation of such a dimer may account for the absorption between 290 and 300 mμ in curve (*f*). More of the extraneous products and less tocopherylquinone were obtained when the γ-tocopherol oxidation products were warmed in acidified ethanol for a longer period than that standardized in the comparative test, showing that the quinone, as well as the oxide, is less stable than the corresponding compound from α-tocopherol.

The instability of the oxide and probably of the quinone is so great in the case of δ-tocopherol that the curves shown in Fig. 6D were obtained. The change from curve (*g*) to curve (*h*) after the isomerization test showed that both the tocopheroxide and the tocopherylquinone exist, although neither is a main product of the reactions. In both curves there is a distinct maximum near 295 mμ possibly due to a non-reducing dimer.

The behaviour of ε-tocopherol was strikingly similar to that of β-tocopherol as can be seen from the results illustrated in Fig. 6E. It was almost completely converted into ε-tocopheroxide by the initial oxidation but a little of the quinone was formed, producing the usual inflexion at

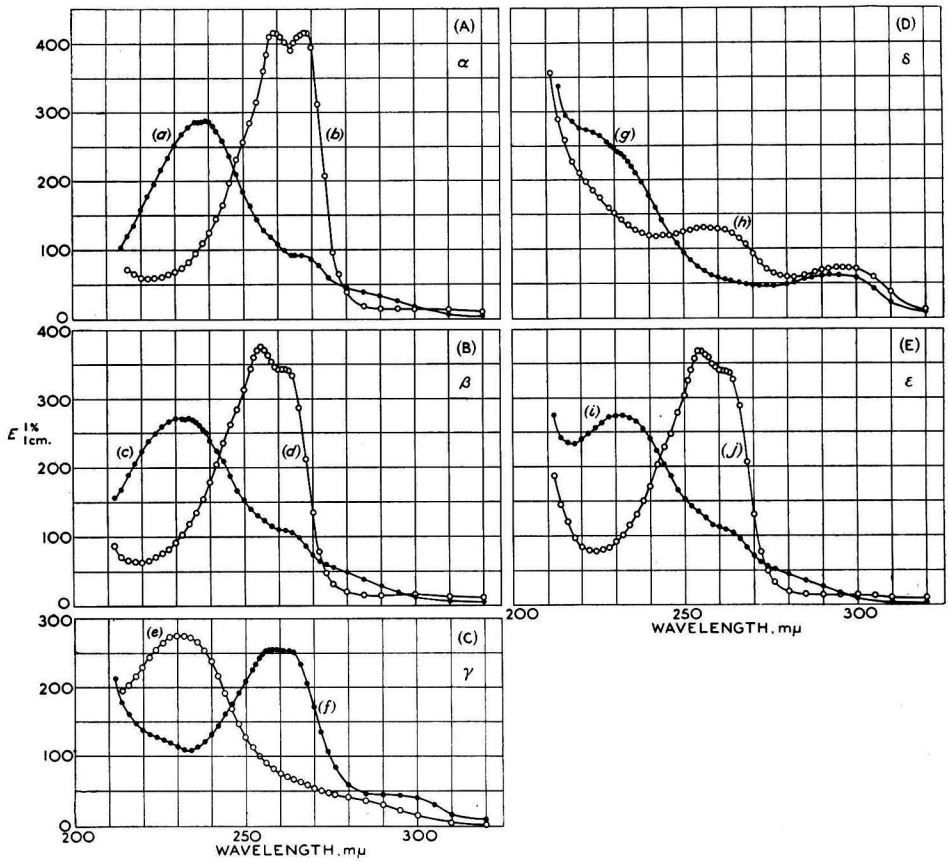

FIG. 6.—*Comparison of the absorption spectra of the products of the reactions between the individual tocopherols and ferric chloride in the presence of αα′-dipyridyl under assay conditions*

Fig. 6A α-tocopherol (200 μg. by weight)
6B β-tocopherol (,, ,, ,,)
6C γ-tocopherol (,, ,, ,,)
6D δ-tocopherol (,, ,, ,,)
6E ε-tocopherol (200 μg. by Emmerie–Engel determination)

Curves *a*, *c*, *e*, *g*, and *i*: spectra of the primary oxidation products from 200 μg. of the tocopherol extracted into 10 ml. of *n*-hexane and washed free of αα′-dipyridyl (mainly tocopheroxide except curve *g*)

Curves *b*, *d*, *f*, *h*, and *j*: spectra after isomerization in acid-ethanol and re-extraction into 10 ml. of *n*-hexane (mainly tocopherylquinone except curve *h*)

265 mμ in curve (*i*). ($E^{1\%}_{1\,cm.}$ at 232 mμ = 275 based on the weight of tocopherol oxidized, as calculated from a separate Emmerie–Engel determination.) In spite of the increase in absorption shown in curve (*i*) below 215 mμ, the proportion of extraneous products formed was relatively small as the subsequent isomerization produced a high yield of the quinone, nearly 90% of that given by α-tocopherol, and very little less than that produced by β-tocopherol. The absorption spectrum of ε-tocopherylquinone is almost a replica of that of β-tocopherylquinone in spectral position, amplitude and shape, including the characteristic single peaked plateau extending from a maximum at 255 mμ to 263 mμ. This suggests that both quinones are methylated at position 5 and unsubstituted at position 7 on the other side of the quinone oxygen group. In view of the chromatographic evidence that ε- and δ-tocopherols are isomers, the results of these experiments point directly to ε-tocopherol being 5-methyltocol.

The relative importance of substitution at the three positions available in the chroman benzene ring in determining the behaviour of a tocopherol, when oxidized with ferric chloride

in the presence of αα′-dipyridyl, has been clarified by this work. α-Tocopherol, possessing three methyl groups in the ring (positions 5, 7 and 8), is oxidized quantitatively to the tocopheroxide which may then be converted cleanly into the quinone. Only a small proportion of extraneous products arise with β-tocopherol (position 7 unsubstituted) and scarcely any more with ε-tocopherol (positions 7 and 8 unsubstituted). Thus, providing a tocopherol is methylated at position 5, it behaves very much like α-tocopherol in these reactions. There is a considerable increase in the extraneous reaction products in the case of γ-tocopherol with the critical methyl group missing, even though the 7 and 8 positions are both substituted. When the reactive positions 5 and 7 on either side of the hydroxyl group are both vacant, as in δ-tocopherol, the extraneous substances become the main products of the reactions. The similarity in behaviour between β- and ε-tocopherols shows that position 8, *meta* to the hydroxyl group, is relatively unimportant so that ζ-tocopherol[3] (5 : 7-dimethyltocol) may be expected to behave exactly like α-tocopherol in these tests and its tocopherylquinone may also possess the bicuspid absorption peak.

The results compared in Fig. 6 can also be related to the numerical factors for converting depth of colour to tocopherol concentration in the modified Emmerie–Engel determination described in an earlier paper.[2] In recent collaborative tests with the Vitamin E Panel of the Society for Analytical Chemistry[18] using this method, the factors have proved to be 96 for α- and β-tocopherols, 90 for γ-tocopherol, and 75 for δ-tocopherol. Green *et al.*[3] have also reported a factor of 96 for synthetic 5 : 7-dimethyltocol. The lower figure for γ-tocopherol shows that, weight for weight, it reduces more ferric iron and produces more colour than can be attributed to the difference in molecular weight. This is reflected in the increased instability of its tocopheroxide and quinone revealed by comparing Fig. 6C with Fig. 6A. δ-Tocopherol illustrates the relation between the factors and the test results more forcibly. In a stoicheiometric reaction, the factors for α-, β-, ζ- and ε-tocopherols would be in the ratio 96 : 93 : 93 : 90. It has been found experimentally, however, that the same factor of 96 applies to α-, β- and ζ-tocopherols when they are determined by the modified Emmerie–Engel method. The close similarity of the results shown in Figs. 6B and 6E obtained by oxidizing β- and ε-tocopherols under the assay conditions shows that the factor for ε-tocopherol is also close to 96, especially as a drop of only 6 units in the case of the γ-factor is related to the considerable distortion of the curves in Fig. 6C. The coincidence between the absorption spectra for nitroso-β- and nitroso-ε-tocopherols in Fig. 5 confirms this figure. The standard β-tocopherol solution was prepared by weighing, whereas the concentration of ε-tocopherol was determined by the Emmerie–Engel assay using the factor 96.

The reversible oxidation of tocopherol to tocopheroxide may form a redox system in nature and Boyer[15] has noted that the ease with which the various tocopherols form oxides and the stability of the oxides is roughly parallel to the respective biological activities of the tocopherols. The above results support the view that ε-tocopherol should have a biological activity approaching that of β-tocopherol.[2, 3] The order of effectiveness of the tocopherols as antioxidants *in vitro*, as judged by their ability to protect carotene, has been reported to be the exact reverse of that for biological activity,[19] and here again ε-tocopherol is likely to behave very like the β-homologue.

The oxidation of ε-tocopherol with nitric acid

(*a*) *In ethanol*

John[20] observed that tocopherols form brilliant red compounds when oxidized with nitric acid, a reaction used by Furter & Meyer[21] in their method for the determination of vitamin E. The reaction proved to be specific for coumarans and chromans which have an hydroxyl group *para* to the bridge oxygen.[22] Smith *et al.*[23] first showed that the red compounds are *o*-quinones and that they are formed from 6-hydroxychromans with the elimination of the group in position 5, providing the group is hydrogen or methyl, but not when it is another chroman ring or a benzyl group.[24] (Reference 24*b* contains a more detailed bibliography on the earlier work on the structure of the red compounds.) The solvent alcohol appears to be involved in the elimination of a methyl group since α-tocopherol forms a red quinone with nitric acid in ethanol, but not in light petroleum, acetic acid, acetone or chloroform.[23, 25] β-Tocopherol, when dissolved in

chloroform, forms a particularly intense but unstable violet colour on shaking with nitric acid, but this is not due to the *o*-quinone.[25] γ-Tocopherol, however, with the 5-position unsubstituted yields the *o*-quinone both in chloroform[25] and in acetic acid[26] under these conditions.

In the hope of obtaining further evidence on the identity of ε-tocopherol with the limited quantities available, it was decided to compare its behaviour with that of the other tocopherols when they are oxidized with nitric acid in ethanol under variations of the conditions described by Furter & Meyer[21] and in chloroform as reported by Kofler.[25] Information was also required on the effect of the presence of ε-tocopherol in samples assayed by the method of Furter & Meyer,[21] including its more recent modifications.

Methods

Reagents: Ethanol, absolute, redistilled from potassium permanganate and potassium hydroxide.
n-Hexane (special for spectroscopy).

(1) 1 ml. of concentrated nitric acid A.R. was pipetted into a 50-ml. conical flask, containing 5 ml. of ethanol and 2 mg. of dissolved tocopherol, with continual swirling to disperse the acid quickly. The flask was fitted with a small reflux condenser (ground-glass joints) and heated in boiling water with gentle shaking. The solution was boiled steadily for exactly 3 minutes and the flask allowed to cool in the dark for 15 minutes. The onset of boiling was sharply defined if a few short lengths of glass capillary were placed in the flask. The absorption spectrum of the acid solution was measured against a blank prepared from 5 ml. of ethanol in the same way (Fig. 7, curves *a*).

(2) Boiling periods of 15 and 30 seconds were also investigated, the flasks being cooled immediately by immersion in iced water (Fig. 7, curves *c*—30 seconds boiling).

(3) The absorption spectra were examined in *n*-hexane so that most of the nitric acid could be removed, permitting measurements at shorter wavelengths. 5 mg. of the tocopherol in 5 ml. of ethanol were oxidized with 1 ml. of nitric acid with 3 minutes' refluxing as before and then 10 ml. of water and 20 ml. of hexane were added immediately to the flask, which was stoppered and shaken vigorously for 1 minute. The solutions were poured into a 50-ml. separating funnel, the aqueous phase run off and the hexane phase washed once with 10 ml. of water again shaking for 1 minute. The reference blank was prepared in exactly the same way (Fig. 7, curves *b*).

β- and δ-tocopherols were also oxidized for only 30 seconds before rapidly cooling, extracting into hexane and washing (Fig. 7, curves *d*).

Results

α- and γ-tocopherols are considered to yield the same *o*-quinone (tocopherol-red) on oxidation with nitric acid with the elimination of the group in the 5-position.[23, 24] Under the Furter–Meyer[21] conditions (method 1) both produced red solutions with absorption maxima at 473 mμ (Figs. 7A and 7C, curves *a*), but when the oxidation products were transferred to *n*-hexane (method 3) orange-yellow solutions were obtained with maxima at 440 mμ in both cases (Figs. 7A and 7C, curves *b*). The spectrum-shift of 33 mμ appeared to be due to the change from a polar to a non-polar solvent, rather than to the removal of the acid, because the addition of an equal volume of ethanol to a washed hexane solution of the oxidation products again produced a red solution with a maximum at 460 mμ, a movement in the reverse direction of 20 mμ. The concentration of acid was, however, found to influence the position of the maximum. Diluting the 6 ml. of acid-ethanol solution resulting from a Furter–Meyer oxidation of α-tocopherol to 20 ml. by adding ethanol, caused the maximum to move from 473 mμ to 467 mμ. The bathochromic effect of increasing the acid concentration was noted by Smith *et al.*,[23] but attention does not appear to have been drawn to the magnitude of the changes in the absorption of the red tocopherol oxidation products with variations in the solvent.

The colour development with γ-tocopherol reached a maximum within 30 seconds on boiling with nitric acid in ethanol (Fig. 7C, curve *c*), but α-tocopherol produced only 78% of the maximum in this time (Fig. 7A, curve *c*), confirming that hydrogen is more easily eliminated from the 5-position than the methyl group.[27]

The serrations which occurred at 345, 357 and 370 mμ in the spectra shown in Fig. 7 were due to traces of ethyl nitrite. The tocopherol reduced a little of the nitric acid to nitrous acid, which then reacted with the solvent.

β-Tocopherol produced considerably less colour in the Furter–Meyer oxidation than the α- and γ-homologues, as observed by Karrer & Reutschler.[28] Curve *a*, Fig. 7B, has an apparent maximum at 450 mμ with an optical density of only 57% of that for the corresponding maximum from α-tocopherol at 473 mμ, but the rapid increase in the absorption below 400 mμ shows that extraneous reaction products distorted the curve considerably from the typical *o*-quinone shape. This distortion also appeared in the spectrum measured in hexane where the maximum occurred at 425 mμ (Fig. 7B, curve *b*). It was observed visually that the red colour reached a maximum intensity after boiling the acid-ethanol solution for about half a minute and then seemed to fade, becoming slightly more orange. This was confirmed by reducing the reaction time to 30 seconds (method 2) when curve *c*, Fig. 7B, was obtained. The maximum then occurred at 465 mμ with 78% of the colour intensity produced by α-tocopherol. The maximum in hexane was then at 437 mμ, very close to that given by both α- and γ-tocopherols (Fig. 7B, curve *d*). The absorption below 400 mμ compared with that from oxidized γ-tocopherol, as well as the lower *o*-quinone maximum, showed that extraneous reaction products contributed significantly to the absorption even with the reduced reaction time. Thus the true absorption maximum for the red quinone from β-tocopherol occurs at, or close to, the same wavelength as that for tocopherol-red. These reactions also indicated that the *o*-quinone from β-tocopherol is less stable in the hot acid-ethanol than the product from α- or γ-tocopherols owing to the lack of a methyl group in the 7-position.

δ-Tocopherol, with both reactive positions *ortho* to the hydroxyl group unsubstituted, behaved quite differently when oxidized with nitric acid. An ethanolic solution became orange-yellow immediately on adding the acid at room temperature, whereas solutions of the other tocopherols remained colourless. The orange-yellow colour persisted on boiling, the usual red pigment not being formed. After 3 minutes' oxidation (method 1) curve *a*, Fig. 7D, was obtained with an inflexion only at 450 mμ and a sharp maximum at 370 mμ ($E^{1\%}_{1\,cm.}$ 42) as has been reported previously by Stern *et al.*[19] A very similar curve Fig. 7D, *c*, was produced by a 30 minutes' oxidation ($E^{1\%}_{1\,cm.}$ at 370 mμ 45). The increased amplitude of the ethyl nitrite serrations suggested that more nitric acid was reduced, than with the other tocopherols, with greater destruction of the tocopherol molecule. The possible presence of some *o*-quinone in the reaction products was indicated by the inflexion at 450 mμ, but extraction into hexane and washing after a 30 minutes' oxidation gave a clear yellow solution with an absorption maximum at 400 mμ. The yellow colour was not due to the *o*-quinone as δ- and β-tocopherols should yield the same product with elimination of the group in the 5-position, and it has been noted already that the β-product had an absorption maximum at 437 mμ in hexane under the same conditions. Moreover, a typical *o*-quinone spectrum with a maximum at 475 mμ is obtained by oxidizing δ-tocopherol dissolved in chloroform (see below).

The hexane curve suggested that some separation of the mixed oxidation products was achieved by partition between aqueous ethanol and hexane. Hence 2 mg. of δ-tocopherol were oxidized in ethanol for 30 seconds, the reaction products were extracted into hexane as in method (3), the hexane phase was washed repeatedly with water and then evaporated to dryness under a stream of nitrogen. The residue, an orange-red oil, was dissolved in 6 ml. of absolute ethanol and the absorption spectrum measured against a blank prepared in the same way. Curve *e*, Fig. 7D, was obtained, revealing that about half the products responsible for the absorption at 370 mμ had been removed with virtually no change in the absorption at 450 mμ, so confirming that the oxidation gave a mixture of products.

The different behaviour of δ-tocopherol, compared with its homologues, was not due to greater instability of its *o*-quinone because the same compound is given by β-tocopherol, but rather to the increased reactivity of the molecule permitting another reaction pathway.

Oxidation of ε-tocopherol by boiling in ethanol with nitric acid for 30 seconds (method 2) produced a red *o*-quinone as can be seen from Fig. 7E, curve *c*. This curve (maximum 465 mμ, $E^{1\%}_{1\,cm.}$ 15·3) is almost a replica of that obtained from β-tocopherol with the same reaction conditions, Fig. 7B, curve *c* (maximum 465 mμ, $E^{1\%}_{1\,cm.}$ 15·8). ε-Tocopherol produced an oxidation spectrum with slightly more extraneous absorption below 400 mμ, but the reactions clearly

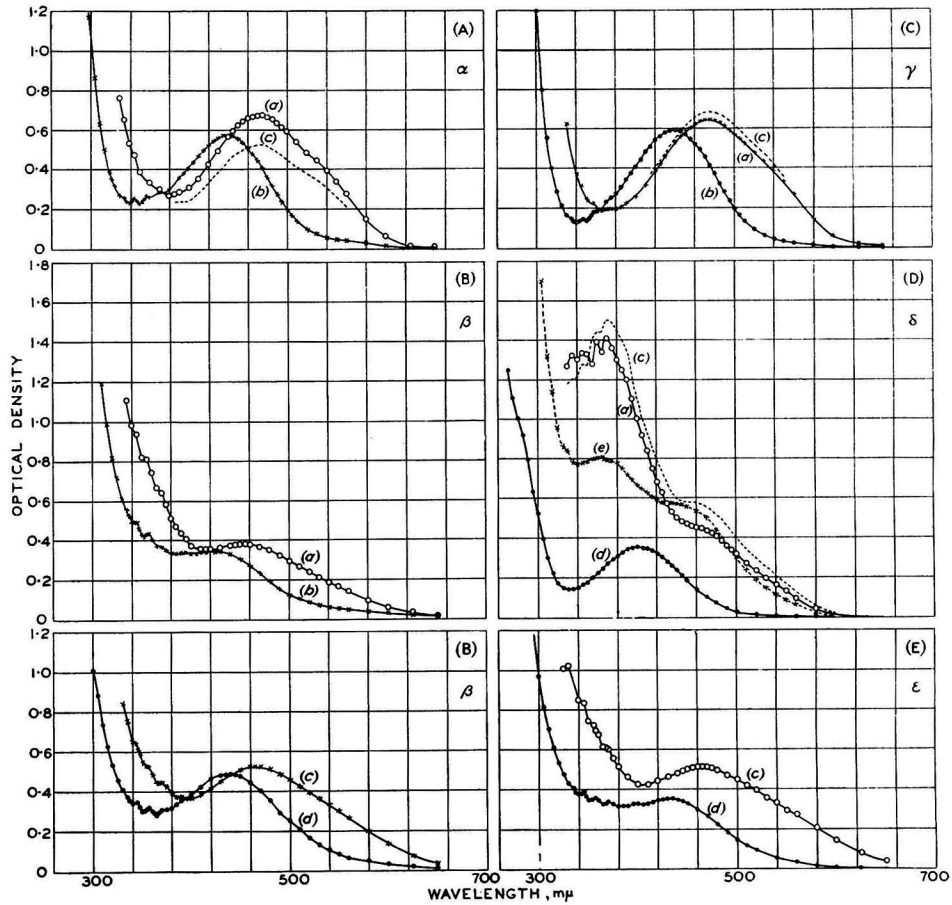

FIG. 7.—*A spectroscopic comparison of the products obtained by oxidizing the tocopherols individually with nitric acid in ethanol under standardised conditions*

Figs. 7A, B, C, D and E from α-, β-, γ-, δ- and ε-tocopherols, respectively
Curves (a) 2 mg. of tocopherol in 5 ml. of ethanol refluxed for 3 min. with 1 ml. of nitric acid. Spectrum measured directly against a blank prepared in the same way
Curves (b) 5 mg. of tocopherol in 5 ml. of ethanol refluxed for 3 min. with 1 ml. of nitric acid, diluted and extracted with 20 ml. of *n*-hexane. The hexane phase washed and examined against a matching blank
Curves (c) As with (*a*) but with refluxing for 30 sec. and rapid cooling
Curves (d) As with (*b*) but with refluxing for 30 sec. and rapid cooling before extraction into hexane
Curve (e) Fig. 7D only. 2 mg. of δ-tocopherol oxidized in ethanol for 30 sec., diluted and extracted into hexane. The latter washed repeatedly, evaporated to dryness, the residue dissolved in 6 ml. of ethanol and the spectrum measured against a matching blank

followed a very similar course. In view of the behaviour of the other tocopherols of known structure, this provides additional evidence that β- and ε-tocopherols are both methylated at the 5-position and unsubstituted at the 7-position. As with the milder oxidation with ferric chloride, a change from a methyl group to hydrogen in the unreactive 8-position *meta* to the hydroxyl group appears to have little effect on the course of the reaction with nitric acid.

Curve *d*, Fig. 7B, was prepared with 5 mg. of β-tocopherol as described in method (3) with 30 seconds' oxidation, the reaction products being extracted into 20 ml. of hexane, whereas curves *c* and *d*, Fig. 7E, were prepared from the same 2 mg. sample of ε-tocopherol in order to conserve supplies of the new compound. Fig. 7E, curve *c*, was obtained first exactly as described in method (2) and, after measuring the spectrum, the ethanolic solution was recombined, diluted, extracted with 8 ml. of *n*-hexane and washed before examining the absorption spectrum

in hexane against the blank treated similarly. Thus Fig. 7E, curve *d*, is not strictly comparable with curve *d* for β-tocopherol. The former is the only curve reproduced in Fig. 7 which was not prepared according to one of the strictly standardized methods given above, but it shows that the absorption maximum of the oxidation products of ε-tocopherol in hexane is near that for β-tocopherol at 437 mμ.

The experiments with nitric acid have indicated that total-tocopherol figures cannot be obtained with mixtures of tocopherols using the Furter–Meyer assay, or other methods depending on the formation of the red quinones, owing to the wide variations in the reactions of the individual tocopherols. The curves also show that it is not possible to determine δ-tocopherol, in the presence of other tocopherols, by making use of its oxidation maximum at 370 mμ as suggested by Stern *et al.*[19]

(*b*) *In chloroform*

Method.—Ten ml. of chloroform A.R. containing 2 mg. of tocopherol were placed in a stoppered 25-ml. measuring cylinder and 1 ml. of nitric acid A.R. blown in from a bacteriological pipette starting a stop-watch at this instant. After a 5-second pause, the cylinder was shaken vigorously for 10 seconds and the contents poured quickly into a 50-ml. separating funnel. After a few seconds to allow the phases to separate, but before the sudden evolution of brown fumes, most of the chloroform phase was run off into a 50-ml. stoppered conical flask. The turbid solution was cleared by shaking with a little anhydrous sodium sulphate and its absorption measured against a chloroform blank prepared in an identical manner.

Blank and test solutions were not always exactly matched with regard to dissolved oxides of nitrogen but the mismatch distorted the absorption spectra only at wavelengths below 400 mμ.

Results

The absorption spectra obtained by applying this method to the individual tocopherols are given in Fig. 8.

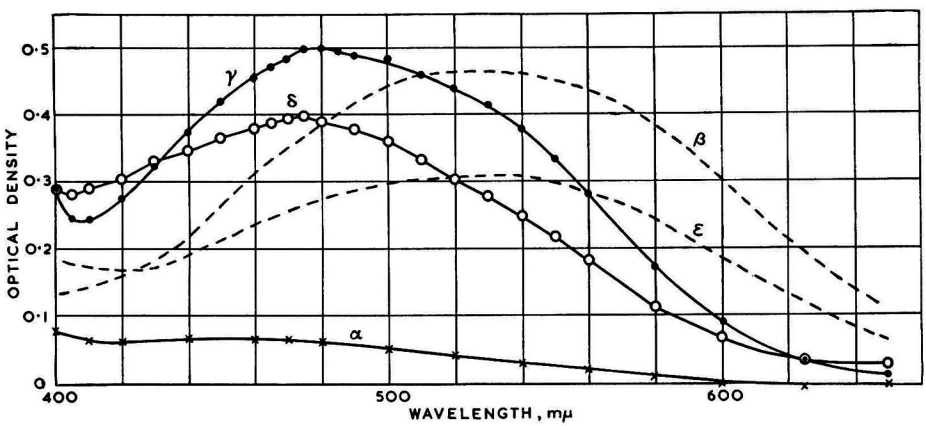

FIG. 8.—*Absorption spectra illustrating the behaviour of the tocopherols in chloroform when shaken with nitric acid*

2 mg. of tocopherol in 10 ml. of chloroform shaken for 10 sec. with 1 ml. of concentrated nitric acid and the spectrum of the chloroform phase measured against a blank prepared in like manner.

α-Tocopherol produced only a very faintly yellow solution whereas γ-tocopherol gave a stable red pigment with an absorption spectrum typical of the *o*-quinone which they both yield in ethanol (maximum 480 mμ). Thus under Kofler's conditions a methyl group in the 5-position is not eliminated.

In contrast to its behaviour in ethanol, δ-tocopherol also gave a stable red solution of its *o*-quinone with an absorption maximum at 475 mμ.

β-Tocopherol behaved very differently. Immediately the nitric acid was added, an intense violet colour was formed. This faded in the course of a few minutes until the solution was almost colourless, with an absorption spectrum coincident with that given by α-tocopherol in the test. By examining a solution in the spectrophotometer immediately after oxidation, taking rapid readings beginning at 650 mμ, the dotted curve in Fig. 8 was obtained. This cannot be regarded as the absorption spectrum of the unstable colour as there was a 20% loss in intensity in the time necessary to take six quick readings with the instrument.

Kofler[25] recognized that the behaviour of β-tocopherol was different from that of the other tocopherols. He thought that the unstable violet colour involved a coloured salt as he found the colour faded if the chloroform solution was shaken with water but was regenerated by shaking with nitric acid or 85% sulphuric acid. Clearly *o*-quinone is not formed and the rule holds good that a methyl group in the 5-position of the tocopherol molecule is not eliminated in this test.

ε-Tocopherol also produced the brilliant unstable violet colour, as shown in Fig. 8 where the dotted curve was obtained in the same way as with β-tocopherol.

It appears that two conditions are necessary for the production of this remarkable colour: (1) the 5-position must be substituted to block the formation of the *o*-quinone; (2) the 7-position, on the other side of the hydroxyl group, must be unsubstituted or the reaction is hindered. In the homologous series of methyltocols these conditions hold only with β-tocopherol and with 5-methyltocol. The reaction thus provides striking evidence as to the identity of ε-tocopherol.

To date, 7-methyltocol remains undiscovered in nature. It would not be separable from ε-tocopherol by either partition or absorption chromatography, but tests, such as coupling with diazonium salts, have failed to detect it in samples of ε-tocopherol. The Kofler oxidations provide additional evidence for its absence. 7-Methyltocol, like γ-tocopherol, would form a stable red quinone with nitric acid in chloroform. The violet oxidized solution of ε-tocopherol fades to an almost colourless solution, however, which then has an absorption spectrum coincident with that given by α-tocopherol in the test.

The methylation of ε-tocopherol

Several methods have appeared in the patent literature for converting γ- and δ-tocopherols into the more valuable α-compound. That described by Weisler & Chechak[29] has been adapted for use on a micro-scale and applied to ε-tocopherol. The method involves chloromethylation with formaldehyde and hydrochloric acid and simultaneous reduction with stannous chloride.

Method

Reagents: (1) Ethyl ether, peroxide-free.
(2) Solution of formaldehyde, B.P. (40%).
(3) Stannous chloride, A.R. (10 g.) dissolved in concentrated hydrochloric acid, A.R. (50 ml.).

About 5 mg. of tocopherol were dissolved in 5 ml. of peroxide-free ethyl ether in a 50-ml. conical flask and 0·1 ml. of 40% formaldehyde and 1·3 ml. of the hydrochloric acid–stannous chloride reagent added. The flask was stoppered and shaken mechanically for 2 hours in the dark. The mixture readily formed a labile, turbid emulsion which quickly clarified, with separation of the phases, when the shaking stopped. It was then diluted with more peroxide-free ether, washed repeatedly with water in a separating funnel, and the ether phase evaporated to dryness under a stream of nitrogen. The residue was dissolved in 5 ml. of peroxide-free ether and the reaction products examined chromatographically using the two-dimensional technique of Green *et al.*[3] Other reaction solvents, such as hexane, were tested, but ethyl ether proved to be the most satisfactory.

The chromatography was conducted exactly as described in detail by Green *et al.*,[3] except that *cyclo*hexane containing 0·5% ethanol by volume was found to give better separation, when used for development in the zinc carbonate-adsorption direction, than pure *cyclo*hexane.

Results

When this procedure was applied to δ-tocopherol a dense spot appeared in the α-tocopherol position on the chromatogram of the reaction products and a less intense spot in the γ-tocopherol position. Some unreacted δ-tocopherol was also present together with unidentified products

which produced spots well away from the tocopherol positions. β-Tocopherol was also converted into α-tocopherol as observed by Weisler & Chechak.[29]

A control chromatogram of the sample of ε-tocopherol used in these tests gave a single spot in the correct position (Fig. 9A). On the reaction chromatogram, a definite spot appeared in

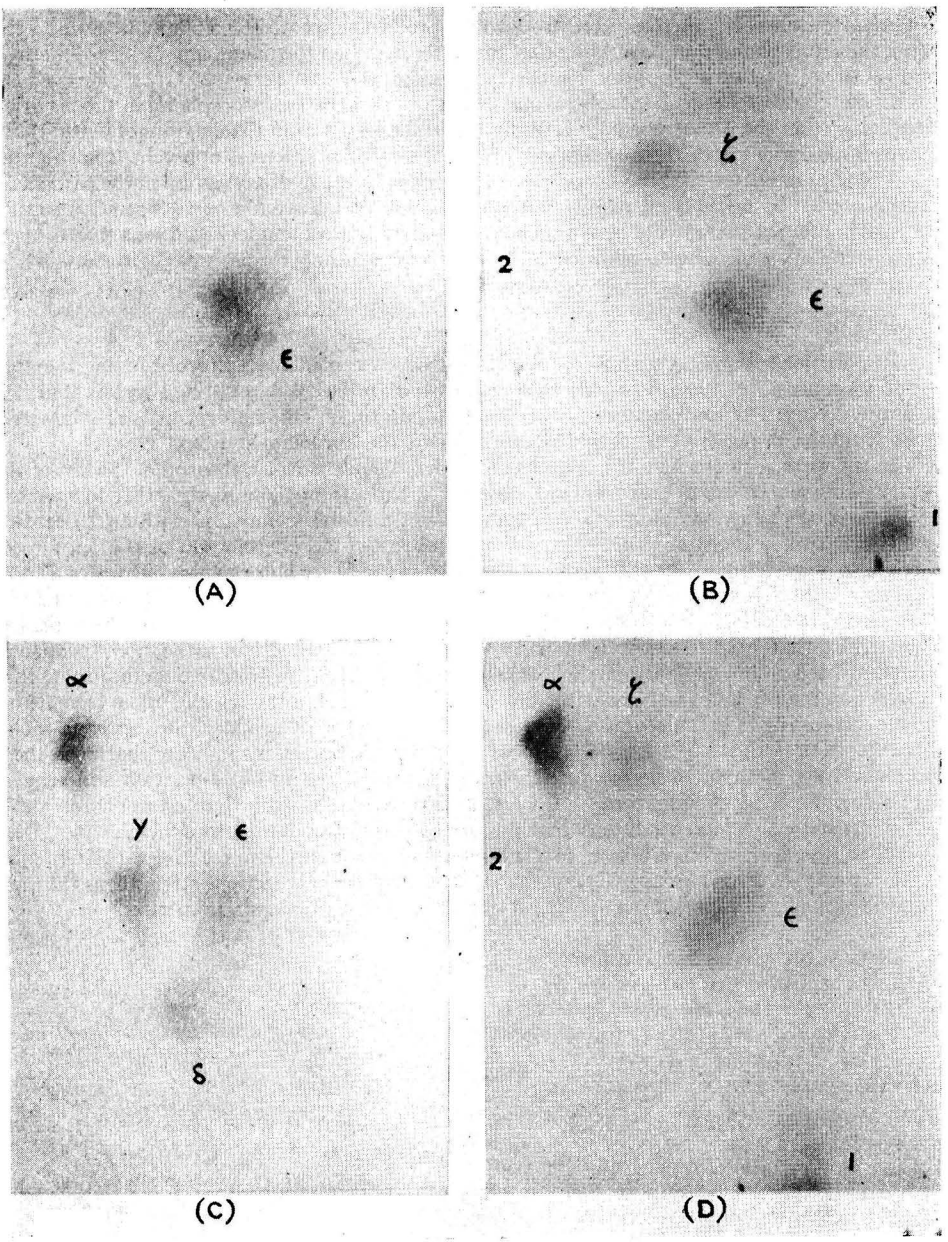

FIG. 9.—*Chromatograms showing the formation of* 5 : 7-*dimethyltocol* (ζ-*tocopherol*) *on methylating* ε-*tocopherol*

exactly the position allocated to 5 : 7-dimethyltocol by Green *et al.*[3] using the synthetic product, together with spots due to unreacted ε-tocopherol and to two unidentified substances (Fig. 9B). The latter were both near the edges of the chromatogram, (1) moving very little in the zinc carbonate-adsorption direction, but rapidly, almost with the solvent front, in the liquid paraffin direction, whereas (2) had an R_F value of about 0·5 on zinc carbonate paper but remained on the origin in the paraffin direction.

An α-tocopherol marker added to the reaction products appeared on a chromatogram to the left of the ζ-tocopherol spot confirming that the latter occupied the unambiguous 5 : 7-dimethyltocol position, as can be seen by comparing Figs. 9C and 9D.

In our chromatograms δ-tocopherol moved slightly slower than ε-tocopherol in the partition direction so that the δ-spot was not vertically below the ε-spot as in the diagram of Green *et al.*[3] Complete agreement with these authors regarding the other positions is apparent from Fig. 9C.

The tests indicated that the 5- and 7-positions, *ortho* to the hydroxyl group in the tocopherol molecule, may be methylated readily under conditions that leave the *meta* 8-position unsubstituted. The fact that ε-tocopherol produces 5 : 7-dimethyltocol under conditions that convert β-tocopherol to α-tocopherol confirms the identity of ε-tocopherol as 5-methyltocol.

General discussion

In partition chromatograms ε-tocopherol behaves as a monomethyltocol running together with δ-tocopherol,[1, 2, 4] and its failure to couple with diazotized *o*-dianisidine suggests that it is 5-methyltocol.[1] The movement of ε-tocopherol on adsorption chromatograms is also in agreement with the presence of a methyl group *ortho* to the chroman hydroxyl group.[3]

In the work reported here it has been shown that the ultra-violet absorption spectra of the β- and ε-compounds are almost identical, differing slightly from those of the other tocopherols in the position of the maximum. ε-Tocopherol forms a nitroso-compound with an absorption spectrum virtually indistinguishable from that of nitroso-β-tocopherol. On oxidation, ε-tocopherol also behaves more like β-tocopherol than any of the other homologues. It forms a tocopheroxide with the Emmerie–Engel reagents and this may be converted into the isomeric ε-tocopherylquinone. The latter has an absorption spectrum with a characteristic single peaked plateau like that of β-tocopherylquinone. With nitric acid in ethanol the new tocopherol produces a red *o*-quinone and some unidentified products, and spectroscopic studies show that the reaction follows a course similar to that with β-tocopherol, although the other tocopherols behave differently. In chloroform, only β- and ε-tocopherols produce the brilliant violet unstable colour with nitric acid. All of these properties and reactions indicate that both these tocopherols are methylated in the 5-position and unsubstituted in the 7-position and suggest that ε-tocopherol is 5-methyltocol. This is confirmed by the fact that ε-tocopherol yields 5 : 7-dimethyltocol under conditions that convert β-tocopherol into α-tocopherol.

ε-Tocopherol therefore differs from the β-homologue only in the unreactive 8-position *meta* to the chroman hydroxyl group. The similarity in their chemical reactions strengthens the view that they should possess similar biological activities and antioxidant properties.

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AMMONIA LIQUOR AS A NITROGENOUS FERTILIZER

By H. TOD and K. SIMPSON

On grassland, raw ammonia liquor from gasworks (0·75–2·30% N) may be a useful source of nitrogen. In a large series of experiments it was not so effective as ammonium sulphate. Precautions must be taken to avoid scorching the grass with the liquor, as such scorch has very serious effects. Experiments with concentrated ammonia liquor (about 12% N) on potatoes and swedes suggest that this material may have toxic effects.

Introduction

This investigation was begun in response to a request from the Scottish Gas Board to explore the possibility of using raw ammoniacal gas liquor as a nitrogenous fertilizer. The work has a twofold origin—the loss of valuable nitrogenous material (the volume in Scotland being around 50 million gallons per annum, equivalent to approximately 10,000 tons of ammonium sulphate), and the problem of disposal of a waste product which was becoming an embarrassment in certain areas.

The results of the first season's work in 1953 and the historical background to the use of this liquor in agriculture have been published elsewhere.[1] In that season, ammonia liquor appeared to be almost as effective as ammonium sulphate applied at an equivalent rate on grass, potatoes and swedes. In view of these results and the fact that, at that time, the commercial application was almost entirely to grass, it was decided to confine work in 1954 to this crop alone. Some trials

had been made elsewhere using a concentrated gas liquor of about ten times the nitrogen content of the raw material on row crops. This work was followed up in 1955 on potatoes and swedes using special cultivator tines to inject the material into the soil.

Experimental

Grassland (Raw liquor)

In 1954 the programme consisted of a comprehensive experiment on grassland with the following objects:

(*a*) the value as a nitrogenous fertilizer of ammonia liquor from four different sources, compared with ammonium sulphate, as to the yield of fresh grass, dry matter and protein;
(*b*) observation of scorch after application of liquor;
(*c*) comparison of the materials at four different centres, Armadale (I), Haddington (II), Hawick (III) and Peebles (IV);
(*d*) comparison of the materials on different types of grassland.

The design of the experiment was 48 randomized blocks each of 6 plots, consisting of 12 blocks at each of the four centres. Two blocks of six plots each were laid down on different fields within reasonable distance of the local gasworks. The treatments per acre were as follows: A—control, no nitrogen; B—56 lb. of nitrogen as ammonium sulphate; C and D, 28 and 84 lb. of nitrogen as ammonia liquor *Type A*;* E and F, 28 and 84 lb. of nitrogen as ammonia liquor *Type B*.†

For all grassland experiments, a basal dressing of 3 cwt. of superphosphate and 2 cwt. of potassium chloride per acre was applied.

The N concentration of the liquors used varied considerably from centre to centre, from 0·75% nitrogen in the Haddington liquor to 2·30% in the Armadale liquor. This meant a three-fold variation in the volume applied. The liquor was applied from watering cans with roses in the 1954 experiments. Ammonium sulphate and the basal dressings of phosphate and potassium were broadcast by hand.

The treatments were applied in late April or early May. At all centres except III the weather was cold, dry and sunny. Visual observations within a week of application of the liquor showed that serious scorch had taken place in many cases. There was little or no scorch from ammonium sulphate treatments. Average visual scores for severity of scorch are shown in Table I where '4' represents extremely severe scorch and '0' no effect.

Table I

Severity of scorch on grass (1954) caused by fertilizer

Centre	Treatment					
	A Without N	B With ammonium sulphate	C Type A ammonia liquor	D Type A ammonia liquor	E Type B ammonia liquor	F Type B ammonia liquor
N supplied, lb./acre	0	56	28	84	28	84
I	0	0	0·4	2·3	0·3	2·2
II	0	0·1	1·0	2·8	1·0	3·0
III	0	0·5	0·2	2·0	0	0·1
IV	0	0	0·7	3·7	1·5	3·0
Average	0	0·15	0·6	2·7	0·7	2·1

* *Type A* was the liquor produced at the local gasworks.
† *Type B* was as follows: at Haddington, Armadale liquor; at Armadale, Haddington liquor; at Peebles, Hawick liquor; at Hawick, Peebles liquor.

Possibly as a result of the dull, showery conditions prevailing at centre III, the scorch was much less than at other centres, where it was more serious than anything that had been experienced in 1953. Most of the severely scorched grasses recovered satisfactorily, but some of the clover was seriously checked. Some plots which had been severely scorched made remarkable recoveries and their final yield was satisfactory.

Four blocks of plots at centre II had to be abandoned because they were grazed after treatment. All other plots were successfully harvested during late June and early July. Table II gives the average results.

Table II

Yield of dry matter, uptake of nitrogen and apparent % recovery of nitrogen from fertilizers in grassland, 1954

Yield of dry matter, cwt./acre

Centre	Treatment						S.E.
	A	B	C	D	E	F	
	Without N	With ammonium sulphate	Type A ammonia liquor		Type B ammonia liquor		
N supplied, lb./acre	0	56	28	84	28	84	
I	35·9	54·9	42·9	46·7	44·3	48·2	± 2·74
II	62·3	80·9	57·4	65·4	63·3	57·5	± 3·61
III	30·3	36·3	33·2	34·8	32·6	35·0	± 1·29
IV	40·6	53·6	36·4	44·0	39·1	39·3	± 1·68
Mean	42·3	56·4	42·5	47·7	44·8	45·0	
			Uptake of N in lb./acre				
I	39·3	66·5	46·9	62·5	51·2	56·7	
II	65·8	102·4	60·8	80·0	67·6	67·4	
III	53·4	59·8	54·9	56·8	52·3	59·0	
IV	52·7	77·3	47·9	70·6	53·8	59·6	
Mean	52·8	76·5	52·6	67·5	56·2	60·7	
			Apparent % recovery of N from fertilizers				
I	—	48·6	27·1	27·6	42·5	20·7	
II	—	65·4	neg.	16·9	6·4	1·9	
III	—	9·6	0·5	4·0	neg.	5·5	
IV	—	43·9	neg.	21·3	3·9	8·2	
Mean	—	42·3	0(?)	17·5	12·1(?)	9·4	

The generally low standard errors indicate that the technique of splitting randomized blocks over a large number of loci—in this case twenty-four—is a highly satisfactory one. It is possible, by this means, to combine the ' farm demonstration ' with good experimental designs.

An attempt, which was seriously upset by the continuous wet weather, was made at two sites (one at centre II and one at IV) to estimate residual effects of these fertilizers, if any. On the centre IV plots, the apparent residual recovery of nitrogen from all fertilizers was negative, possibly due to the high proportion of clover on the control plots which gave rise to a very high percentage of nitrogen in the dry matter (2·42). At centre II the Type A liquor gave satisfactory residual recoveries, while the ammonium sulphate, most of which had been removed by the first cut, and the Type B liquor gave much lower uptakes.

To overcome the setbacks due to scorch, a method was devised during the season of applying the liquor treatments from jets, seven inches apart, following the local commercial practice. This system was tried out in a late season experiment at centre IV and gave encouraging results. Little or no scorch was observed within one week after the application of treatments. The average percentage recoveries from 42 lb. of nitrogen per acre as ammonia liquor, ' nitro-chalk ' and ammonium sulphate were 15·8, 2·4 and 9·7 respectively.

This method, the application of the liquor from jets, was employed in the 1955 experiments on grassland.

Two experiments were carried out at the same farms at Haddington as in 1954. The design, in each case, was four randomized blocks each of 6 plots. Treatments per acre were as follows: A—no nitrogen; B—56 lb. of nitrogen as ammonium sulphate; C and D, 28 and 84 lb. of nitrogen as ammonia liquor; E and F, 28 and 84 lb. of nitrogen as 'nitro-chalk'. Visual observations showed moderate scorch on the liquor plots at site I but little at site II. The nitrogen content of the ammonia liquor was 0·78% and this necessitated the use of a large volume for each plot. At both sites, it was necessary to run over each plot twice with the jet apparatus to distribute the liquor adequately, but at site I the surface of the field was so rough as to make uniform distribution of the liquor impossible. It was noticeable that the plots which had received ammonia liquor, whether or not scorch had previously occurred, showed a deep blue green colour when compared with other treatments, while the control plots showed very thin, pale growth. At site II considerable scorch was observed on the plots treated with ammonium sulphate.

The plots were harvested, nine weeks after the application of fertilizers, in the last days of June. A second cut was taken at site II during the third week in September, but at site I, the grass was so short that it was not practicable to do so.

The results are summarized in Table III.

Table III

Yield of dry matter, uptake of nitrogen and apparent % recovery of nitrogen from fertilizers in grassland, 1955

	Treatment						S.E.
	A Without N	B With ammonium sulphate	C With ammonia liquor	D	E With 'nitro-chalk'	F	
Site I							
N supplied, lb./acre	0	56	28	84	28	84	
Wt. of dry matter (lb./acre)	3015	3705	2839	2686	3405	3289	± 150
Uptake of N (lb./acre)	20·2	40·5	24·3	29·2	28·5	39·3	± 2·2
Apparent % recovery	—	36·3	14·8	10·7	29·6	22·7	
Site II							
Wt. of dry matter (lb./acre)							
First cut	5145	4818	5326	4717	4883	5200	± 238
Second cut	494	721	602	852	558	753	36
Uptake of N (lb./acre)							
First cut	40·2	48·3	46·6	58·2	50·2	64·3	± 5
Second cut	4·4	7·3	5·5	7·7	4·8	7·0	0·4
Apparent % recovery (2 cwt.)	—	19·7	26·8	25·4	37·1	31·8	

Potatoes and swedes (concentrated liquor)

In 1955, concentrated ammonia liquor, with a total nitrogen content of 12·7%, was used for these row crops. This material was injected into the soil, at a depth of about four inches, using cultivator tines with jets at the back. A uniform design (4 randomized blocks, each of 5 plots) was adopted for three experiments with potatoes and one with swedes. The plots were four drills 27 inches wide and 15 yards long, so that fairly accurate distribution was possible.

The treatments per acre for potatoes were as follows: A—control, no nitrogen; B and C, 56 and 112 lb. of N as ammonia liquor; D and E, 56 lb. and 112 lb. of N as ammonium sulphate. For swedes all treatments were halved. The basal treatments for potatoes and swedes, respectively, were 5 cwt. and 4 cwt. superphosphate, 2 cwt. and 1·6 cwt. of potassium chloride. The above treatments were applied for both swedes and potatoes during the last week in April. Potatoes were lifted in the third week of September and swedes in the second week of November.

The results are shown in Table IV.

Table IV

Effect of fertilizer treatment on yield of potatoes and swedes—1955

Potatoes (average of 3 experiments)

Treatment	A Without N	B With ammonia liquor	C	D With ammonium sulphate	E	S.E.
N supplied, lb./acre	0	56	112	56	112	
Yield in cwt./acre	117·0	116·1	99·3	127·8	121·8	± 6·58

Swedes (1 experiment)

Treatment	A Without N	B With ammonia liquor	C	D With ammonium sulphate	E	S.E.
N supplied, lb./acre	0	28	56	28	56	
Yield of roots cwt./acre	258·5	294·5	270·5	277·7	300·1	± 8·38
% recovery of N	—	61·0	36·1	60·6	37·1	

Discussion

The most notable feature of the 1954 grassland experiments was the overall superiority of ammonium sulphate. This was most marked at centres I and II—from 10 to 20 cwt. of dry matter per acre increase being obtained. In one case, the low dressing of local ammonia liquor (C) at centre IV, there was a significant decrease in yield from that of the control plots. The scorch on the C plots at this centre was not severe and it is necessary to seek another explanation, e.g., toxic materials in the liquor. The only centre at which the ammonia liquor gave generally superior yields to the control plots was centre I. A comparison of the effects of the four different liquors, applied at an average rate of 56 lb. of N per acre, showed no significant differences in yield of dry matter per acre. Likewise it was not possible to detect any differences in the responses of the different types of grassland to added nitrogen, except at centre III. Here the recovery of nitrogen was extremely low, and this may be due to the use of long leas rich in clover, as well as to the fact that the grass is grazed by sheep for a period in the spring before being allowed to grow away. The low uptake of nitrogen from the control plots at centre I gives the explanation for the large increases in crop due to all forms of nitrogen applied.

For all centres, the mean production of crude protein in lb./acre from treatments was: ammonium sulphate, 148; ammonia liquor at 28 lb. of N/acre, 10; ammonia liquor at 84 lb. of N/acre, 71. The percentage recovery of nitrogen from nitrogen from fertilizers was of the same order for ammonium sulphate in 1954 as in earlier experiments in 1953, but the recovery from ammonia liquor was much lower and a number of instances were recorded of ' negative uptakes '.

The overall spraying method of application of ammonia liquor has, because of the severe scorch produced, contributed greatly to the low response of grass to this material. This finding is supported by the results obtained in 1955. During this season, scorch by ammonia liquor at site I and by ammonium sulphate at site II appears to have been responsible for decreases in the yield of dry matter. In both of these cases, however, the nitrogen content of the grass was sufficiently high to counteract this effect and at site II the plots with ammonium sulphate recovered sufficiently to give as good a yield as other treatments in the second cut.

The average crude protein production in lb., for the two 1955 experiments, from 56 lb. of nitrogen per acre in three different forms was: ammonia liquor, 68; ammonium sulphate, 103; ' nitro-chalk ', 103.

The potato and swede experiments with concentrated ammonia liquor in 1955 were rather inconclusive. In this very dry season, responses to nitrogen in any form were generally poor. The ammonium sulphate treatments did not produce significant crop increases in potatoes and barley did so in the longer growing swede crop. The higher dressing of ammonia liquor (C) produced a significant depression in the yield of potatoes and the swede yield produced by treatment C (56 lb. of N per acre supplied as ammonia liquor), was lower than that by treatment B (28 lb. of N per acre of the same material). These facts strongly suggest that toxic materials in the liquor are having an effect, presumably on the soil, as the plant population was not altered.

The uptake of nitrogen by swedes, however, is virtually identical from ammonia liquor and

ammonium sulphate plots, so that the depression in total yield caused by treatment C is not reflected in the amount of crude protein per acre produced by this treatment.

Summary

Ammonia liquor has been compared with ammonium sulphate and 'nitro-chalk' as a nitrogenous fertilizer for grassland, potatoes and swedes, during three seasons, 1953–55. The results of the 1953 work are reported elsewhere.[1]

The following tentative conclusions may be drawn:

(1) Ammonia liquor has not generally been so effective as ammonium sulphate or 'nitro-chalk', although in 1953 the results were more promising than in the other two seasons.

(2) Scorching of herbage, by ammonia liquor, appears to be the dominant factor in the comparatively poor results obtained from this material. The importance of this factor was emphasized by the fact that where scorch occurred following the use of ammonium sulphate a similar decrease in yield was obtained.

(3) Scorch can be largely overcome by the use of jets at 6-9-inch intervals instead of spray nozzles.

(4) In the case of the *concentrated* liquor used in 1955 it is likely that some toxic material contained in the liquor was responsible for the depression of yield of potatoes obtained. In 1953, raw liquor proved satisfactory with this crop,[1] this depression not being shown.

In 1954, at one centre, there was some evidence of toxic effects from the raw liquor from one source.

(5) If the raw liquor is made available cheaply, it represents a useful source of fertilizer nitrogen, provided that full precautions are taken to minimize the effects of scorch.

Acknowledgments

The authors wish to express their thanks to Mr. David Elgin of the Scottish Gas Board for his unfailing help and the very full co-operation of his colleagues, and to Messrs. Thos. Ward & Co., Sheffield, for supplying the four special coulters used with the concentrated liquor. Part of the expenses of the investigation were met by a generous grant from the Scottish Gas Board (South Eastern Area). Thanks are also due to Dr. Smith for his interest and guidance and to all members of the Chemistry Department who helped so much both in the field and laboratory.

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Reference

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ERRATA

The following amendments should be made in the papers entitled 'Studies on the Effects of Treatment with Chlorine Dioxide on the Properties of Wheat Flour' in *J. Sci. Fd Agric.*, 1956, **7**, May issue:

Part I, p. 362, line 20 should read 'Flour 3 was prepared . . . of chlorine dioxide per sack.'

Part III, p. 376. The formula for relative testes weight should read

$$\text{'}\frac{\text{Wet weight of testes in g.}}{\text{Wet weight of animal in g.}} \times 1000\text{'}$$

p. 377, Table III. *For* 'α-tocopherols' *read* 'α-tocopherol.'

J. Sci. Fd Agric., 1956, **7**, Suppl. Issue, on p. S116 line 17–18 this sentence should read: 'No one could possibly survive the quantity of alcohol involved in drinking enough beer to contain a quantity of dimefox that would produce dangerous results.'

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1956

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

INDEX OF AUTHORS' NAMES

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1956

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Characteristics of some forest soils from the grey-brown podsolic–podsol transition zone in Northeastern Minnesota. R. F. Holt and P. R. McMiller (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 84—87).—In the transition zone there was an intermingling of podsols, brown podsolic, grey-brown podsolic and grey wooded soils. Influence of climatic and biotic factors on an interlacing of parent materials from several different glacial drifts has given rise to some rather distinctive profile differentiations in this zone. A. H. CORNFIELD.

Internal standards in the excitation of soil and plant ash. A. C. Oertel (*Aust. J. appl. Sci.*, 1955, **6**, 467—475).—A theoretical study of the increase in precision of measurement of line intensity obtainable by the use of an internal standard has shown that an internal standard is of real value only when the coeff. of correlation of the line pair is $\pm 0{\cdot}8$ and when the individual errors of the two lines are approx. equal. A practical examination of the arc excitation of soil and plant ash has shown that precision may be increased when the line pair consists of corresponding lines of two elements that have very similar volatilisation curves. If such a line pair is not available precision can be increased more efficiently by replication with no internal standard. The use of an unsuitable internal standard can result in a loss of precision. O. M. WHITTON.

Taking soil samples. R. O. Woodward (*Okla. agric. Exp. Sta., Circ.* 513, 8 pp.).—Details of the method used for taking representative top- and sub-soil samples are described. A. H. CORNFIELD.

Factors affecting soil sampling. C. D. Welch and J. W. Fitts (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 54—56).—There was no significant difference between results of analysis for samples collected with a tube, spade or trowel. The pH and org. matter values were significantly lower for samples collected with an auger than for those taken with other tools. The available P and K and org. matter content of soils decreased with depth down to 9 in. where both sod or row crops were grown. Soil pH decreased with depth only where sod crops were grown. A comparison of results obtained from 0—3-in. and 0—6-in. depth cores indicated generally that the shallow sampling is more desirable for soils in sod crops. With row crops the deeper sampling usually gave the same results as the shallow sampling, although significantly lower values were obtained in some areas with the 0—6-in. sampling depth. A. H. CORNFIELD.

Soil characteristics which affect root penetration and timber site quality of Douglas fir in Western Washington. F. E. Schlots, W. J. Lloyd and C. E. Deardorff (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 101—105).—Profile characteristics of five soil types are presented and discussed in relation to tree growth. The highest timber site quality was produced on sites with deep friable soils of moderate to high fertility level. Progressively lower timber site quality was obtained on sites with increasing strong profile development or degree of horizonation. There was a close relationship between timber site quality and soil depth over claypan horizons. Soils having similar profiles, though developed from different parent materials, had similar site qualities. A summary of genetic soil properties and woodland site indices on 203 sites representing 14 soil types is presented. A. H. CORNFIELD.

Influence of soil crusts on gaseous diffusion. C. W. Domby and H. Kohnke (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 1—5).—The effects of different degrees of crusting produced by varying rainfall under laboratory conditions on the gaseous diffusion rate of soils was studied. Differences in degree of crusting had no effect on diffusion rate of air-dried soils. Most of the diffusion occurring through a crusted cracked silt loam was through the crust and not through the cracks. Surface crusts restricted diffusion only at low moisture tension ($pF > 2{\cdot}5$). Diffusion rates increased approx. linearly with soil pF from pF 2·5 to 6·0, values for crusted and uncrusted soils being very similar. The rate of gaseous diffusion through soils is determined by the total vol. and not by the size of the air-filled pores. A. H. CORNFIELD.

Adsorption of carbohydrates and related compounds on clay minerals. D. L. Lynch, L. M. Wright and L. J. Cotnoir, jun. (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 6—9).—The extent of adsorption of a no. of sol. carbohydrates (dextrins, substituted cellulose, inulin, starch, etc.) by montmorillonite and kaolinite was studied. The amount of carbohydrate adsorbed increased with the amount present and reached a max. the value of which depended on the material. Both Ca- and H-montmorillonite adsorbed larger quantities of all materials than did Ca-kaolinite. Ca- and H-montmorillonite showed nearly equiv. amounts of adsorption for most of the materials. Ethyl cellulose and starch were adsorbed to the greatest extent by montmorillonite and inulin by kaolinite.. The materials were adsorbed between the interplanar spacings of montmorillonite. Cation and anion exchange were not involved in the process, although H^+ bonding appeared to play a part. A. H. CORNFIELD.

Available phosphorus in soils of Ohio. P. F. Pratt, N. Holowaychuk and H. H. Morse (*Ohio agric. Exp. Sta., Res. Circ.* 27, 16 pp.).—Soil samples from the 0—6-in. and 6—12-in. and in some cases even lower, layers of soils from 235 locations were analysed for available P (Bray–Kurtz 0·3N-NH_4F in 0·025N-HCl and 0·03N-NH_4F in 0·1N-HCl reagents). Available P in the soils was not related to soil series but the ratio of P sol. in one reagent to that sol. in the other was related to soil series. In most profiles available P decreased with depth and was low below 20 in. Soils maintained at high pH contained relatively high 0·1N-NaOH-extractable P in comparison with acid-extractable P. The reverse was true for neutral soils. A. H. CORNFIELD.

Effects of mineral phosphates upon the organic phosphorus content of organic soils. W. W. McCall, J. F. Davis and K. Lawton (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 81—83).—Addition of $Ca(H_2PO_4)_2$ to peat and muck soils prior to incubation resulted in increased mineralisation of org. P during incubation. None of the added inorg. P was fixed during incubation. After four months' incubation (at the moisture equiv. at 26·7°) available P (Bray–Kurtz acid-sol. + adsorbed P) increased by 31—292% in six and decreased in two of the soils. The reduction in available P occurred only in soils of pH 6·3 or greater. A. H. CORNFIELD.

Residual availability in the soil of various sources of phosphorus as measured by plant absorption of ^{32}P and by soil tests. A. C. Caldwell, A. Hustrulid and F. L. Hammers (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 25—28).—When the fertilisers were applied at the rate of 40 lb. of P_2O_5 per acre for six years yields of legume hay and the % of P in the plant on a silty clay loam were increased by ordinary and conc. superphosphate, $Ca(PO_3)_2$, H_3PO_4 and fused $Ca_3(PO_4)_2$, but not by rock phosphate or colloidal clay phosphate. Yields of flax, oats, wheat and maize were unaffected by any of the treatments, although the % of P in the plant was usually increased. Application of ^{32}P-labelled superphosphate in the seventh year and the uptake of P in this year indicated a definition residual effect from all materials, with the exception of rock phosphate and colloidal clay phosphate, applied during previous years. There was a close correlation between Bray's adsorbed soil P and available soil P as indicated by adsorption of ^{32}P. Bray's adsorbed + acid-sol. P and Thornton's P values were not consistently good indicators of available P. A. H. CORNFIELD.

Residual soil phosphorus from various fertiliser phosphates extracted by different solvents. M. Salomon and J. B. Smith (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 33—36).—The P status of a silt loam which had received different types of P fertiliser for 48 years followed by 10 years without any treatment was determined by eight chemical methods and the results were compared with yields of and P uptake by Sudan grass and hay. Bray's 0·1N-HCl + 0·03N-NH_4F, Thornton's 0·1N-HCl + $(NH_4)_2MoO_4$, Peech's NaOAc + AcOH and Truog's 0·002N-H_2SO_4 methods were generally satisfactory indicators of P availability for the soils as a whole. Rubins' 0·1N-NaOH, Olsen's 0·5M-$NaHCO_3$, Bray's 0·1N-HCl and Morgan's NaOAc + AcOH methods were not quite as satisfactory. The Truog and Thornton methods extracted too much P from rock phosphate, whilst Rubins' method extracted large amounts of org. P. A. H. CORNFIELD.

Sub-irrigation and plant nutrition: II. Utilisation of phosphorus by lucerne from the soil surface to the water table. R. C. Lipps and R. L. Fox (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 28—32).—A comparison of the available and total P contents of nine profiles (down to 3- to 15-ft. depths) growing lucerne for 18—45 years with control

profiles not growing lucerne showed that the soils had been depleted of much of their available P where lucerne had been grown. The greatest reduction in available P had occurred in the 6—12-in. depth. The depth to the water table had influenced root activity as measured by P utilisation of subirrigated lucerne. Root activity (with respect to P uptake) of lucerne growing on soils with shallow water tables (10 ft. or less) was greatest in the surface soil and gradually decreased with depth. In these soils water was at a relatively low tension throughout the profile. In soils with water table at depths greater than 10 ft. root activity was high near the surface and also just above the water table but was relatively low in the intermediate layers.
A. H. CORNFIELD.

Relationship of available potassium to soil moisture. R. E. Luebs, G. Stanford and A. D. Scott (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 45—50).—The exchangeable K content (neutral N-NH_4OAc extract) of 13 Iowa soils increased after air-drying. Uptake of K by maize in pot tests increased when the soils were air-dried prior to being cropped. The extent of increased uptake was equiv. to applying 30—60 p.p.m. of K to the soils. There was an inverse relationship between exchangeable K content and v.p. of the laboratory air with which the soil had come into equilibrium. The exchangeable K content of the surface-inch field soils varied with time of sampling, whilst the exchangeable K content of the soil below this depth remained virtually const. Air-drying of soils prior to determining exchangeable K may give an erroneous indication of their K-supplying power to plants. The K uptake by maize in pot tests with a variety of soils was correlated better with the exchangeable K content of undried than that of dried soil samples. A. H. CORNFIELD.

Subsidence of organic soils in the Florida Everglades. J. C. Stephens (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 77—80).—Subsidence of peat soils was directly related to depth to the water table. An equation relating the two factors is presented. The average annual subsidence was about 1·25 in. over 40 years. The subsidence rate of Florida peat soils was about double that of Indiana peat soils. Assuming that the more or less uniform rate of subsidence which has taken place will continue 88% of the original peat would have disappeared by A.D. 2000. To reduce subsidence the water table should be kept as high as crop and field requirements will tolerate and drained land should not be allowed to remain fallow.
A. H. CORNFIELD.

Development and present problems of soil microbiology. H. G. Thornton (*J. Sci. Food Agric.*, 1956, **7,** 93—101).—A review, mainly of recent work, covering: components and localised distribution of the soil micropopulation, effects of microbes on soil structure, Mn cycle, oxidation and reduction of Fe compounds, trace elements, S cycle, N cycle, nodule bacteria and leguminous crops, antagonism between micro-organisms in soil in relation to root disease, and the problem of altering the soil population, e.g. by inoculation, partial sterilisation, etc. (104 references.) J. S. C.

Influence of method of storage on the microbiological properties of soil samples. C. N. Acharya and S. P. Jain (*J. Indian Soc. Soil Sci.*, 1955, **3,** 91—95).—Both the ammonifying and N-fixing power of air-dried soils stored for 6—12 months were similar whether aërobic or anaërobic storage conditions were used. The nitrifying power of the soils [$(NH_4)_2SO_4$ test] was lower and the amount of NO_3' produced during incubation was higher after storage under anaërobic than under aërobic conditions. A. H. CORNFIELD.

Carbon–nitrogen relationships in soil. I. Immobilisation of nitrogen in the presence of carbon compounds. II. Quantitative relationships between nitrogen immobilised and carbon added to the soil. G. W. Winsor and A. G. Pollard (*J. Sci. Food Agric.*, 1956, **7,** 134—141, 142—149).—I. Immobilisation of N in starch- and sucrose-treated soils was studied. Max. conversion of inorg. N→org. N was on incubation at 23·5° for ~2 days. The active organisms showed a marked preference for NH_3 rather than NO_3', although NO_3' was invariably assimilated at the higher levels of added C compounds. Assimilation of NH_3 lowers soil pH and that of NO_3' increases it markedly. (26 references.)

II. Quant. relationships between N immobilised and C compounds added to soil (in the form of sucrose) were studied. The relationship between C added and N immobilised was found to be linear. At max. N immobilisation (23·5° incubation, 2 days), one unit of N was immobilised for every 8·3—10·8 units of C added. Part of the C was evolved as CO_2 and the ratio of retained added C to N immobilised was ~6. (10 references.) J. S. C.

Molybdenum deficiencies in the United States. E. J. Rubins (*Soil Sci.*, 1956, **81,** 191—197).—A review of work chiefly concerned with lucerne, citrus and brassicas. T. G. MORRIS.

Factors affecting molybdenum availability in soils. E. B. Davies (*Soil Sci.*, 1956, **81,** 209—221).—A review. Mo deficiency, availability and excess are considered. T. G. MORRIS.

Molybdenum in Everglades peat. J. G. A. Fiskel, G. A. Mourkides and N. Gammon, jun. (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 73—76).—The water-sol. Mo of samples from eight Everglades peat fields ranged from 0·18 to 0·32 p.p.m. Exchangeable Mo was less than 0·07 p.p.m. The Mo extractable by 0·05% quinol in 50% alcohol was less than 0·121 p.p.m., whilst most of the Mo present was extracted by N-aqNH_3. Peat samples from fields cropped for 12 years still showed high available Mo at all depths. Of various materials added to pasture areas with a view to reducing Mo availability to the forage (and thus preventing toxicity in cattle) only attapulgite, Krilium, and bauxite reduced the uptake of Mo by the forage. Kaolin, vermiculite, ball clay, colloidal phosphate and Mn and Cu compounds had no effect on Mo uptake.
A. H. CORNFIELD.

Methods of soil and plant analyses for molybdenum. E. R. Purvis and N. K. Peterson (*Soil Sci.*, 1956, **81,** 223—228).—Working details are given of the methods employed by the authors for the determination of Mo in plant and soil materials. The method employed utilises the thiocyanate colour. T. G. MORRIS.

Reclamation of an alkali soil of the Hacienda series. R. Overstreet, J. C. Martin, R. K. Schulz and O. D. McCutcheon (*Hilgardia*, 1955, **24,** 53—68).—$CaSO_4.2H_2O$ at 4, and 93% H_2SO_4 at 3·5 tons per acre, in conjunction with swamp-culture pre-treatment, showed marked beneficial effects on the subsequent growth of lucerne.
E. G. BRICKELL.

Reclamation of a saline and high-boron soil in the Coachella valley of California. R. C. Reeve, A. F. Pillsbury and L. V. Wilcox (*Hilgardia*, 1955, **24,** 69—91).—Flushing as a reclamation procedure proved ineffective. Leaching at the rate of 1 ft. of water for each ft. depth of soil removed 80% of the initially high salt accumulation. To leach a similar proportion of B required three times as much water. E. G. BRICKELL.

Soil test summaries for predicting fertiliser ratios and type of lime needed. J. W. Fitts, C. D. Welch and W. L. Nelson (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 36—40).—The interpretation and use of chemical soil test summaries for preparing general fertiliser recommendations on an area basis is described. The Mg–Ca ratio of North Carolina soils was fairly constant and of the order of about 1–5, regardless of region or type of crop grown. This wide ratio indicates that dolomitic limestone should generally be used where liming is necessary. A. H. CORNFIELD.

Fertiliser and lime recommendations for New Jersey. Anon. (*New Jersey agric. Exp. Sta.*, 1955, *Circ.* 568, 16 pp.).—Details of recommendations for liming and for fertilisation of field, fruit and vegetable crops are given. A. H. CORNFIELD.

Urea as a fertiliser for sandy soils. G. M. Volk (*Agric. Chemicals*, 1955, **10,** 41—42, 133, 135).—Yields of and % of N in three species of grasses and losses of N by leaching from a sandy soil were of the same order whether urea, NH_4NO_3 or $NaNO_3$ were used as N carriers. The pH of the surface soil was reduced to a greater extent by NH_4NO_3 than by urea. Both fertilisers reduced the pH of the surface soil to a greater extent than that of the subsoil.
A. H. CORNFIELD.

Crop responses to deep tillage with lime and fertilisers. L. E. Englebert and E. Truog (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 50—54).—Deeper penetration of lucerne roots on a strongly acid soil having a compacted subsoil was obtained by subsoiling with deep placement of lime and fertilisers, but not by subsoiling alone. Yields of 2nd- to 4th-year lucerne hay were increased by subsoiling + deep placement of lime and fertilisers, but not by subsoiling or subsoiling + deep placement of lime. Ploughing to 12 in. (in combination with lime and fertiliser applications) had no effect on yields of lucerne–bromegrass hay in the first year but increased the yields in the second year in comparison with 6 in. ploughing. Maize and oats made no response to subsoiling even when lime and fertilisers were applied.
A. H. CORNFIELD.

Fertiliser placement for small grains in relation to crop stand and nutrient uptake in Nebraska. R. A. Olson and A. F. Dreier (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 19—24).—Yields of oats and wheat and the utilisation of applied P in greenhouse and field tests were greater where superphosphate was applied with the seed than where it was broadcast or mixed with the soil. Reduced emergence due to placement of fertiliser with seed was most severe at low levels of available soil moisture. Under these conditions even the application of fertiliser containing 10 lb. of N per acre resulted in significant stand reduction, whilst emergence was reduced to zero with 160 lb. of N per acre. With 40 lb. of N per acre $CaCN_2$ and aq.NH_3 reduced stands completely, whilst NH_4NO_3 was the least damaging of the N carriers tested. Superphosphate, Rhenania phosphate and $Ca(PO_3)_2$ reduced stands slightly, whilst $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$ reduced stands to a fair extent. There was less stand reduction with pelletted than with powdered P fertilisers. Rye and

oats were less susceptible to stand reduction due to fertiliser injury than were wheat and barley. A. H. CORNFIELD.

Plant Physiology, Nutrition and Biochemistry

Products of starch hydrolysis and their metabolism. P. V. Vittorio, G. Krotkov, C. D. Nelson and R. G. S. Bidwell (*Canad. J. Bot.*, 1956, **34**, 209—213).—^{14}C-labelled tobacco-leaf starch was digested with N-H_2SO_4 at 100° but was not completely hydrolysed to glucose after 24 hr. Paper partition chromatography, with butanol–ethanol solvent, showed the presence (after 3 hr. hydrolysis) of four ^{14}C-labelled products with R_F values lower than glucose. These bands, fed individually to tobacco leaves in light, became incorporated into sucrose, glucose, fructose and starch, and were better starch formers than glucose, glucose-1-phosphate, or maltose. J. S. C.

Biochemical and nutritional studies of potassium. I. Effect of potassium on respiration rates of higher plants. II. Potassium in relation to carbohydrate metabolism of higher plants. A. Fugiwara and S. Iida (*Tohoku J. agric. Res.*, 1955, **6**, 57—66, 67—74).—I. Respiration in rice, barley and tomato plants was inversely related to the K supply in the nutrient. The increase in respiration resulting from additions of glucose to the nutrient was associated with increased intake of K by roots; with the decline in respiration as the glucose was consumed K was excreted by the roots. The increased respiration of K-deficient plants occurred with NH_4^+ or NO_3^- as N source. The O_2 uptake per unit wt. in roots supplied with N as NH_4^+ was greater than when as NO_3^- whether the K supply was deficient or abundant, values for roots exceeding those for tops in all cases. Na had no apparent influence on respiration.

II. The transpiration rates of rice, barley and tomato plants in K-deficient water-culture exceeded those of plants grown with adequate K. The increased loss of water and of respiratory substrate accelerated the wilting of deficient plants. A. G. POLLARD.

Physiology of potassium applied by foliar spray to the vine, studied by use of radioactive potassium. P. Lecat (*C. R. Acad. Agric. Fr.*, 1955, **41**, 712—713).—Using foliar sprays of 0·5% aq. KNO_3 or $KHCO_3$, labelled with ^{42}K, on vines, the effects on the quantity of K stored in the foliar system, its rate of penetration into the leaf, its rate of migration from the leaf into the other parts of the plant (particularly the grape) and the quant. effects of K nutrition on seed growth were investigated. Preliminary results show that storage of K in foliar systems is extremely variable in spite of very complete spraying of both sides of the leaves. Penetration from leaves to the plant is rapid and complete within five days, and is slightly more rapid for older plants than for younger ones. The use of KNO_3 as against $KHCO_3$, in foliar spray applications, appears to have some relative advantage. J. S. C.

Nutrient composition of leaf samples from North Carolina orchards. A. C. McClung and W. L. Lott (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 10—15).—The major and trace element contents of leaves from peach trees from 19—46 orchards collected between August 10th and 25th over two years were determined and compared with values obtained from other surveys. Levels of P, K and Mn were well above deficiency level. Levels of Zn and Mg were generally in the deficiency region, whilst Fe, B and Cu levels were also low compared with values reported from other regions. There was no clear relationship between Fe content of leaves and occurrence of chlorosis. Values for Ca, Mg, Fe and Al, were roughly correlated with predictions of uptake based on soil type. A. H. CORNFIELD.

Interaction of kind of soil colloid, fertility status and seasonal weather variation on the cation content of turnip leaves. T. J. Army and E. V. Miller (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 57—59).—The concn. of Ca and Mg in turnip leaves decreased, whilst that of Na and K increased, from spring to autumn. Varying soil temp. had little effect on the cation content of the leaves. In both spring and autumn there was a greater absorption of Ca and Mg from a kaolinitic than from a montmorillonitic soil, whilst the reverse was true of K absorption. There was a greater reduction in the uptake of Ca from spring to autumn in the kaolinitic than in the montmorillonitic soil. A. H. CORNFIELD.

Occurrence of antibacterial substances in seed plants with special reference to *Mycobacterium tuberculosis*. A. Frisbey, S. Geis, R. Y. Gottshall and E. H. Lucas (*Mich. agric. Exp. Sta. Quart. Bull.*, 1955, **38**, 279—287).—Further results on the screening of extracts of seed plants are reported. Species (81) were found to contain principles inhibitory *in vitro* to *M. tuberculosis*; 65 of them had specific activity. E. G. BRICKELL.

Cytochemical localisation of acid phosphatase in root tip cells. W. A. Jensen (*Amer. J. Bot.*, 1956, **43**, 50—54).—Intercellular distribution in *Allium cepa*, *Vicia faba* and *Pisum sativum* was studied by a modification of the Gomori technique. The sites of activity are spherical (0·6μ.) or rod-shaped (0·6 × 1·2—2·0μ.) cytoplasmic particles containing small amounts of ribonucleic acid and large amounts of lipins. E. G. BRICKELL.

Copper requirements and deficiency symptoms of field and vegetable crops. L. G. Nelson, K. C. Berger and H. J. Andries (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 69—72).—In field tests on a Cu-deficient peat soil (pH 5·0) yields of red beetroots and oat grain were greatly increased by application of CuO (25 lb. per acre). Yields of carrots, cabbage, potatoes and maize were also increased to a fair extent. Similar results with these and other species were obtained in pot tests; lettuce, peas and tobacco made no response to Cu applications. The Cu content of the plants usually increased with the treatment even where no yield increases were obtained. The treatment greatly increased the 0·1N-HCl-extractable Cu in the soil. Yields of oats grain on a no. of mineral soils were not affected by applying CuO (20 lb. per acre) although straw yields were significantly increased on some of the silt loam and gravel soils tested. Cu deficiency symptoms of a no. of species are described. A. H. CORNFIELD.

Rôle of molybdenum in plant nutrition. H. J. Evans (*Soil Sci.*, 1956, **81**, 199—208).—A review. T. G. MORRIS.

Molybdenum deficiency in horticultural and field crops. P. R. Stout and C. M. Johnson (*Soil Sci.*, 1956, **81**, 183—190).—A review. T. G. MORRIS.

Symptoms of molybdenum deficiency in plants. E. J. Hewitt (*Soil Sci.*, 1956, **81**, 159—171).—A review. The effects of Mo deficiency on 13 families of plants are described. T. G. MORRIS.

Molybdenum deficiencies in legumes in Australia. A. J. Anderson (*Soil Sci.*, 1956, **81**, 173—182).—A review of work in Australia chiefly on clover and lucerne. T. G. MORRIS.

Molybdenum content of lucerne in relation to deficiency symptoms and response to molybdenum fertilisation. H. M. Reisenauer (*Soil Sci.*, 1956, **81**, 237—242).—Lucerne was grown on a soil deficient in Mo, fertilised with KNP, gypsum and Mo in some cases. Plants deficient in Mo were stunted and of poor colour with chlorotic spots, but these symptoms were indicative more of N starvation than of Mo deficiency. The Mo and N contents of the leaves were examined and as the symptoms became more severe the level of N fell but that of Mo did not. Yields of lucerne were increased by Mo treatments of 0·8 lb. per acre, this level giving almost the max. yield provided there was satisfactory nodulation. The N content of the leaves also increased under these conditions. Mo (0·5 p.p.m.) in the leaves was associated with max. yields and max. N content. T. G. MORRIS.

Protection effect of various chemical compounds against damage to chromosomes by gamma radiation. H. P. Riley (*Amer. J. Bot.*, 1955, **42**, 765—769).—Protection is conferred by Na hydrosulphite and BAL (2 : 3-dimercaptopropanol) in concn. of 2×10^{-3} and 2×10^{-4}M respectively. Root tips from germinated onion seeds were protected against the formation of anaphase bridges by BAL, Na hydrosulphite and pyrosulphate but not by glucose or sodium persulphate. E. G. BRICKELL.

Estimates of consumptive-use and irrigation water requirements of crops in Oklahoma. J. E. Garton and W. D. Criddle (*Okla. agric. Exp. Sta.*, 1955, *Tech. Bull.* 57, 26 pp.).—Total consumptive use of water by and net irrigation requirements of a no. of crops, including pasture, are presented for various areas in Oklahoma. A. H. CORNFIELD.

Rooting of cuttings in air-cooled mist-chamber. D. V. Sweet and R. F. Carlson (*Mich. agric. Exp. Sta. Quart. Bull.*, 1955, **38**, 258—267).—A polyethylene-enclosed mist-chamber, equipped with a suitable exhaust fan, in which cuttings were held at 83°F. when the outside temp. exceeded 100°F., was more satisfactory than chambers without air-circulation. Cuttings were also comparatively free from disease organisms. E. G. BRICKELL.

Seed fat of *Mallotus philippinensis* (Kamala oil). N. C. Hancox and H. H. Hatt (*Qd J. agric. Sci.*, 1955, **12**, 57—60).—Yield and quality of fat from Australian-grown seed of *Mallotus philippinensis* was comparable with those of fat from Indian-grown samples. The kamlolenic acid content of the fat (on which drying properties depend) varied considerably with location and year of growth. A. H. CORNFIELD.

Promotion of floral initiation by auxin. D. de Zeeuw and A. C. Leopold (*Amer. J. Bot.*, 1956, **43**, 47—50).—Naphthylacetic acid (1—5 p.p.m.) applied before induction had a pronounced promotive effect on the floral initiation of short-day species such as cocklebur and Biloxi soya-bean. The effect follows a two-phase concn. curve similar to that for the other growth responses to auxin. E. G. BRICKELL.

Effects of ozonated hexene on photosynthesis and respiration of *Lemna minor* L. L. C. Erickson and R. T. Wedding (*Amer. J. Bot.*, 1956, **43**, 32—36).—Exposure for periods up to 24 hr. resulted in

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severe visible injury to the plants; O_2 evolution was decreased and chlorophyll content reduced. Respiration was however stimulated by exposures up to 12 hr. E. G. BRICKELL.

Some anatomical and cytological responses of barley to maleic hydrazide. E. M. Gifford, jun. (*Amer. J. Bot.*, 1956, **43,** 72—80).—The following changes were reported after spraying with 0·2% maleic hydrazide (as the diethanolamine salt). Plants were stunted and leaves became thicker, brittle and more green in colour. Mitoses were inhibited, nuclear aberrations appeared in the cells of the protoderm and ground meristem, and there was a precocious growth of axillary buds (tillers). Cytoplasmic particulates became clumped, cell walls thickened particularly in the ground parenchyma and phloem, and there was some evidence of necrosis in mesophyll parenchyma cells. After 14 days, extensive necrosis took place in the pith and this continued, together with general necrosis, after 28 days. E. G. BRICKELL.

Effects of 4 : 6-dinitro-o-cresol on water influx and oxygen uptake of excised onion roots. J. R. Lott and H. F. Rosene (*Amer. J. Bot.*, 1956, **43,** 69—72).—Both water influx and O_2 uptake were increased by exposure to low concn. of DNOC and decreased by stronger concn. Probably these processes are related and dependent upon cell metabolism. E. G. BRICKELL.

Crops and Cropping

Mineral nutrition of wheat grown under field conditions. N. G. Potapov (*Dokl. Akad. Nauk SSSR*, 1955, **105,** 529—532).—The nitrate-N content of the sap of wheat grown in various soils and climatic conditions varied from 0 to 317 mg./l. without addition of NPK fertilisers, and from 0 to 562 mg./l. with them. Phosphorus compounds were represented exclusively by phosphates, the PO_4''' content varying from 51 to 213 mg./l., for different localities, growth stages, and manuring conditions. Probably the root system can, under conditions of low environmental N- and P-supply, convert all the absorbed inorg. N into nitrogenous org. products, but that when excess of inorg. N is available NO_3' and PO_4''' are supplied unchanged to the leaves and flowers. R. TRUSCOE.

Effects of fertilisers and stand on maize and of stand on soil moisture. F. E. Shubeck and A. C. Caldwell (*Minnesota agric. Exp. Sta.*, 1955, *Tech. Bull.* 214, 23 pp.).—There was an increase in the no. of poor ears and a decrease in ear size with increasing stand of maize. Height of plants and shelling % were little affected by stand, but silking was delayed in the higher stands. Although there were differences in yield responses to fertilisers, varying rates and ratios and N–P–K had little effect on nubbins, ear size and height of plant. Yield increases from side-dressed N were not consistent, although N content of the grain was always increased. Application of urea sprays (7·5—30 lb. in 100 gal. of water per acre) to the plants either had no effect on or reduced the yields of maize. Available soil moisture decreased with increasing stand. A. H. CORNFIELD.

Antigo : a new white, medium-maturing potato variety resistant to common scab. G. H. Rieman and D. A. Young (*Amer. Potato J.*, 1955, **32,** 407—410).—The pedigree and characteristics of the new variety are described. A. H. CORNFIELD.

Effect of nitrogen, potassium and organic matter on utilisation of superphosphate phosphorus by potatoes. P. M. Smirnov and B. P. Pleshkov (*Dokl. Akad. Nauk SSSR*, 1955, **103,** 673—675).—Assimilation of superphosphate-P by potato plants is lower in the early stages of growth when N and K fertilisers are also present than with P alone or with compost. The favourable effect of composts is ascribed to formation from them of org. acids, which by combining with soil sesquioxides prevent binding of phosphate ions. At later stages of growth the uptake of superphosphate P is greatest for plants receiving P + N manuring. R. TRUSCOE.

Effects of soil moisture tension and of potassium chloride or sulphate upon yields, composition and bacterial soft rot of Irish potatoes. M. E. Harward, W. A. Jackson, L. W. Nielsen, J. R. Piland and D. D. Mason (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 59—65).—The effects of K (180 lb. K_2O per acre) applied as SO_4'' or as Cl′ and varying soil moisture levels (soil was re-saturated when moisture tensions ranging from 300 to 10,000 cm. were attained) on growth and quality of potatoes grown in tubs in a mixture of fine sandy loam soil and sand were studied. Yields of U.S. No. 1 tubers at all moisture levels were somewhat higher whilst yields of vines were somewhat lower where K_2SO_4 than where KCl was used. Yields of tubers decreased to about the same extent with increasing soil moisture tension whether SO_4'' or Cl′ was used. The K content of the leaves was similar with both sources of K, but that of tubers was greater with SO_4'' than with Cl′. The Ca and Mg contents of leaves and stems were lower, whilst the N content of leaves, stems and tubers, the dry matter content of leaves and stems and the Mg content of tubers were higher with SO_4'' than with Cl′. The sp. gr. of tubers decreased with increasing soil moisture tension. The extent of wound infection by bacterial soft rot organisms increased, whilst the rate of bacterial decay of tubers decreased, with increasing soil moisture tension. A. H. CORNFIELD.

Effect of 2 : 4-D and maleic hydrazide on sprouting, yields and colour of Red McClure potatoes. J. L. Fults and M. G. Payne (*Amer. Potato J.*, 1955, **32,** 451—459).—In a year in which available soil moisture was low early in the season application of 2 : 4-D alkanolamine salts (0·5 lb. acid equiv. per acre) alone or in combination with maleic hydrazide (3 lb. per acre) either in late July or late Aug. had no effect on the skin colour of Red McClure potatoes. In a year of normal soil moisture the only treatment which increased skin colour was 2 : 4-D applied in late July. Combination of maleic hydrazide and 2 : 4-D produced no better sprout inhibition during storage than did maleic hydrazide applied alone. Late July treatment with maleic hydrazide or maleic hydrazide + 2 : 4-D gave better sprout inhibition than did late Aug. treatment only in the year of normal rainfall. A. H. CORNFIELD.

Seed production studies. I. Effect of nitrogenous fertiliser, applied at different rates and dates, on seed production in cocksfoot (*Dactylis glomerata* L.). II. Effect of grazing on seed production in cocksfoot (*Dactylis glomerata* L.). J. P. Lambert (*N.Z. J. Sci. Tech.*, 1956, **37,A,** 432—442, 443—450).—I. The application of nitrogenous fertiliser to cocksfoot seed crops depends on differences in soil fertility and on the age of the crop; in good conditions it may not be necessary at sowing time, or in the first preharvest spring; after four or five harvests at least 6 cwt. per acre may be needed for continued high production. Spring applications resulted in the greatest wt. per 1000 seeds, and greatest no. and wt. of viable seeds per panicle. Increasing rates of application are needed as the seed-production area ages; dividing the heavier applications between autumn and spring may be advisable. (18 references.)

II. In cocksfoot, grazing increased the no. of panicles but reduced the wt. per 1000 seeds, the no. and wt. of viable seed per panicle and the wt. of viable seed per acre. Spring applications of N increased the no. and wt. of viable seed per panicle, but wt. per 1000 seeds was not affected significantly by time of application. Grazing of cocksfoot may reduce the seed yield from intercultivated rows, but a greater reduction may occur where crop litter accumulates. E. M. J.

Effect of placement and rate of phosphate, potash and limestone on the growth of lucerne and lespedeza. W. W. Woodhouse, jun. (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 15—18).—The effects of two levels of P, K or $CaCO_3$ applied in three ways (broadcast, mixed with surface 4 in., or applied at a 4-in. depth through plough-sole placement) on yields of the legume component of lucerne–orchard grass and lespedeza–Dallis grass swards over three years on a sandy clay loam were studied. Lucerne yields were increased to a fair extent in all years by P and $CaCO_3$ but only slightly by K. Lespedeza yields were increased to a much smaller extent. Increased yields due to P were similar for all methods of fertiliser placement. Mixing $CaCO_3$ with the soil was much more effective in increasing yields of lucerne than was surface placement, whilst yields from plough-sole placement were intermediate. There were little differences in yields of lespedeza due to method of fertiliser placement. A. H. CORNFIELD.

Effects of various rates and frequencies of applications of rock and superphosphate on the yield and composition of forage on a Lake Charles clay loam soil. R. L. Cheaney, R. M. Weihing and R. N. Ford (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 66—68).—Yields of forage (from a Dallis grass–white, Persian, and hop clovers pasture) over four years were somewhat greater where 30 lb. P_2O_5 (superphosphate) per acre was applied each year or where 60 lb. was applied every two years than where 120 lb. was applied initially. With initial dressings of 240 or 480 lb. P_2O_5 forage yields were only slightly higher than with the lighter dressings. Where rock phosphate was used forage yields increased somewhat as applications of rock phosphate were changed from 30 lb. of P_2O_5 annually to 480 lb. initially. Total uptake of P and N by the forage was roughly correlated with yields. A. H. CORNFIELD.

Efficiency of various nitrogen sources for pasture grasses in large lysimeters of Lakeland fine sand. G. M. Volk (*Proc. Soil Sci. Soc. Amer.*, 1956, **20,** 41—45).—The effects of different N carriers on the growth of four species of grasses over three years in lysimeters containing a fine sandy soil is described. There were no consistent differences in yields or N content of the foliage from N applied as urea, NH_4NO_3 or $NaNO_3$. Loss of N by leaching was low where Pensacola Bahia grass, Pangola grass or coastal Bermuda grass were grown but was appreciable where carpet grass was grown. Recovery of applied N by the forage ranged from 50 to 80%. The pH

of both top- and subsoil after three years was lower where NH_4NO_3 than where urea had been used. With all treatments subsoil pH was higher than topsoil pH. A. H. CORNFIELD.

Effects of fertiliser on yields of mountain meadow hay. C. S. Cooper (*Ore. agric. Exp. Sta.*, 1955, *Bull.* 550, 7 pp.).—Mountain meadows (Nevada bluegrass or rush–sedge-grass) which are subjected to annual flooding gave economical returns of forage when treated with N fertilisers (60—80 lb. of N per acre) in late autumn or early spring. Baltic rush (wire grass) meadows which are flooded for long periods should not be treated with N. Application of P (40—60 lb. P_2O_5 per acre) greatly increased the growth of clovers, where these were present. Harvesting should be delayed to allow natural re-seeding of clovers. A. H. CORNFIELD.

Effect of trace elements on lucerne and oats in Minnesota. J. M. MacGregor and J. F. Mulvehill (*Minnesota agric. Exp. Sta.*, 1955, *Tech. Bull.* 213, 16 pp.).—Yields of lucerne and oats on a variety of soils over a no. of years were unaffected by application of B, Cu, Mn, or Zn. Heavy applications of B increased the B content of oats and lucerne in the year of application, but not in the following year. All the soils contained sufficient available B (hot water-sol. B). Available B decreased with increasing depth of soil. A. H. CORNFIELD.

Pasture grazing trials on various land types. J. Q. Lynd, W. C. Elder and R. Totusek (*Okla. agric. Exp. Sta.*, 1955, *Bull.* 445, 22 pp.).—The greatest annual average animal gain per acre over four years on Bermuda grass–legumes pastures occurred on alluvial bottom land soils receiving CaO and P. Pastures on claypan prairie, upland prairie and permeable upland prairie soils produced about half the animal gain. Liming and fertilisation increased gains on all soils. A. H. CORNFIELD.

Effect of phosphate nutrition of tomatoes on the quality of the seed. A. A. Anismov and O. N. Popova (*Agrobiologiya*, 1954, No. 5, 110—111).—Application of small amounts of P fertiliser (5 g. per 2 sq. m. of frame) improved seed quality. Seed from treated plants produced larger plants, earlier yields and fruit having higher sugar and vitamin C and lower acid contents. HORT. ABSTR. (A. G. P.).

Boron requirements of young apple trees in sand cultures. J. M. Beattie (*Ohio agric. Exp. Sta.*, 1955, *Res. Bull.* 754, 46 pp.).—Young Rome Beauty apple trees were grown in sand culture through two seasons with three levels each of Ca and B and two levels each of K and Mg. The Ca, K and B content of the foliage was relatively high in the first and low in the second season, whilst the reverse was true for Mg. Leaf B increased with the B content of the nutrient solution (0—5 p.p.m.). Varying the K supply from 30 to 166 p.p.m. had no effect on B accumulation. Total terminal growth increased with Ca, Mg and K supply but not with B supply. The interaction of Ca, Mg and K supply on B accumulation was highly significant in both years. A. H. CORNFIELD.

Cranberries and cranberry products. C. F. Fellers and W. B. Esselen (*Massachusetts agric. Exp. Sta.*, 1955, *Bull.* 481, 62 pp.).—The chemical composition of cranberries and cranberry products (bottled cranberries, juices, etc.) as affected by various factors are presented and reviewed. A. H. CORNFIELD.

Seedbed for cotton. J. Porterfield and E. M. Smith (*Okla. agric. Exp. Sta.*, 1955, *Bull.* 449, 14 pp.).—A new type of seedbed for cotton combines furrow and bed planting. Equipment for producing the seedbed is described. The new type of seedbed gave better stands of cotton than did other types tested. A. H. CORNFIELD.

Influence of light and nutrition on colour and growth of red cedar seedlings. R. E. McDermott and P. W. Fletcher (*Missouri agric. Exp. Sta.*, 1955, *Res. Bull.* 587, 15 pp.).—The effects of three different light intensities and varying levels of N, Ca, P and K fertilisers on growth and colouring of red cedar seedlings are reported. Varying fertiliser levels had no effect on growth or foliage colour at any light intensity. Growth in 10% of full sunlight was less than that in full or in 33% of full sunlight. During summer foliage colour varied with light intensity. Anthocyanin formation in autumn occurred only under full sunlight. A. H. CORNFIELD.

Huller-cleaner for castor bean harvesters. L. G. Schoenleber (*Okla. agric. Exp. Sta.*, 1955, *Bull.* 461, 10 pp.).—A large-capacity huller-cleaner adaptable for mounting on to castor bean harvesters is described. A. H. CORNFIELD.

Pest Control

Fifteen years' progress in crop protection. T. Dugdale (*J. Sci. Food Agric.*, 1956, **7,** Suppl. Issue, s1—s5).—A survey of crop protection developments during a 15-year interval from 1940 is presented, covering mechanical methods, biological methods and the use of chemical sprays and dusts; chemical control, divided into four sections (i) insecticides and ascaricides, (ii) fungicides, (iii) nematocides and (iv) herbicides is discussed. E. M. J.

Crop Protection Products Approval Scheme. R. de B. Ashworth (*J. Sci. Food Agric.*, 1956, **7,** Suppl. Issue, s78—s81).—The administrative procedure involved in opening a new group of chemicals for the approval of proprietary products within the ambit of the scheme is described. E. M. J.

General survey of the foliage diseases of arable crops. H. E. Croxall (*J. Sci. Food Agric.*, 1956, **7,** Suppl. Issue, s13—s15).—In this survey the fungal diseases confined principally or entirely to the foliage are considered: potato blight, cereal mildew and cereal yellow rust. In potato crops, tuber growth ceases when 75% of the foliage is destroyed. Every season some crops of oats, wheat or barley are attacked by their particular strains of *Erisyphe graminis*. In barley, mildew was responsible for a decrease in yield of 13% in an early sown and 22% in a later sown crop. Black stem rust of wheat and yellow rust (*Puccinia glumarum*) in wheat and some barley crops are discussed. Other diseases mentioned include chocolate spot of beans, leaf spots of peas caused by *Ascochyta* and *Mycosphærella*, and the powdery mildew of swedes and turnips. E. M. J.

Control of pests of arable crops. J. L. Hunt (*J. Sci. Food Agric.*, 1956, **7,** Suppl. Issue, s25—s32).—The range of insecticides and methods of application are discussed: 14 pests are dealt with, including wireworm, leatherjackets, bean seed fly, pea and bean weevil etc. A summary of control measures of the 14 pests, form of application (whether as dust or spray etc.), insecticide and rate of application is presented. E. M. J.

Seed treatment for plant disease control. W. Chaffin (*Okla. agric. Exp. Sta.*, 1955, *Circ.* 615, 12 pp.).—Materials and methods used for controlling plant diseases by chemical treatment of seeds are described for cereals, sorghum, maize, groundnuts, soya-beans, castor beans, flax and cotton. A. H. CORNFIELD.

Spray deposits. I. Effect of spray supplements on the tenacity of a copper fungicide. E. Somers (*J. Sci. Food Agric.*, 1956, **7,** 160—172).—The influence of 47 adhesive materials on the tenacity of CuO on a cellulose acetate surface was determined. Seven of the most promising of these were then evaluated as supplements to CuO in a potato blight field trial with mainly negative results. Assays *in vitro* against *Alternaria tenuis* are reported and discussed. Polyvinyl acetate, at sufficiently high concn., should be a suitable sticker for Cu fungicides. (23 references.) J. S. C.

Problem of toxic residues [of pesticides]. J. M. Barnes (*J. Sci. Food Agric.*, 1956, **7,** Suppl. Issue, s60—s61).—Risks of acute or subacute poisoning from the single or repeated ingestion of the poisonous material and of chronic poisoning from long-continued ingestion of small amounts of pesticides are discussed. E. M. J.

Persistence and fate of DDT on foliage. II. Comparative rates of loss of DDT deposits from glass plates and growing leaves. J. Ward and P. E. Burt (*Bull. ent. Res.*, 1956, **46,** 849—868).—A method of applying even deposits of insecticide to leaves by means of an air-operated paint-spray gun under controlled conditions, is described. Deposits were more toxic on leaves than on plates. In greenhouse tests deposits were lost more rapidly from plates than from living leaves and the lower the rate of deposit the greater was the % lost. Volatility is the main cause of loss of *pp'*-DDT from crystalline deposits of the pure material exposed to sunlight; loss of toxicity resulting from penetration of DDT into the leaf is not of practical importance. E. G. BRICKELL.

Hazards during application of pesticides. E. F. Edson (*J. Sci. Food Agric.*, 1956, **7,** Suppl. Issue, s54—s60).—This review covers general hazards, dangers of the chemicals, dangers of different application techniques, equipment and worker habits, dangers from increasing duration of exposure. An appendix contains a summary of main requirements of the Agriculture (Poisonous Substances) Regulations, 1955, for the use of scheduled substances. E. M. J.

Taint problem. M. Cohen (*J. Sci. Food Agric.*, 1956, **7,** Suppl. Issue, s73—s77).—Alteration of flavour caused by the use of crop protectants is discussed. Reference is made to BHC, DDT, aldrin, dieldrin and a few other materials less widely used. Two tasting tests are briefly described. E. M. J.

Toxicologic considerations of 2 : 2-bis-(*p*-chlorophenyl)-1 : 1-dichloroethane (TDE, DDD). H. B. Haag and C. Kampmeier (*Agric. Chemicals*, 1955, **10,** 85, 123—126).—A review. A. H. CORNFIELD.

Malathion and its formulations. J. F. Yost, I. B. Frederick and V. Migrdiehian (*Agric. Chemicals*, 1955, **10,** 43—45, 137, 138).—The stability of malathion emulsifiable concentrates, the effects of adding various chemicals on this stability and the effects of pH, moisture,

temp. and type of filler on the stability of malathion in wettable powder formulations are reported. A. H. CORNFIELD.

Effects of diluents on the acaricidal action of malathion and aramite in dusts. F. F. Smith, E. L. Gooden and E. A. Taylor (*J. econ. Ent.*, 1955, **48**, 762—763).—When tested against two-spotted spider mites, *Tetranychus telarius*, Aramite (1·5%) was highly effective with $CaCO_3$, talc or pyrophyllite: these dusts gave higher mortalities after 24 hr. than did those prepared with other diluents. However, after one week dusts containing diatomite, montmorillonite and a special fine attapulgite gave higher mortalities, especially of newly hatched larvæ. The same diluents were effective with malathion (4%) as well as kaolin, diatomite, and special fine attapulgite. Malathion with normal particle-size attapulgite or with montmorillonite gave lower mortalities. There was little correlation of effectiveness with particle size of diluent, its bulk density or its pH, although both Aramite and malathion were more toxic to mites when diluted with the special fine attapulgite than with the normal particle-size grade. A. A. MARSDEN.

Dieldrin seed dressings. A. J. A. Pearson (*J. Sci. Food Agric.*, 1956, **7**, Suppl. Issue, s66—s72).—The position of dieldrin as a seed dressing is surveyed. The very low phytotoxicity of the insecticide coupled with its persistent and high biological activity are appropriate for this purpose. E. M. J.

A surface aliquot masking technique for the bioassay of lindane. B. Davidow and E. P. Lang (*J. econ. Ent.*, 1955, **48**, 659—661).—This method is based on the presumption that excessive concn. of lindane deposited on a surface may be assayed by taking an aliquot of the surface area. This was prepared by masking the remaining portion of the experimental area thus limiting the mortality of flies under count. A. A. MARSDEN.

A magnetically suspended insect cage. G. H. Kaloostian (*J. econ. Ent.*, 1955, **48**, 756—757).—A small, light-weight cage for confining aphids used in virus-vector investigations is described and illustrated. The cage is held on a leaf by means of a small magnet placed on the opposite side of the leaf. A. A. MARSDEN.

Translocation of streptomycin and tetracycline in cuttings of *Pyracantha*. S. M. Alcorn and P. A. Ark (*Phytopathology*, 1955, **45**, 692—693).—The length of cutting remaining healthy after inoculation with *Erwinia amylovora* followed by immersion in a solution of the antibiotic serves as a measure of the translocation of the latter. A. G. POLLARD.

Daphnids help to screen systemics. E. H. Wollerman and L. S. Putnam (*J. econ. Ent.*, 1955, **48**, 759—760).—The use of a small arthropod (*Daphnia pulex* de Greer) as a biological indicator in the detection of systemic insecticides is described. Daphnid tests made with the foliage from black locust trees grown in soil treated with 200—800 ml. of Systox (32% demeton) showed 100% mortality in a 1-hr. test carried out 11 days after treatment. Applications of 2 and 10 ml. of Systox made to each tree resulted in little or no daphnid mortalities on the foliage in a 1½-hr. test 49 days after treatment but a dose of 50 ml. killed 88% of the daphnids after this time. A. A. MARSDEN.

Reaction of small grain varieties to greenbug attack. R. G. Dahms, T. H. Johnston, A. M. Schlehuber and E. A. Wood (*Okla. agric. Exp. Sta.*, 1955, *Tech. Bull.* **55**, 61 pp.).—Many of the barley varieties tested showed a high degree of resistance to greenbug (*Toxoptera graminum*, Rond). attack. Most of the highly resistant varieties originated in China, Korea and Japan. Resistance to greenbug attack is inherited. Resistance was in nearly all cases dominant to susceptibility. None of the wheat and rye varieties tested showed a high degree of resistance, although several were more resistant than varieties now grown in the hard winter area. Although there was some variation in the reaction of oat varieties to greenbug attack, none showed a high degree of resistance. A. H. CORNFIELD.

Timing treatments for control of snow mould of winter wheat. R. Sprague (*Phytopathology*, 1955, **45**, 696).—The mould was controlled by Ceresan if applied at the first appearance of basidiospores (Nov. 1—15). Later applications (Dec.) were much less effective. A. G. POLLARD.

Effect of 2 : 4-dichlorophenoxyacetic acid on wheat, oats and barley. W. C. Shaw, C. J. Willard and R. L. Bernard (*Ohio agric. Exp. Sta.*, 1955, *Res. Bull.* **761**, 17 pp.).—The cereals were most tolerant to 2 : 4-D applications after the plants were well-tillered but before the two nodes of the stem were visible above the rosette and again after the plants had blossomed until harvested. The most susceptible stages were prior to the appearance of tillers and again after the second node of the stem appeared until after bloom. When 2 : 4-D was applied, at rates normally used for weed control, to weed-free grains at the most tolerant stages of growth no injurious effects were noted. When applied at susceptible stages there were reductions in yields, increases in protein content, reduction in germination % of oats and in germination vigour of wheat and impairmant of milling and baking quality of wheat. A no. of morphogenic abnormalities arising from the treatment are described. At equiv. rates of 2 : 4-D acid the Bu ester was more phytotoxic than was the amine salt formulation. Legume stands with under-seeded small grains were reduced when the cereals were treated with 2 : 4-D although there was not always a reduction in yields of the legume. A. H. CORNFIELD.

Insecticides in mineral oil emulsions for control of the maize earworm and fall armyworm in sweet maize. R. A. Blanchard, G. P. Wene, W. A. Douglas and H. K. Gouck (*J. econ. Ent.*, 1955, **48**, 714—718).—DDT and endrin in mineral oil emulsions gave the best control of maize earworms, whilst toxaphene, TDE and heptachlor were almost as effective. Aldrin, the F analogue of DDT, pyrethrum, lindane, allethrin and Isodrin were unsatisfactory. Although $C_2H_4Cl_2$ was highly toxic it was too volatile for use in an oil emulsion spray. Toxaphene caused burning of the maize. DDT, lindane and TDE all gave excellent kill of the fall armyworm, whilst the F analogue of DDT and allethrin gave 85 and 62% reduction, respectively, of this pest. A. A. MARSDEN.

Immunity to virus X in potato: selection of immune plants in the breeding programme. R. G. Timian, C. E. Peterson and W. J. Hooker (*Amer. Potato J.*, 1955, **32**, 411—417).—Methods used for selecting plants immune to virus X are described. A. H. CORNFIELD.

Radiation and resistance of tubers to rot. P. E. Waggoner (*Amer. Potato J.*, 1955, **32**, 448—450).—Exposure of tubers to γ-radiation in doses sufficient to inhibit sprouting had no effect on the resistance to infection of wounds by *Erwinia atroseptica* and *E. carotovora*. The treatment had no effect on diffusion of nutrients from wounds or on maceration, but inhibited periderm formation somewhat. A. H. CORNFIELD.

Stem streak necrosis of potato in Prince Edward Island. D. B. Robinson and L. C. Callbeck (*Amer. Potato J.*, 1955, **32**, 418—423).—Stem streak necrosis was most severe at low soil pH (~4·5) and was decreased by liming and by application of complete fertiliser. Application of Mn increased the severity of the disease whilst application of Zn, Mo and Mg had no effect. There were varietal differences in susceptibility to the disease. A. H. CORNFIELD.

Effects of pesticides on yields and specific gravity of potatoes. R. Bonde and M. Covell (*Amer. Potato J.*, 1955, **32**, 399—406).—Sp. gr. of tubers was lowered by factors (defoliation by late blight or insect attacks) which caused premature death of foliage. Sprays (e.g. Bordeaux mixture and tribasic $CuSO_4$) which controlled pests generally increased both yields and sp. gr. of tubers. DDT spraying of foliage had no effect on sp. gr. of tubers. Fungicide 1124 (dinitrophenyl thiocyanate) and Fungicide 1189 (oxygenated hexachlor-*cyclo*pentadiene) were more effective than nabam, Bordeaux mixture and tribasic $CuSO_4$ in increasing yields but produced tubers with lower sp. gr. A. H. CORNFIELD.

Effect of powdery scab on the resistance of potato tubers to late blight rot. R. Bonde (*Maine agric. Exp. Sta.*, 1955, *Bull.* 538, 11 pp.).—When a no. of varieties of potato were planted in soil infected with powdery scab, there was a positive relationship between the % of scab sori and the extent of late-blight infection. Some of the varieties became infected with late-blight only through scab sori. There were varietal differences in susceptibility to scab infection. Recommendations for control are given. In general, soil infected with scab should not be planted to potatoes. A. H. CORNFIELD.

Relation of soft rot development to protective barriers in Irish potato slices. W. L. Smith, jun. and H. F. Smart (*Phytopathology*, 1955, **45**, 649—654).—Slices of potatoes removed from cool storage (40°F.) were kept at various temp. (40—80°F.) for 1—4 days in moist chambers and then inoculated with *Erwinia atroseptica*. After four days at 70—80°F. inoculation produced little or no decay in the slices. After four days at 50—60°F. the rot produced by inoculation was similar to that occurring after keeping for two days at 70—80°. The rate of formation of a protective barrier (suberin and periderm) was minimal at 40°F. and no inhibition of infection was apparent; barrier development increased with rise in temp. above 40°F. at rates which showed varietal differences A. G. POLLARD.

Selection of sugar-beets for resistance to *Cercospora*. J. Margara and H. Touvin (*C. R. Acad. Agric. Fr.*, 1956, **42**, 71—73).—Breeding trials with sugar beet varieties, carried out with the object of producing strains which will combine resistance to *Cercospora* with good yields of sugar, are discussed and some typical results tabulated. J. S. C.

Yellows: a virus problem in sugar beet. R. Hull (*J. Sci. Food Agric.*, 1956, **7**, Suppl. Issue, s20—s24).—The occurrence and control of yellows in Great Britain is reviewed. Since 1945 the average

annual loss of yield on 400,000 acres of sugar beet has been 311,000 tons. The incidence of the disease, carried by overwintering *Myzus persicæ*, could be reduced by spraying, but infection by winged aphids coming from nearby virus sources is not prevented by this means. E. M. J.

Control of the sweet potato weevil and several insects attacking roots of sweet potatoes in the field. E. H. Floyd (*J. econ. Ent.*, 1955, **48**, 644—648).—Weekly applications of Ca arsenate dust for eight weeks significantly reduced sweet potato weevil infestations. Aldrin (1—3 lb. per acre) and chlordane (1—10 lb. per acre) gave considerable protection to the roots from sweet potato weevils and other soil insects. The method of application used had little effect on the control obtained. No off-flavours were detected in potatoes grown in treated soils. A. A. MARSDEN.

Control of the spotted lucerne aphid on lucerne in Southern California. H. T. Reynolds and L. D. Anderson (*J. econ. Ent.*, 1955, **48**, 671—675).—This pest was very easily killed by many insecticides, but control was often difficult because of mass migration from neighbouring fields. The following materials gave excellent results when used as sprays: Systox (2—4 oz. per acre), Meta-Systox (4 oz.), parathion (2—4 oz.), malathion (8—16 oz.), diazinon (11 oz.), American Cyanamid 12008 (3·2 oz.), endrin (0·3 lb.), and in most cases, toxaphene (3 lb.), and TEPP (1 pint)–DDT (1 lb.). Good results were obtained with TEPP, rotenone, and DDT. Nicotine, amongst other materials, gave poor control. A toxaphene (15)–DDT (5%) dust at 24 lb. per acre gave excellent control and was superior to toxaphene dust alone or a parathion dust. A. A. MARSDEN.

Physiology and nature of disease development in winter crown rot of lucerne. J. B. Lebean and J. G. Dickson (*Phytopathology*, 1955, **45**, 667—673).—A low-temp. basidiomycete associated with the crown rot is examined in culture. Mycelial growth was considerable at 4° and was optimal at about 12°. HCN was produced by the organism and its absorption in lethal concn. by the crown tissue of lucerne was associated with the development of crown rot. Conditions favouring the production of HCN were also those in which crown rot occurred with particular severity. A. G. POLLARD.

Control of common lucerne insects in Wisconsin. J. T. Medler (*J. econ. Ent.*, 1955, **48**, 718—723).—No single insecticide was completely effective against the injurious insects potato leafhoppers, mirids and grasshoppers attacking lucerne whether grown for forage or for seed production. A DDT/dieldrin or similar mixture gave the best control of these pests in seed lucerne. For forage production, parathion was effective but its toxicity limited it for practical uses. A mixture of methoxychlor or Perthane for leafhoppers, and aldrin or heptachlor for mirids and grasshopper control is recommended. A. A. MARSDEN.

Control of major hay crop pests in New Jersey. L. G. Merrill and R. S. Filmer (*New Jersey agric. Exp. Sta.*, 1955, *Ext. Bull.* 287, 8 pp.).—Details of the methods used for controlling meadow spittlebug, pea aphid, lucerne weevil and potato leafhopper in lucerne and clover are described. A. H. CORNFIELD.

Apple insects in Maine. F. H. Lathrop (*Maine agric. Exp. Sta.*, 1955, *Bull.* 540, 88 pp.).—Characteristics, types of injury caused and control of the important insects attacking apples are presented. A. H. CORNFIELD.

Summer control of the apple aphid. E. H. Glass and P. J. Chapman (*J. econ. Ent.*, 1955, **48**, 695—697).—The 20 insecticides tested were toxic to *Aphis pomi* but most of them had little residual activity: TEPP, parathion, malathion, chlorthion, diazinon, lindane, and nicotine sulphate were included in this group. Some systemic insecticides such as demeton, Meta-Systox, Isolan and G-22870 [3-methylpyrazol-5-yl *NN*-dimethylcarbamate] gave very effective kill and also prevented reinfestation for up to three weeks. A. A. MARSDEN.

Stone fruit virus investigations. I. Inoculation studies of the ring spot virus complex in sweet cherry. D. F. Millikan, jun. (*Missouri agric. Exp. Sta.*, 1955, *Res. Bull.* 582, 23 pp.).—The ring spot complex found on sweet cherry, although closely related to that found on sour cherry, produced symptoms somewhat different from the latter. Sweet cherry varieties fell into two groups with respect to the type of response to ring spot inoculation. A. H. CORNFIELD.

Emergency measures directed against Mexican fruit fly threat to California. H. M. Armitage (*J. econ. Ent.*, 1955, **48**, 657—659).—Eradication measures include the establishment of a protective barrier involving the frequent use of an attractive poison-bait (sugar–tartar emetic) spray to all host trees lying within five miles of the Californian border. Isodrin (4 lb. per acre) was also added to the upper 2 in. of soil under all host trees to control mature larvæ entering the soil for pupation, and/or emerging adults. A. A. MARSDEN.

Control of peach catfacing insects in Illinois. S. C. Chandler (*J. econ. Ent.*, 1955, **48**, 635—638).—Tarnished plant bugs and stinkbugs were killed with DDT, dieldrin or parathion. Only the two last-named insecticides killed both plum curculios and the sucking insects, and of these, dieldrin was superior both as a spray and as a dust. Orchard tests showed that early applications gave the best results, preferably at the pink bud stage. A. A. MARSDEN.

Ovicidal action of parathion to eggs of the peach tree borer. E. H. Smith (*J. econ. Ent.*, 1955, **48**, 727—731).—Eggs treated with parathion at all stages of development respired normally until the seventh day and this rate then declined for several days beyond the normal time of hatching. The depressed respiratory rate is probably a secondary effect of poisoning. Parathion entered the egg as a vapour, younger eggs being more permeable. The toxicant taken up at any stage remained in the egg as such or was metabolised to a more active anticholine-esterase which persisted in the egg: its inhibitory action was lethal only when embryonic development neared completion. A. A. MARSDEN.

Peach insect investigated at Fort Valley, Georgia, during 1954. O. I. Snapp (*J. econ. Ent.*, 1955, **48**, 734—736).—Aldrin, dieldrin or heptachlor worked into the soil were all highly effective against immature stages of plum curculios. Parathion, malathion, aldrin, dieldrin and heptachlor all controlled curculios, but endrin was unsatisfactory. Residue analyses showed that aldrin, dieldrin and heptachlor should be used only in the early part of the spray season: harvest residues of malathion were negligible. Strobane, chlorthion, Perthane alone or with Darvan gave promising results in preliminary cage tests. $C_6H_6Cl_6$ and DDT were superior to parathion in trunk sprays to control peach tree borers, *Sanninoidea exitiosa*, but trunk sprays alone were inadequate in heavily infested orchards. $C_2H_4Cl_2$ and trichlorobenzene emulsions gave excellent control of this pest, although the latter material caused some injury to young trees. Against the lesser peach tree borer, *Synanthedon pictipes*, parathion and EPN were more effective than malathion for preventing infestation of peach trees: applications in the spring and autumn are recommended. A. A. MARSDEN.

Control of insect pests, diseases and deficiencies in citrus. F. J. Stofberg and F. C. Loest (*Fmg S. Afr.*, 1955, **30**, 436—438).—Commercial insecticides and fungicides and formulæ are reviewed. E. G. BRICKELL.

Field screening tests with various materials against the European brown snail on citrus in California. J. L. Pappas and G. E. Carman (*J. econ. Ent.*, 1955, **48**, 698—700).—Of 32 prep. tested against *Helix aspersa*, isolan was the most effective, followed by Compound G-341 (1-ethyl-3-methylpyrazol-5-yl dimethylcarbamate) and pyrolan. Preliminary tests with baits and sprays of Isolan gave promising results against this pest. A. A. MARSDEN.

Fumigation trials with ethylene dibromide for the control of eggs and larvæ of *Ceratitis capitata* (Wied.) in citrus fruit. A. Grunberg, K. Polac'ek, — Eng and J. Peleg (*Bull. ent. Res.*, 1956, **46**, 803—811).—Jaffa and Valencia oranges can tolerate a concn. of 18 g./m.^{3} for 2½ hr.; for grapefruit it is 15 g./m.^{3} and a 75% kill of the larvæ of *Ceratitis* inside the fruit is obtained. The treatment for this purpose ethylene dibromide (**I**) is compatible with the customary use of Decco (NCl_3) and with an **I** concn. of 6 g./m.^{3} for 6 hr. the combined treatment gave a kill of 93·5% to 96·4%. E. G. BRICKELL.

Control of flower thrips on blackberries. H. H. Tippins and L. L. Hyche (*J. econ. Ent.*, 1955, **48**, 769—770).—Parathion and malathion treatments gave highly significant control of the flower thrips, *Frankliniella tritici*, on blackberries. TEPP was only moderately effective, whilst nicotine sulphate failed to give adequate control. Residues on blackberries picked four days after a second application of parathion or malathion were $<0·5$ p.p.m. A. A. MARSDEN.

Insect pests of strawberries in Ohio. R. B. Neiswander (*Ohio agric. Exp. Sta.*, 1955, *Res. Bull.* 763, 31 pp.).—The characteristics of the seven important and 10 lesser species of insects attacking strawberries in Ohio and methods for their control are described. A. H. CORNFIELD.

Effects of soil treatments with insecticides on residues and fruit quality of strawberries. G. G. Gyrisco, W. G. Evans, R. H. Burrage and A. M. Briant (*J. econ. Ent.*, 1955, **48**, 700—703).—The flavour and odour of the fresh or frozen fruit was satisfactory in strawberries grown in soil treated with aldrin, dieldrin, lindane or heptachlor (1, 2 or 4 lb. per acre). However, the flavour of jam made from fruit grown on aldrin-treated plots had a significant off-flavour: this did not apply to jams prepared from fruit grown in soil treated with the other three insecticides. All residues in the fresh frozen fruit were $<0·1$ p.p.m. A. A. MARSDEN.

Effects of cycloheximide on powdery mildew of grapes and brown rot of peach fruits in the laboratory. J. M. Ogawa and C. Vergara

(*Phytopathology*, 1955, **45**, 695).—Spraying or dusting the rapidly growing shoots of vines with *cyclo*heximide (**I**) in concn. 0·5—2·0 p.p.m. protected them from infection by the mildew, *Unicula necator*. On vines already infected, **I** sprays (2 p.p.m.) eradicated the disease with only slight damage to the infected tissue. Dusts were ineffective for this purpose. Peaches were protected from brown rot infection (*Sclerotinia fructicola*) by spraying with **I** (100 p.p.m.). In concn. of 50 p.p.m. **I** eradicated the fungus in infected fruit. A. G. POLLARD.

Control of pineapple scab. A. R. Brimblecombe (*Qd J. agric. Sci.*, 1955, **12**, 81—94).—Repeated applications of white oil–nicotine sprays were of little value in controlling scab. Application of 0·1% parathion sprays gave better control than did 0·1% γ-$C_6H_6Cl_6$ or 0·5% chlordane sprays. Fruit picked within five weeks of applying $C_6H_6Cl_6$ had definite off-flavours. Infestations varied considerably in density from year to year. A. H. CORNFIELD.

Marked suppressing action of schradan on the walnut aphid. A. E. Michelbacher and E. Oatman (*J. econ. Ent.*, 1955, **48**, 768—769).—One spray treatment of schradan at 1·5 to 2·5 lb. of toxicant per acre, thoroughly applied in early May, resulted in excellent aphid suppression for a full year. Schradan treatments at the above dosages had also a residual controlling action against crawlers of the frosted scale, *Lecanium pruinosum*, and were effective against the Pacific spider mite, *Tetranychus pacificus*, but not the European red mite, *Metatetranychus ulmi*. A. A. MARSDEN.

Control of the pecan weevil. H. A. Hinrichs and H. J. Thomson (*Okla. agric. Exp. Sta.*, 1955, *Bull.* 450, 12 pp.).—The life history and nature of damage caused by the pecan weevil (*Curculio caryæ*, Horn) are described. Satisfactory control was obtained by spraying with DDT (4 lb. 50% wettable powder per 100 gal.) in early August (when the " tree-shaking " test brought down more than five insects per tree) followed by a second spray after the first heavy rain, or when rain did not fall, on September 1st. A. H. CORNFIELD.

Effect of acrylonitrile fumigation on diapause in the walnut husk fly. D. L. Lindgren and C. Gammon (*J. econ. Ent.*, 1955, **48**, 752—753).—Pupæ of *Rhagoletis completa* of varying ages (10—193 days old) were fumigated with acrylonitrile at a dosage of 2·5 and 3 lb. per 1000 cu. ft. for 3 hr. The emergence of adult flies observed 30 to 280 days after treatment was always greater for the treated pupæ than for untreated controls. Some mortality of pupæ from this treatment occurred but only at a dosage of 3 lb. for 3 hr. A. A. MARSDEN.

Effect of ingredients in insecticides on the behaviour of the Japanese beetle. J. R. Foster (*J. econ. Ent.*, 1955, **48**, 703—706).—Field tests of the attractant and repellent qualities of various ingredients used in insecticidal treatments against Japanese beetles are reported. The response of the beetles to these test materials was so erratic that it was difficult to determine the repellency or attractiveness, if any, of the materials. Results of trapping experiments showed that beetles were not apparently attracted into maize fields by the presence of DDT in the insecticide. A. A. MARSDEN.

Tomato spraying in the absence of late blight. W. S. Beach (*Pa agric. Exp. Sta.*, 1955, *Bull.* 603, 9 pp.).—Results of various spraying and dusting treatments on yields of tomatoes over five years when late blight was not present are presented. Maneb and zineb alone, or ziram and maneb in combination with tribasic $CuSO_4$ or with each other gave very similar average increases in yields. These increases represented control of early blight, anthracnose and certain fruit rots. Spray treatments were somewhat more effective than were corresponding dust treatments. A. H. CORNFIELD.

Control of potato aphid on tomatoes. E. F. Taschenberg and A. W. Avens (*J. econ. Ent.*, 1955, **48**, 685—688).—Of seven org. P aphicides tested against *Macrosiphum solanifolii*, Systox (1 oz. per 100 gal.) was the most effective material. Parathion gave a high degree of control, followed by malathion and EPN. Malathion and parathion residues on tomato fruits decreased rapidly and were $<1{\cdot}0$ p.p.m. within seven days. A. A. MARSDEN.

Some factors affecting substrate colorisation and growth of the *Verticillium* wilt fungus in soil. S. Wilhelm (*Phytopathology*, 1955, **45**, 696).—In autoclaved soils *Verticillium* grew vigorously. In soils treated with chloropicrin (400 lb. per acre) growth was less and in those treated with ethylene dibromide only slight development occurred. A. G. POLLARD.

Reductions of insecticidal residue on mature green-wrap tomatoes. D. O. Wolfenbarger and C. H. Van Middelem (*J. econ. Ent.*, 1955, **48**, 744—746).—Analyses of residues on tomato fruits sprayed with DDT, demeton, Dilan, EPN, malathion, parathion and TDE were all <2 p.p.m. at 0·2 day after the final spray application. The rates of residue reductions were plotted on semilogarithmic paper and showed straight-line relationships. A. A. MARSDEN.

Survey of soil pests affecting vegetable crops. D. W. Wright (*J. Sci. Food Agric.*, 1956, **7**, Suppl. Issue, s9—s12).—A summary of present-day methods of control of cabbage root fly, carrot fly and cabbage stem weevil is presented, including results of recent research: improvement in yield of summer cauliflowers; treatment of seed beds of brassica and Primo cabbage; root dips for Primo cabbage; control of carrot fly and of stem weevil by lindane seed dressings. Careful timing of the insecticidal treatment, method of application, dosage rate and type of material used are important in preventing damage to useful predatory insects. E. M. J.

Application of materials to control pests and diseases of pea crops. R. W. Shorrock (*J. Sci. Food Agric.*, 1956, **7**, Suppl. Issue, s37—s46).—Of insect pests attacking peas, three are important: the pea weevils, the pea aphis or greenfly and the pea moth, but thrips, midge and leaf miners cause damage also. Control measures are discussed. Aërial spraying against these pests is unreliable. Downy mildew (causing serious losses in some seasons) and powdery mildew are the main diseases affecting the crop. No effective control is available. Seed dressing with thiram reduces losses from air-borne fungi. E. M. J.

Treatment of pea, snap bean and lima seed with insecticides and fungicides. L. P. Ditman, C. E. Cox and J. G. Kantzes (*J. econ. Ent.*, 1955, **48**, 688—693).—Results of all seed treatments were very variable, depending to a large extent on weather and soil conditions. Within these limits good results were obtained with all seed treatments. Captan and thiram were more satisfactory than chloranil. Lindane, chlordane, aldrin and dieldrin emulsions all gave about equally good results for control of seed-corn maggot. A. A. MARSDEN.

Effectiveness of certain residual insecticides in preventing emergence of the bean weevil from infested bean seeds. R. F. Ruppel (*J. econ. Ent.*, 1955, **48**, 757—758).—Heptachlor and aldrin were the most promising materials for the residual insecticidal treatment of bean seed infested by bean weevils, *Acanthoscelides obtectus*. A dosage of 1·0 and 0·5 kg., respectively, of the active material per metric ton of seed gave good results. $C_6H_6Cl_6$ retarded the germination of the seeds, whilst chlordane and dieldrin reduced the % germination of the seeds. Isodrin, although highly effective, was so toxic that its general use for seed treatment was impractical. A. A. MARSDEN.

Control of pests of brassica seed crops. S. G. Jary (*J. Sci. Food Agric.*, 1956, **7**, Suppl. Issue, s33—s36).—Three major pests are concerned with direct loss of seed on brassica crops: the blossom beetle *Meligethes æneus* (F.), the seed weevil *Ceuthorrhynchus assimilis* (Payk) and the bladder-pod midge *Dasyneura brassicæ* (Winn); there are also the mustard beetle *Phaedon cochleariæ* (F.) and the mealy cabbage aphis *Brevicoryne brassicæ* (L.). Control measures are described; precautions against injury to honey bees are discussed. E. M. J.

Anthracnose in water melons. H. Boellma (*Fmg S. Afr.*, 1955, **30**, 444, 452).—Symptoms, damage and conditions for attack by *Colletotrichum lagenarium*, (Pass.) Ell. and Halst. are described. Zineb (sprayed or dusted), captan and TMTD give control. E. G. BRICKELL.

Control of hop aphid and two-spotted spider mite in Oregon. H. E. Morrison and B. G. Thompson (*J. econ. Ent.*, 1955, **48**, 706—710).—Although TEPP and parathion were effective on both hop pests they were also very toxic to their natural enemies. Demeton was superior to schradan, parathion, malathion, diazinon and Meta-Systox in hop pest control. Demeton (1—4 oz. per acre) gave good control of both species of hop pests, and at the rate of 8 oz. per acre gave control for 33 days. The successful application of demeton to hops through a sprinkler irrigation system is described. A. A. MARSDEN.

Mode of action of the wildfire toxin. A. C. Braun (*Phytopathology*, 1955, **45**, 659—664).—Biological tests using *Chlorella*, together with chemical evidence, establish the toxin of tobacco wildfire as a structural analogue of methionine towards which it acts as an anti-metabolite. A. G. POLLARD.

Fumigation of agricultural products. XIII. Trials of onion seed treated with methyl bromide, and an improved method for its analysis. O. F. Lubatti and R. E. Blackith (*J. Sci. Food Agric.*, 1956, **7**, 149—159).—The fumigation of onion seed with MeBr, over a range of moisture contents, was studied. An improved catalytic combustion unit for MeBr determination is described. Germination of seeds in soil gives clearer and better evidence of the extent of damage to moist seed by fumigation than laboratory tests on paper pads. Seed dry enough to be stored with unimpaired germination should not contain $>10\%$ of moisture and will then withstand concentration–time products of 1100 mg. hr./l., whether applied at high doses for a short time or low doses for a longer period. Dry seed properly

fumigated shows a slightly enhanced but also slightly delayed total germination which is of small practical interest. (20 references.) J. S. C.

Seed disinfection. XII. Glasshouse tests for control of damping off of *Pinus radicata* seed. H. Jacks (*N.Z. J. Sci. Tech.*, 1956, **37A,** 427—431).—Captan, various dithiocarbamates, org. Hg compounds and quinones were used to control damping-off of seedlings of *Pinus radicata*, Betanal (2·8% Hg as methoxyethyl Hg silicate), captan and chloranil giving the most successful seed treatments. Post-emergence infection was not completely controlled, but was substantially reduced. E. M. J.

Biology and control of the European pine shoot moth. W. E. Miller and R. B. Neiswander (*Ohio agric. Exp. Sta.*, 1955, *Res. Bull.* 760, 31 pp.).—Characteristics of the pest and nature of injury to pine are described. Sprays of org. pesticides were more effective than dusts in controlling the pest and better results were obtained with power ground equipment than with aëroplanes. One application of DDT (1—2 lb. per 100 gal.) in mid-April, late June or early July gave excellent control. A. H. CORNFIELD.

Control of black locust insects by systemics. E. H. Wollerman, C. R. Reese and A. S. Kiefer (*J. econ. Ent.*, 1955, **48,** 760—761).—Application of demeton (200—400 ml.) to the soil around black locust trees was rapidly translocated to the foliage, as shown by the best animal responses. Data from these tests indicated a relationship between the responses of *Enchenopa binotata* and *Daphnia pulex* to foliage from the same treated tree. Foliar toxicity persisted over a long period of time, 100% mortality of *E. binotata* resulting 14 days after soil applications of 200 and 450 ml. of demeton to black locust trees. A. A. MARSDEN.

Effect of wood moisture content on the emergence of southern lyctus beetle. R. H. Smith (*J. econ. Ent.*, 1955, **48,** 770—771).—Approx. 34% more beetles, *Lyctus planicollis*, emerged from wood (red and white oak, and hickory) having a 12% moisture content than from wood with an 8% moisture content. Increase of moisture content of the wood did not accelerate emergence of the beetles. A. A. MARSDEN.

New mosquito repellents. I. H. Gilbert, H. K. Gouck and C. N. Smith (*J. econ. Ent.*, 1955, **48,** 741—743).—Of the eleven repellents tested against *Aedes ægypti*, *A. tæniorhynchus*, *A. sollicitans*, *Psorophora confinnis* and *Anopheles quadrimaculatus*, the most consistently effective compound was *NN*-diethyl-*m*-toluamide (20218), which was at least equal to the standard repellent used against each species. Its *ortho*-isomer, 20217, was rather less effective against several other species but still as effective as the standard. Several other compounds were repellent to one or more species of the insects under test. A. A. MARSDEN.

Control of mosquito larvæ in rice fields with water-soluble phosphorus insecticides. J. B. Gahan and J. R. Noe (*J. econ. Ent.*, 1955, **48,** 665—667).—Water-sol. parathion (0·01 p.p.m.) was completely effective against floodwater *Psorophora* mosquitoes after flowing $>\frac{1}{2}$ a mile through a canal and 400 ft. through a rice field. Very good control also occurred in plots treated at the rate of 0·005 p.p.m., but some live larvæ were found. Bayer L13/59 and Shell OS 2046 (2-carbethoxy-1-methylvinyl dimethyl phosphate) gave 100% control at 0·05 and 0·1 p.p.m., and DDVP (dimethyl 2 : 2-dichlorovinyl phosphate) was highly effective at 0·05 and 0·1 p.p.m. The residual toxicity of these treatments was insufficient to control *Anopheles quadrimaculatus* and *Culex erraticus* larvæ. A. A. MARSDEN.

Acaricides in insect vector virus research. H. R. Wolfe (*J. econ. Ent.*, 1955, **48,** 749—750).—When required for the control of spider mites without harming those insects used in insect vector virus research, sprays of chlorobenzilate, methyl-chlorobenzilate and Mitox (*p*-chlorobenzyl *p*-chlorophenyl sulphide) caused no apparent harm to leafhoppers and aphids under greenhouse conditions. Chlorbenzilate ($\geq$2 lb. per 100 gal.) and Mitox ($\geq$4 lb.) had no significant effect on survival of all stages of the geminate leafhopper, *Colladonus geminatus*. At the dosages used, most greenhouse plants tested were not damaged by these materials. A. A. MARSDEN.

The elegant grasshopper. E. K. Hartwig (*Fmg S. Afr.*, 1955, **30,** 430—432, 450).—Distribution, life cycle and habits of *Zonocerus elegans*, Thunb. are described. Control by E605 and malathion sprays or a mealie meal–porridge bait containing Na_2SiF_6 or a phosphorus ester is recommended. E. G. BRICKELL.

Chemical control of wireworms (*Agriotes* spp.). I. The direct and residual effects of BHC, DDT, D-D and ethylene dibromide. C. Potter, M. J. R. Healy and F. Raw (*Bull. ent Res.*, 1956, **46,** 913—923).—All treatments were effective but BHC broadcast and combine-drilled, and DDT combine-drilled gave the best results. BHC applied to the soil at rates of 1·96 lb./acre and upwards may taint root crops, at least in the second year after treatment. E. G. BRICKELL.

The khapra beetle, *Trogoderma granarium*, Everts. D. L. Lindgren, L. E. Vincent and H. E. Krohne (*Hilgardia*, 1955, **24,** 1—36).—Distribution, description and habits of the khapra beetle are described. Increases in temp. decreases average length of generation but to obtain a 95% kill at 50% RH a temp. of 118°F. for 8 min. is required. HCN and acrylonitrile were the most toxic fumigants to all three immature stages and of contact insecticides it was found that the methyl analogue of parathion, Compound 4124 [*OO'*-dimethyl *O*-(2-chloro-4-nitrophenyl) thiophosphate], parathion, malathion, Pyrocid 175, Compound 12008 (*OO*-diethyl *S-iso*propylmercaptomethyl dithiophosphate) and chlorthion were the most toxic to fourth-instar larvæ. E. G. BRICKELL.

Cutworms of Pennsylvania. S. W. Frost. **Control of cutworms and army worms.** J. O. Pepper (*Pa agric. Exp. Sta.*, 1955, *Bull.* 596, 1—28, 28—29).—The characteristics of cutworms are described and a field key to the cutworms of Pennsylvania is presented. Cutworms and army worms are controlled by applying 5% chlordane, DDT or toxaphene dusts at 30 lb. per acre or the same materials as sprays at 1 lb. per acre. A. H. CORNFIELD.

General survey of eelworm problems. B. G. Peters (*J. Sci. Food Agric.*, 1956, Suppl. Issue, s6—s8).—Of the approx. 100 species of eelworms known, about a dozen are of economic importance. On account of the differences in structure, life history and behaviour of these different eelworms, each eelworm problem should be dealt with on its own terms. The genera *Hederodera*, *Ditylenchus*, *Anguinea* and *Aphelenchoides* infesting root, shoot, inflorescence (of *Graminæ*) and leaf tissues respectively, the method of attack of eelworms on the plant cells, within or from the outside, and measures used in control are discussed. E. M. J.

Use of maleic hydrazide [as weedkiller]. G. F. Harding and G. O. P. Eaton (*J. Ph. Adm.*, 1955, **19,** 371—375).—Maleic hydrazide (**I**) applied at the rate of 4 or 6 lb. in 40—100 gal. of water per acre effectively retarded the growth of grasses, no varietal differences in response being apparent. 2 : 4-D did not alter the effect of **I** on grasses and remained fully effective against broad-leaved weeds when used in combination with **I**. HORT. ABSTR. (A. G. P.).

Control of weeds in strawberries. Anon. (*Agric. Chemicals*, 1955, **10,** 47).—DNPB (8 lb. per acre) was the most satisfactory pre-planting treatment for controlling weeds in strawberries. Seven post-planting treatments with SES (3 lb. per acre) at monthly intervals gave good control. Excellent control of chickweed was obtained by applying CIPC (3 lb. per acre) in mid-Nov. or mid-Feb. A. H. CORNFIELD.

Chemical weed control in rose nursery fields. B. E. Day and R. C. Russell (*Hilgardia*, 1955, **23,** 597—612).—3-(3 : 4-Dichlorophenyl)-1 : 1-dimethylurea, 3-(*p*-chlorophenyl)-1 : 1-dimethylurea, phenyl carbamates, dinitro-*o-sec.*-butylphenol and 3 : 6-endoxyhexahydrophthalate show promise. E. G. BRICKELL.

The slangbos problem. I. B. J. Smit (*Fmg S. Afr.*, 1955, **30,** 479—481, 488).—The invasion of natural pastures by *Stœbe vulgaris* is discussed together with aspects of its eradication. 2 : 4-D and 2 : 4 : 5-T ethyl esters are effective. E. G. BRICKELL.

Control of gorse. D. D. Hill (*Ore. agric. Exp. Sta.*, 1955, *Bull.* 553, 16 pp.).—Application of a mixture of 2 : 4-D and 2 : 4 : 5-T esters (3—5 lb. acid equiv. per acre) has given effective control of gorse for at least one year. Soil treatment with CMU (40—80 lb. per acre) or Ammate (3 lb. per rod) has also given good control. Cultural methods of control are also described. A. H. CORNFIELD.

Control of big sagebrush, *Artemisia tridentata*. D. N. Hyder and F. A. Sneva (*Ore. agric. Exp. Sta.*, 1955, *Tech. Bull.* 35, 16 pp.).—In general 2 : 4 : 5-T was more effective than was 2 : 4-D in controlling big sagebrush, although the latter material was more effective towards the end of the growing season. Spraying at any time during the growing season gave effective control. Application of 1 lb. of acid equiv. killed 78% and of 2 lb. of acid equiv. per acre killed 87% of individual plants. The Bu ester and the propylene glycol Bu ether ester of 2 : 4-D were equally effective. Solvents of water, diesel oil, or diesel oil emulsion were equally effective. Somewhat better control was obtained with 6 gal. than with 3 gal. of solution per acre. A. H. CORNFIELD.

Animal Husbandry

Antibiotics in animal nutrition. P. Van Dijck (*Agricultura*, 1955, **3,** 427—450).—A review. The mode of action of antibiotics in the animal system and factors concerned therein are considered. (64 references.) A. G. POLLARD.

Antibiotics and growth. R. Février, A. François, M. Michel, R. Péro and E. Salmon-Legagneur (*C. R. Acad. Agric. Fr.*, 1955, **41,**

698—708).—The mechanisms of antibiotic action on animal growth were studied. Aureomycin and penicillin increased the average daily growth of pigs by 13% and 8%, respectively. In chicks, the effect of penicillin depends on its antibiotic power. In the pig, the antibiotics are partially inactivated in the digestive tract but a part always remains active. The antibiotics, whilst having no effect on the utilisation coeff. of foods by the pig, inhibit deamination by intestinal flora, as shown by a direct relation between growth increase indices and deaminating effect for each antibiotic. $CuSO_4$ and 3-nitro-4-phenylarsonic acid have a similar effect on both deamination and growth. A mixture of aureomycin and chloramphenicol stimulates the growth and reduces the mortality of suckling pigs. Antibiotics, notably aureomycin, inhibit choline degradation by the bacterial flora of the digestive tract of the pig. Even if fed at double the normal rate, the storage of antibiotics in pig tissues is feeble or nil. (27 references.) J. S. C.

Molybdenum in animal nutrition. A. T. Dick (*Soil Sci.*, 1956, **81**, 229—236).—A review of work in Australia, chiefly with sheep. T. G. Morris.

Preservation of grass silage with sodium metabisulphite. J. W. Bratzler R. L. Cowan and R. W. Swift (*Pa agric. Exp. Sta.*, 1955, *Bull.* 597P, 6 pp.).—Details of the method are described. The material was much more effective than was molasses in reducing losses of total digestible nutrients during ensiling. A. H. Cornfield.

Hay crop silage treated with sodium metasulphite for winter feeding of dairy cows. C. J. Little (*Mich. agric. Exp. Sta. Quart. Bull.*, 1955, **38**, 252—257).—No significant advantages are reported. E. G. Brickell.

Nitrogen metabolism of the ensilage process. A. R. Kemble (*J. Sci. Food Agric.*, 1956, **7**, 125—130).—Overall changes in N distribution in laboratory silage, and, in particular, determinations of most of the monoamino-monocarboxylic acids were studied. In good silage, the free amino-acid content was less than the expected value calculated from the extent of protein breakdown, whereas, in bad silage, a considerable excess of alanine above that calculated on the assumption of uniform proteolysis developed during the first three weeks of fermentation and, after eight weeks, α-aminobutyric acid appeared. Ensilage of grass free from micro-organisms gave rise to extensive proteolysis and excess alanine but no free NH_3 was formed. The formation of NH_3 from amino-acids during ensilage may be due to anaërobic bacteria rather than to plant enzymes, but there is evidence to the contrary. Initial rapid proteolysis during ensilage is caused by enzyme action. The enzymes do not produce substances which change the pH from that of fresh grass. (10 references.) J. S. C.

Loss of carotene from dried green crop during storage. The gradient of loss through a stack. V. H. Booth (*J. Sci. Food Agric.*, 1956, **7**, 114—119).—Stacks of dried green crop meal were examined for carotene losses at various locations. Dry meal at the outer edges of stacks and at the open ends of tubes, each exposed to air, took up moisture and lost relatively little carotene during storage, whereas the inner contents of both stacks and tubes remained dry and lost more carotene. The greatest distance that measurable amounts of moisture penetrated through meal during six months was 22 cm. or half a sack width. The conclusion drawn is that preservation of carotene in storage depends on leaving an optimum moisture content in the green crop in drying. (11 references.) J. S. C.

Winter disorders in cows and ewes fed low-quality roughages. C. K. Whitehair and W. D. Gallup (*Okla. agric. Exp. Sta.*, 1955, *Tech. Bull.* 53, 31 pp.).—A no. of winter disorders (symptoms described) of cows and ewes fed on low-quality roughages were traced to lack of protein in the diets. A. H. Cornfield.

Producing and dehydrating sweet potatoes for livestock feed. F. B. Cross (*Okla. agric. Exp. Sta.*, 1955, *Bull.* 447, 7 pp.).—Sweet potatoes grown on sandy soils (which are not suitable for other types of carbohydrate feed crops) produced an amount of feed equivalent to that produced by maize on river bottom soils. Dehydration was a suitable method of producing a product suitable for storage. Dehydrated sweet potatoes satisfactorily replaced half of the maize in fattening rations for steers. A. H. Cornfield.

Switchback [double-reversal] trials for more than two treatments. H. L. Lucas (*J. Dairy Sci.*, 1956, **39**, 146—154).—Detailed statistical analysis is given of experimental designs that allow the double-reversal technique to be applied to comparisons of three to nine treatments. Designs of reduced size are discussed, together with the introduction of a blocking feature. Missing value formulæ are also given. S. C. Jolly.

Effects of feeding grass and of sanitation on growth of young dairy calves under two systems of management. G. E. Hawkins, jun. and K. M. Autrey (*J. Dairy Sci.*, 1956, **39**, 196—203).—The growth of calves raised in a conventional calf barn and that of calves raised in portable outside pens located on clean soil were similar; when pens were on ground frequented by older cattle, wt. gains and height at withers were smaller. Supplementing the ration with 1 lb. of grass daily increased total digestible nutrient (TDN) intake but did not affect feed utilisation or wt. gains. Wt. gains per lb. of TDN consumed were greater for calves in barns than for those in pens. S. C. Jolly.

Dairy calf research in Louisiana. L. L. Rusoff and J. B. Frye, jun. (*Louisiana agric. Exp. Sta.*, 1955, *Bull.* 494, 26 pp.).—Work over the previous 10 years in raising dairy calves in Louisiana is presented and reviewed. Nutritional and management practices are discussed and the mineral requirements of calves summarised. Particular attention is given to studies with antibiotics. A. H. Cornfield.

Dairy calf losses in the Kentucky Agricultural Experiment Station dairy herd. C. A. Lassiter and D. M. Seath (*Kentucky agric. Exp. Sta.*, 1955, *Bull.* 622, 8 pp.).—Calf losses averaged 14·43% over a 24-year period, the principal causes being abortions and calves born dead. Scours and pneumonia were the main causes of death after birth. Calf losses were highest in January of each year and averaged 7·6% higher during October–March than during the rest of the year. A. H. Cornfield.

Distillers' grain solubles in calf starter rations. C. A. Lassiter, D. M. Seath, R. F. Elliott and G. M. Bastin (*Kentucky agric. Exp. Sta.*, 1955, *Bull.* 623, 8 pp.).—Maize or milo distillers' dried solubles could replace an equal amount of dried skim milk in calf starter rations containing hay and a limited amount of whole milk. Growth rate of calves fed rye distillers' solubles was less than that of calves fed dried skim milk and maize or milo distillers' solubles. Palatability of maize and milo solubles was satisfactory whilst that of rye solubles was relatively poor. A. H. Cornfield.

Effects of supplementing a calf ration with trace minerals, aureomycin and other dietary constituents as measured by growth and feed consumption. W. G. Jones, E. E. Bartley, M. J. Swenson, G. K. L. Underbjerg, F. W. Atkeson and H. C. Fryer (*J. Dairy Sci.*, 1956, **39**, 188—195).—Addition of trace minerals (Fe, Cu, Co, I, Mn and Zn) to a standard basal ration significantly increased the growth of calves during the first eight weeks after birth, and the increase was maintained at a comparatively slower rate up to 24 weeks of age. Additional supplementation of the ration with major minerals (Ca, P, Mg and NaCl), vitamins (A, B-complex, C, D, E and K) and aureomycin further improved growth rate. Feed consumption and efficiency of gain were not significantly affected by the various rations. S. C. Jolly.

Lucerne *versus* prairie hay for dairy calves. S. D. Musgrave, J. B. Williams, C. L. Norton and W. D. Gallup (*Okla. agric. Exp. Sta.*, 1955, *Bull.* 443, 9 pp.).—Calves showed better wt. gains and feed efficiency with respect to total digestible nutrients when fed lucerne hay than when fed prairie hay either from birth or from eight weeks of age. Feed efficiency was slightly higher in winter than in summer trials. A. H. Cornfield.

Use of dried buttermilk as a feed for dairy calves. D. S. Flux and M. R. Patchell (*N.Z. J. Sci. Tech.*, 1956, **37, A**, 451—457).—Two types of dried buttermilk were used containing respectively 16 and 8% of fat, each reconstituted milk being fed to dairy calves at the rate of 10 and 12·5% of body wt. The calves were given whole milk for the first four weeks, this being gradually replaced by buttermilk, and from 6—10 weeks of age they were given only buttermilk; they grazed good quality pasture during the whole period. There was no significant difference between growth rates of the calves fed the two types of powder, but those receiving reconstituted milk at 12·5% of body wt. had better wt. gains than had those receiving 10%. E. M. J.

High-roughage system for raising calves based on early development of rumen function. VI. Influence of hay-to-grain ratio on calf performance, rumen development and certain blood changes. J. W. Hibbs, H. R. Conrad, W. D. Pounden and N. Frank (*J. Dairy Sci.*, 1956, **39**, 171—179).—Wt. gains (*W*), total-digestible-nutrient (*N*) intake, efficiency of feed utilisation and % of protein digested in rumen-inoculated calves fed until 12 weeks of age on a high-roughage ration containing mixed clover–timothy hay and grain in the ratios of 4 : 1, 3 : 2 and 2 : 3 increased as the proportion of grain increased. Calves fed an all-milk ration showed greater skeletal growth than did those on the high-grain ration, although *W* was not greater and *N* was less. Hay-to-grain ratios had no effect on total volatile fatty acid content of rumen juice. Rumen pH increased with age, but was maintained at a lower level on high-grain rations. The rating of Hay II group of rumen bacteria was markedly reduced as the proportion of grain increased. Blood-glucose and -cholesterol were not affected by hay-to-grain ratios: high levels were maintained in calves fed an all-milk ration. S. C. Jolly.

Antibiotics for young dairy calves. C. A. Lassiter, T. W. Denton, G. M. Bastin, J. W. Rust and D. M. Seath (*Kentucky agric. Exp. Sta.*, 1955, *Bull.* 624, 12 pp.).—Aureomycin, Terramycin or a combination of the two antibiotics had no significant effect on the growth rate, feed efficiency or incidence of scours of Jersey and Holstein calves. Results were similar whether the starter ration was made up of plant or animal protein. A. H. CORNFIELD.

Supplemental phosphorus requirement of range beef cattle. A. B. Nelson, W. D. Gallup, O. B. Ross and A. E. Darlow (*Okla. agric. Exp. Sta.*, 1955, *Tech. Bull.* 54, 29 pp.).—In an area in which soils were bordering on P deficiency beef cattle grazing native grasses during summer and fed locally-grown hay supplemented with maize gluten meal during winter benefited slightly from a mineral P supplement, as indicated by growth of heifers, wt. changes of cows, reproductive performance, and plasma P levels. Where soils were definitely deficient in P, supplementation of feed with mineral P definitely increased the P levels of plasma, particularly during winter, and also increased the growth rate of calves and wt. of cows. A. H. CORNFIELD.

Effect of protein content and vitamin and mineral additions on wintering bred yearling heifers. A. B. Nelson, R. W. MacVicar, W. D. Campbell and O. B. Ross (*Okla. agric. Exp. Sta.*, 1955, *Bull.* 460, 11 pp.).—Yearling heifers fed a 40% protein supplement made better wt. gains during the winter than did those receiving a 20% protein supplement. Gains of heifers through the year and both birth wt. and weaning wt. of calves were similar with both treatments. Addition of a commercial mineral + vitamin supplement to the feed had no effect on growth rate. A. H. CORNFIELD.

Dried potato pulp for daily cattle. H. C. Dickey (*Maine agric. Exp. Sta.*, 1955, *Bull.* 539, 7 pp.).—Dried potato pulp could be mixed with dairy grain mixtures to constitute 25% of the feed. Since the material is low in fat and vitamins, it should be used in combination with grains high in fat and should also be supplemented with vitamins A and D unless good quality hay and silage are also being fed. The material was a satisfactory grass silage preservative, being added at the rate of 150 lb. per ton of grass. A. H. CORNFIELD.

Protein production in the bovine. Comparison of daily protein, fat and milk production during the entire lactation period. B. L. Larson, G. D. Rolleri and K. A. Kendall (*J. Dairy Sci.*, 1956, **39,** 204—213).—Max. daily milk and fat production by dairy cows was reached after about the first month postpartum; protein production was a max. on day of parturition and either remained constant or decreased during this period. Max. casein production was reached in a few days, but serum protein output decreased at the same time. The ratio of protein to fat production varied considerably during a lactation period. S. C. JOLLY.

Fluorimetric estimation of œstrogens in bovine urine. E. P. Smith, W. M. Dickson and R. E. Erb (*J. Dairy Sci.*, 1956, **39,** 162—170).—A fluorimetric method is described for the extraction and determination of œstrogens in bovine urine based on the methods of Stimmel (*J. biol. Chem.*, 1946, **162,** 99) and of Friedgood *et al.* (*ibid.*, 1948, 523). Biological assays indicate that only about 25% of the fluorescence is due to biologically active œstrogens. The total fluorescence-producing substances in the urine of pregnant cattle increased with advancing pregnancy. S. C. JOLLY.

Processing, storing and shipping frozen bull semen. J. P. Mixner (*New Jersey agric. Exp. Sta.*, 1955, *Circ.* 573, 16 pp.).—The methods used are described. A. H. CORNFIELD.

Metabolism of bovine semen. III. Uptake and metabolic utilisation of glycerol-1-^{14}C by bovine spermatozoa. W. T. O'Dell, R. J. Flipse and J. O. Almquist (*J. Dairy Sci.*, 1956, **39,** 214—218).—In diluted bull semen, labelled glycerol entered the spermatozoa in measurable quantities and was converted into $^{14}CO_2$ during anaërobic fermentation. Oxidation of glycerol is due to metabolic processes of the spermatozoa and not to enzyme activity of the seminal plasma. The presence of seminal plasma inhibited the uptake and utilisation of glycerol by separated spermatozoa. S. C. JOLLY.

Diluters for bovine semen. VIII. Effects of alterations of some physical factors of a milk diluter on the livability of bull spermatozoa. P. E. Johnson, R. J. Flipse and J. O. Almquist (*J. Dairy Sci.*, 1956, **39,** 180—187).—The livability of bovine spermatozoa is unlikely to be improved by altering the pH, osmotic pressure or buffering capacity of a diluter prepared from cysteine-treated skim milk powder, although slight improvement resulted from the addition of 0·5N-NaCl (5—10 ml. per 100 ml. of diluter). S. C. JOLLY.

Unknown growth factors in distillers' dried solubles. H. M. Scott, W. D. Morrison and P. Griminger (*Poultry Sci.*, 1955, **34,** 1446—1447).—Addition of 10% of dried distillers' solubles to a purified diet containing all known nutrients increased the growth rate of non-depleted chicks to four weeks of age. The ash of dried distillers' solubles, added in amount ≡10% solubles, had no effect on growth rate. A. H. CORNFIELD.

Nutritive value (for chicks) of herring meals. III. Effects of heat treatment and storage temperature as related to oil content. J. Biely, B. E. March and H. L. A. Tarr (*Poultry Sci.*, 1955, **34,** 1274—1279).—The nutritive value of commercial flame-dried herring meal and low-temp. dried herring meal was not improved by extracting the oil with hexane. Hexane-extracted low-temp. dried meal showed lower stability of its folic acid during storage than did the non-extracted material. Meals stored at −25°, 21° and 37° for one year had very similar nutritive values. A. H. CORNFIELD.

Comparison of some sources of unidentified chick growth factors with fish meal. H. Morimoto, S. Ariyoshi and H. Hoshii (*Poultry Sci.*, 1955, **34,** 1392—1395).—Autolysed cuttle fish, autolysed sardines, and fish solubles were not such potent sources of unidentified growth factor(s) for chicks as was fish meal. A mould grown on distillers' residue contained negligible amounts of the factor. Brewers' yeast and two out of six Koji feeds (mould grown on starch cake and $(NH_4)_2S$) tested were almost as effective as was fish meal in supplying the factor. A. H. CORNFIELD.

Effect of chlortetracycline (aureomycin) and fish meal on the growth and thyroids of chickens. A. D. Keeling, C. H. Hill, H. W. Garren and J. W. Kelly (*Poultry Sci.*, 1955, **34,** 1453—1454).—Addition of chlortetracycline (0·1 g. per lb. of feed) (**I**) to an all-vegetable diet increased the growth rate of chicks to four weeks of age but had no effect on thyroid wt. per unit of body wt. or on the histology of the thyroid. Addition of 5% of fish meal to the all-vegetable diet increased the growth rate and decreased thyroid wt. Addition of **I** to a diet containing 5% fish meal had no effect on growth rate or thyroid wt. A. H. CORNFIELD.

Production of depleted chicks by feeding maternal diets deficient in unidentified growth factors. P. E. Waibel, A. B. Morrison and L. C. Norris (*Poultry Sci.*, 1955, **34,** 1322—1329).—Chicks from hens fed a purified diet containing all known nutrients were depleted of unidentified growth factors. These factors were present in maize–soya-bean meal, desiccated liver and fish solubles. A. H. CORNFIELD.

Arginine requirement of the growing chicken. P. Griminger, H. Fisher and H. M. Scott (*Poultry Sci.*, 1955, **34,** 1247—1249).—Growth rate of male chicks (New Hampshire × Columbian) from 1 to 21 days of age was greater with 1·75% than with 1·25% of arginine in the feed (purified diets containing 22% of casein). Results were similar whether 66% of cerelose or dextrin were used as the main source of carbohydrate. A. H. CORNFIELD.

Protein quality and "available lysine" in animal products. K. J. Carpenter and G. M. Ellinger (*Poultry Sci.*, 1955, **34,** 1451—1452).—The "gross protein value" (determined by chick assay) of a no. of animal products was highly correlated with the "available lysine value" (determined chemically) of the materials. The "total lysine value" was not as well correlated with the "gross protein value." A. H. CORNFIELD.

Effect of productive energy level of the diet on the methionine requirement of the chick. J. T. Baldini and H. R. Rosenberg (*Poultry Sci.*, 1955, **34,** 1301—1307).—The methionine requirement of the chick for optimum growth and feed efficiency from 0—8 weeks of age increased from 0·350% to 0·497% as the productive energy level of the diet increased from 798 to 1002 kg.-cal. per lb. A. H. CORNFIELD.

Concomitant use of fat and methionine in broiler diets. H. R. Rosenberg, J. T. Baldini, M. L. Sunde, H. R. Bird and T. D. Runnels (*Poultry Sci.*, 1955, **34,** 1308—1313).—Addition of 0·05% DL-methionine to a simple maize–soya-bean oil meal broiler diet resulted in slight improvement in wt. gains and feed efficiency, whilst addition of 3—6% vegetable or animal fat resulted in somewhat greater wt. gains and much greater feed efficiency. Addition of both supplements together improved wt. gains and feed efficiency to a greater extent than would be expected if the effects were simply additive. This fat-methionine interaction occurred in other tests at other locations using somewhat different basal diets. A. H. CORNFIELD.

Utilisation of carotene and vitamin A by chicks during the first week after hatching. J. D. Harvey, D. B. Parrish, P. E. Sandford and J. S. Hughes (*Poultry Sci.*, 1955, **34,** 1348—1357).—The chick was able to convert carotene to vitamin A even during its first day of life. Vitamin A was utilised for physiological purposes at least by the fifth day of life or probably earlier. A. H. CORNFIELD.

Application of deutectomy for reducing initial vitamin-A reserves in newly-hatched chicks. J. D. Harvey, D. B. Parrish and P. E. Sanford (*Poultry Sci.*, 1955, **34,** 1314—1321).—Deutectomy resulted in a reduction in the initial vitamin-A reserves in newly-hatched chicks. The reduction was not so marked where the dam's intake

of vitamin A was restricted. The variations in reserves of vitamin were less after than before deutectomy. Vitamin A in the yolk at hatching is absorbed into the chick body largely during the first week. Liver vitamin A was depleted sharply during the first two weeks after hatching. When chicks were fed diets containing 1000—2000 i.u. of vitamin A per lb., liver storage of the vitamin began at two weeks of age. Deutectomy did not impair the ability of chicks to store the vitamin at this stage. A. H. CORNFIELD.

Utilisation of vitamin A by normal and deutectomised chicks. D. H. Laughland and W. E. J. Phillips (*Poultry Sci.*, 1955, **34,** 1359—1362).—Normal chicks were able to store dietary vitamin A in their livers at eight days of age or possibly earlier. When β-carotene was supplied, no storage of vitamin A occurred at eight days, but significant storage occurred at 15 days, of age. Similar results were obtained with deutectomised chicks, although the quantity of vitamin deposited in the liver was not quite as great. A. H. CORNFIELD.

Effect of thyroprotein on the growth of Egyptian chicks. M. M. Oloufa (*Poultry Sci.*, 1955, **34,** 1292—1294).—Addition of thyroprotein (5—10 g. per 100 lb. of feed) to the diet of chicks from birth to 12 weeks of age reduced growth rate of the birds and increased their mortality rate. A. H. CORNFIELD.

Effect of exogenous gonadal hormones on Single Comb White Leghorn pullets. R. B. Herrick and J. L. Adams (*Poultry Sci.*, 1955, **34,** 1362—1367).—Birds were given, twice weekly, injections of diethylstilbœstrol (0·004—0·016 g.), testosterone propionate (0·005—0·020 g.) and progesterone (0·010—0·040 g. per bird per week). Egg production was severely depressed by all treatments whilst body wt., egg wt., yolk wt., albumin quality, blood spots, hatchability and shell thickness were not affected. Meat spots were reduced by all treatments. Progesterone depressed fertility significantly. Birds in which production was inhibited by progesterone moulted heavily. A. H. CORNFIELD.

Effect of ration, caponising and hormone treatment on broiler strain New Hampshire cockerels. J. L. Adams (*Poultry Sci.*, 1955, **34,** 1295—1297).—Birds made better growth on a modern-type (1951) than on an old-type (1936) broiler ration. Caponising birds at six weeks of age improved the growth of birds receiving the old, but not of those receiving the modern, ration. Implantation with diethylstilbœstrol pellets improved the growth rate, conformation and finish of birds receiving both types of rations. A. H. CORNFIELD.

Effects of growth hormones on chickens. D. A. Libby, J. Meites and P. J. Schaible (*Poultry Sci.*, 1955, **34,** 1329—1331).—Addition of Protamone (10 g. per 100 lb. of feed) to the diet of cockerels or injection of somatotrophin (**I**), testosterone or diethylstilbœstrol (**II**) had no effect on growth rate or feed efficiency. The fat content of the thighs was increased and comb size was reduced by **II**, whilst **I** had no effect on comb size or on fat, protein or water content of the thigh. Growth of chicks from birth to two weeks of age was unaffected by **I**. A. H. CORNFIELD.

Effects of growth hormones on pullets and mature hens. R. D. Carter, R. N. Risner and H. Yacowitz (*Poultry Sci.*, 1955, **34,** 1407—1414).—Injection of pullets and mature hens with bovine or porcine growth hormone (0·001—0·002 g. per bird per day) for 25 days had no effect on body wt. but reduced egg production somewhat. Some of the treated birds moulted. Injection with ACTH (0·08—0·32 units per day) for 21—53 days had no effect on body wt. When both ACTH and bovine growth hormone were injected the body wt. dropped rapidly for three weeks and then increased again. Feed consumption was reduced by all treatments. The high level of ACTH increased egg production and reduced thyroid wt., whilst the low level of ACTH + growth hormone reduced egg production and increased spleen wt. A. H. CORNFIELD.

Effect of testosterone and dienœstrol diacetate on hæmoglobin levels in cockerels and capons. T. Tanaka and M. M. Rosenberg (*Poultry Sci.*, 1955, **34,** 1429—1437).—Addition of testosterone (**I**) (0·909—1·818 g. per 100 lb. of mash) to the diet of capons from 10 to 18 weeks of age significantly increased the hæmoglobin levels in the birds. Addition of dienœsterol diacetate (**II**) (0·310—0·620 g. per 100 lb. mash) had no significant effect on the hæmoglobin levels in capons, whilst neither material had any effect on the hæmoglobin levels in cockerels. When those capons which had received **II** for eight weeks were then given **I** for a further eight weeks there was a rapid increase in hæmoglobin level, whilst capons transferred from **I** to **II** showed no change in hæmoglobin levels. Cockerels made no response to the reversed treatments. A. H. CORNFIELD.

Hæmatological changes in cockerels after ACTH and cortisone acetate treatments. J. Hublé (*Poultry Sci.*, 1955, **34,** 1357—1359).—Administration of ACTH (4 i.u. per day per 100 g.) or cortisone acetate (0·005 g. per day per 100 g.) produced a relative lymphopenia, a relative and abs. rise in heterophils and a relative and abs. eosinopenia. The treatments had no effect on the abs. no. of lymphocytes and erythrocytes. A. H. CORNFIELD.

Effect of different levels of dietary free gossypol supplied by different cottonseed products on growth rate of chicks. B. W. Heywang and H. R. Bird (*Poultry Sci.*, 1955, **34,** 1239—1247).—Chick diets were supplemented with raw decorticated cottonseed, screw-press hydraulic solvent-extracted, or pre-press solvent-extracted cottonseed meals or pure gossypol so as to supply 0·008—0·075% of free gossypol in the diet from one day to 5—6 weeks of age. Satisfactory growth, feed consumption and feed efficiency were obtained when the diet contained >0·016% gossypol in the case of White Leghorns and >0·020% in the case of New Hampshires. A depression of growth rate resulting from feeding a very high level of cottonseed meal was not due to the gossypol content of the meal. A. H. CORNFIELD.

Comparison of the chick growth inhibition of unheated linseed hull and cotyledon fractions. K. F. Schlamb, C. O. Clagett and R. L. Bryant (*Poultry Sci.*, 1955, **34,** 1404—1407).—Linseed hulls produced less inhibition of chick growth when added to their feed than did heated or unheated linseed meal or linseed cotyledons. Unheated meal and cotyledons caused considerable reductions in growth rate. A. H. CORNFIELD.

Effect of low levels of furazolidone in poultry feeds upon early growth of chickens. D. A. Libby and P. J. Schaible (*Mich. agric. Exp. Sta. Quart. Bull.*, 1955, **38,** 241—251).—Growth rate and feed efficiency were slightly improved by addition of approx. 7·5 of 3-*N*-(5-nitro-2-furfurylidene)amino-2-oxazolidone per ton of feed with no adverse effect on livability and the treatment was more effective in heavily contaminated than in cleaner environments. Whey products enhanced the value of the substance. E. G. BRICKELL.

Grading eggs. S. G. Skelton (*Okla. agric. Exp. Sta., Circ.* 549, 11 pp.).—Colour photographs illustrating candled appearance and broken-out appearance of eggs of varying grades are presented. Equipment and methods used for candling and grading of eggs are described and factors determining egg grades are discussed. A. H. CORNFIELD.

Effects of two sulphonamides on the formation of egg shells. A. L. Mehring, jun., H. W. Titus and J. H. Brumbaugh (*Poultry Sci.*, 1955, **34,** 1385—1389).—When laying pullets were supplied with Diamox (2-acetamido-1 : 3 : 4-thiadiazole-5-sulphonamide, 0·05—0·10 g.) or benzenesulphonamide (0·035 g. per bird per day) they laid eggs with thin shells or no shells beginning almost immediately after commencement of treatment. Some of the birds stopped laying. Egg laying and shell thickness returned to normal within a few days of discontinuing the treatments. A. H. CORNFIELD.

Cleaning eggs with detergents and detergent-sanitisers. A. R. Winter, B. Burkart, P. Clements and L. MacDonald (*Ohio agric. Exp. Sta.*, 1955, *Res. Bull.* 762, 42 pp.).—A 0·3% concn. of most of the commercial detergent and detergent-sanitisers tested was effective for removing dirt and stain. Hand washing was somewhat more effective than machine washing. Detergent-sanitisers were more effective than detergents in reducing the no. of bacteria on the egg shell and within the egg. Eggs washed within a few hours of collection kept better than did those washed after holding for several days. Soaking eggs for even 30 min. at 37·7° in 0·3% aq. solutions of a no. of detergents and detergent-sanitisers had no effect on egg white quality as measured ay appearance, odour, flavour and cooking properties. A. H. CORNFIELD.

Tolerance of the chicken embryo to periods of low temperature exposure. R. E. Moreng and R. L. Bryant (*Poultry Sci.*, 1955, **34,** 1342—1348).—Embryonic mortality occurred at low temp. when ice formed in the embryo. Eggs held at 0° for up to 76 hr. (after one day of incubation) had fairly high % survival. A slow rate of cooling to 0° was less detrimental to embryonic survival than was a fast rate of cooling. A. H. CORNFIELD.

Increasing shelf-life of fresh chicken by chlorination. F. Ziegler and W. J. Stadelman (*Poultry Sci.*, 1955, **34,** 1389—1391).—Addition of Cl_2 (10—20 p.p.m.) to the cooling water used after scalding and evisceration of carcasses resulted in significantly increased storage life at 1·1° as indicated by length of time required for off-odours to develop. The extent of increase of storage life was of no practical importance. The treatments had no effect on the time required for slime to appear. A. H. CORNFIELD.

Turkey raising. H. H. Kauffman (*Pa agric. Exp. Sta., Circ.* 456, 20 pp.).—A detailed description of the confinement and range methods of rearing turkeys. A. H. CORNFIELD.

Heritability of some body measurements and reproductive characteristics in turkeys. P. A. Kondra and R. N. Shoffner (*Poultry Sci.*, 1955, **34,** 1262—1267).—Studies on the heritability of live body

wt., breast width, keel length, body depth and shank length of three breeds of turkeys and crosses of two of the breeds are reported. A. H. CORNFIELD.

Whole barley compared with hulled barley for turkeys on range. H. J. Almquist and J. B. Merritt (*Poultry Sci.*, 1955, **34,** 1449).—Both turkey toms and hens receiving a limited mash allowance consumed less hulled barley than whole barley when given unlimited amounts of one or other of the grains. The ratio of the amounts of the two kinds of barley consumed was inversely related to the productive energy of the two kinds of grain. A. H. CORNFIELD.

Seven years of managed turkey grazing. P. H. Margoff and J. B. Washko (*Pa agric. Exp. Sta.*, 1955, *Bull.* 608, 9 pp.).—An average of 150 turkeys per acre per annum were reared on the same pasture for seven successive years. Grazing intensity was not related to mortality. Under heavy grazing the proportion of ladino clover in the sward decreased earlier each year and finally disappeared. Orchard grass continued to thrive even under heavy grazing. Daily moving of shelters, feeders, and water fountains aided recovery of the herbage. A. H. CORNFIELD.

Heritability of egg production in White Holland Turkeys. M. G. McCartney (*Poultry Sci.*, 1955, **34,** 1280—1283).—Selection based on the combination of individual performance and full-sister family averages was the most efficient method of improving egg production. A. H. CORNFIELD.

Effect of holding time of turkey semen on fertilising capacity. J. A. Harper (*Poultry Sci.*, 1955, **34,** 1289—1291).—Holding turkey semen at 12·6—15·5° for up to 4 hr. after collection was not detrimental to fertility for hens inseminated during the period 16th Feb. to 9th March. Later in the season degree and duration of fertility decreased with length of holding period (1—4 hr.) of the semen. A. H. CORNFIELD.

Effect of penicillin, aureomycin and 3-nitro-4-hydroxyphenylarsonic acid on the growth of goslings. G. S. Lindblad, W. G. Hunsaker and J. R. Aitken (*Poultry Sci.*, 1955, **34,** 1259—1261).—Addition of procaine penicillin (25—100 p.p.m.) or aureomycin HCl (100 p.p.m.) to the diet of goslings improved their growth rate, in comparison with controls, up to two weeks of age, but at five weeks of age there was no significant difference in wt. between treated and control birds. In general, the treatments had no effect on feed efficiency. Addition of 3-nitro-4-hydroxyphenylarsonic acid (50 p.p.m.) to the diet had no effect on the growth rate of male goslings but reduced that of female goslings up to four weeks of age. A. H. CORNFIELD.

Nutritional requirements of white-tailed deer for growth and antler development. C. E. French, L. C. McEwen, N. D. Magruder, R. H. Ingram and R. W. Swift (*Pa agric. Exp. Sta.*, 1955, *Bull.* 600, 50 pp.).—Details of the studies carried out are presented. A. H. CORNFIELD.

Dieldrin as a systemic against cattle grubs. A. R. Roth and J. B. Johnson (*J. econ. Ent.*, 1955, **48,** 761—762).—A high mortality of cattle grubs, *Hypoderma lineatum* and *H. bovis*, was caused by the subcutaneous injection in the backs of the cattle of dieldrin (5% suspension in peanut oil) at 25 mg./kg. No adults emerged from the specimens collected from treated animals but several emerged from pupæ taken from untreated animals. Dieldrin failed to prevent larvæ from encysting or cutting holes in the backs of the cattle. A. A. MARSDEN.

Tests with repellents against the American dog tick. M. M. Cole and G. W. Lloyd (*J. econ. Ent.*, 1955, **48,** 772—773).—Of 10 repellent mixtures tested on clothing against *Dermacentor variabilis*, M-1960 (2-butyl-2-ethylpropane-1 : 3-diol–benzyl benzoate–*N*-butylacetanilide) was the most effective mixture giving an average repellency of 93% after 7—17 days of ageing. The following three mixtures M-2059 [M-1960 (99%)–lindane 1%], M-2061 (2-butyl-2-ethylpropane-1 : 3-diol–dibutyl adipate–*N*-butylacetanilide) and dibutyl adipate gave average repellencies of 86—89% over the same period. A. A. MARSDEN.

Triiodothyroxine and thyroxine for chick goitre prevention. C. J. Shellabarger (*Poultry Sci.*, 1955, **34,** 1437—1440).—Triiodothyroxine was four times more potent than was thyroxine in reducing the thyroid response of male albino rats to thiouracil treatments. There was no difference between the ability of the two compounds in reducing the response of the thyroid gland of chicks to thiouracil. A. H. CORNFIELD.

Endocrine and lymphatic gland changes occurring in young chickens with fowl typhoid. H. W. Garren and C. W. Barber (*Poultry Sci.*, 1955, **34,** 1250—1258).—Changes occurring over a two-week period in 33—48-day-old New Hampshires and Rhode Island Reds inoculated with *Salmonella gallinarum* are reported. A. H. CORNFIELD.

Incidence of *Salmonella pullorum* in wild pheasants in Southern Michigan. R. C. Belding (*Poultry Sci.*, 1955, **34,** 1441—1444).—Of 65 wild pheasants caught in Southern Michigan five were infected with *S. pullorum*. The organism could not be recovered from any of 36 eggs examined. No *Salmonella* types other than *S. pullorum* were recovered. A. H. CORNFIELD.

Synthetic medium for propagation and maintenance of virulent strains of *Salmonella pullorum*. R. F. Gilfillan, D. F. Holtman and R. T. Ross (*Poultry Sci.*, 1955, **34,** 1283—1288).—The medium is described. Max. growth of the organism occurred in 36 hr. and infectivity was as high from the synthetic medium as from animals. A. H. CORNFIELD.

Vaccination procedures for Newcastle disease. J. G. Wadsworth and F. Young (*Poultry Sci.*, 1955, **34,** 1454—1455).—Two vaccinations (one at four days and the other at four weeks of age) of chicks via the drinking water resulted in much better immunity than did a single vaccination in a vaccinating machine (at one day of age), as indicated by mortality resulting from challenge with live virus at nine weeks of age. A. H. CORNFIELD.

Administration of Newcastle disease and infectious bronchitis vaccines through the drinking water. R. E. Luginbuhl, E. L. Jungherr and T. W. Chomiak (*Poultry Sci.*, 1955, **34,** 1399—1403).—Chickens could be infected with Newcastle disease and infectious bronchitis through the digestive tract by feeding the birds with capsules containing the viruses. Birds drinking water containing 10^5 embryo infective doses per 0·2 ml. water developed significant levels of sp. antibodies. A. H. CORNFIELD.

Use of "incomplete" Newcastle disease virus as an experimental vaccine. M. K. Nadel and A. Eisenstark (*Poultry Sci.*, 1955, **34,** 1298—1301).—Day-old chicks were inoculated interocularly with an "incomplete" form of Newcastle disease virus (obtained from allantoic fluid of infected chick embryos at a stage when the fluid contained hæmagglutination units but was devoid of infective power) and challenged with Newcastle disease three weeks later. The "incomplete" virus was safe when inoculated into the chicks and was as efficient in eliciting anti-body response as was a normal live-virus vaccine. A. H. CORNFIELD.

Helminth infections in relation to restricted feeding on range. R. L. Tugwell (*Poultry Sci.*, 1955, **34,** 1372—1375).—The extent of nfection by different species of helminth of birds on free range of pasture but receiving varying amounts of mash was studied. The helminths found in all birds consisted largely of *Heterakis gallinæ*, although fair no. of *Ascaridia galli* and *Hymenolepsis carioca* were found. There was a tendency towards heavier helminth infection as the supply of mash was reduced. A. H. CORNFIELD.

Rôle of the infected egg in the transmission of visceral lymphomatosis. B. R. Burmester and N. F. Waters (*Poultry Sci.*, 1955, **34,** 1415—1429).—When hatched and brooded in isolated units, there was no significant difference with respect to the incidence of visceral lymphomatosis between families of dams that shed the virus and those of dams that did not shed the virus. When disease-free birds were exposed during hatching and brooding to chicks of an infected stock there was a high rate of transmission of the disease during the brooding period but not during the hatching period. The introduction, into a large commercial hatching unit during the hatching period, of an aërosol of either infectious oral washings or lymphomatous liver filtrate did not significantly increase the incidence of gross lesions of the disease. A. H. CORNFIELD.

Relative activity of drugs used as coccidiostats. R. L. Tugwell (*Poultry Sci.*, 1955, **34,** 1368—1371).—Mortality of birds from 4—7 weeks of age infected with cæcal coccidiosis was considerably reduced by addition of 0·15% Parabis-90 [2 : 2-methylenebis-(4-chlorophenyl)] (**I**), 0·24% of a mixture of sulphaquinoxaline : micronised S 1 : 18 parts, micronised S 0·23%, or nitrofurazone 0·0056% to the diets. Addition of 0·0125% of sulphaquinoxaline (**II**) or 0·0125% of nitrophenide (**III**) was only partially effective whilst 0·25% of sulphamethazine (**IV**) was ineffective in reducing mortality. Wt. gains of non-infected birds up to 42 days of age were unaffected by **I**, **II**, **III** or **IV**, whilst the other treatments reduced wt. gains. A. H. CORNFIELD.

***In vitro* comparison of some antibacterial agents on a strain of avian pleuropneumonia-like organisms.** C. H. Domermuth and E. P. Johnson (*Poultry Sci.*, 1955, **34,** 1395—1399).—*In vitro* tests of 12 drugs for inhibiting the growth of avian pleuropneumonia-like organisms (Massachusetts chronic respiratory disease strain A5967) showed that the effectiveness of the drugs was in the order magnamycin > Terramycin, streptomycin, furazolidone > aureomycin, chloromycetin > neomycin > penicillin. The first four drugs killed the organism at concn. of magnamycin 10^{-8} g. per ml. and of the other three drugs 10^{-5} g. per ml. of culture solution. There was no tendency for the organism to develop resistant strains after treatment with sublethal concn. of these four drugs. Four "sulpha" drugs were ineffective even at 10^{-2} g. per ml. A. H. CORNFIELD.

Waterproofing of agricultural land at soil-based grounds. Imperial Chemical Industries, Ltd. (Inventors: P. J. Askey and S. W. Hawkins) (B.P. 735,725, 24.12.52. Addn to B.P. 680,921, 22.12.50). —A compound [NHR·CH:CH·CH:C(OH)·CH:NHR]$^+$X$^-$ (R is SO_3H-free aryl, e.g., Ph, X is anion of a strong acid), optionally admixed with 10—50 pt. of inert carrier (sand), is incorporated (5—500 lb. per acre) in the top 8 in. of the land, to inhibit accumulation of mud and water. F. R. BASFORD.

Preparation of fodder for domestic animals. T. A. Pedersen (B.P. 739,862, 29.3.54).—A mixture of $CaCO_3$ 5000, pulverised oak-bark 4500, $FeSO_4$ 490 and I 2 g. is added to animal fodder, to prevent diarrhœa. F. R. BASFORD.

2.—FOODS

Presence of lævoglucosan in maize starch hydrolysates. L. D. Ough and R. G. Rohwer (*J. agric. Food Chem.*, 1956, **4,** 267—271).—Lævoglucosan (1 : 6 anhydro-D-glucose) was quant. determined by paper chromatographic method in a study of maize starch acid hydrolysates at equilibrium; 2·3% on a total carbohydrate basis was found. The lævoglucosan was also isolated on a cellulose column, the triacetate was prepared and its identity verified by mixed m.p. with known material. Maize starch even in dilute solutions, cannot be hydrolysed completely to D-glucose by acid. Other components were detected: hydroxymethylfurfuraldehyde, glycerol, xylose, arabinose, fructose, *iso*maltose and gentiobiose. Lævoglucosan, when subjected to reaction conditions similar to those for hydrolysing starch, results in essentially the same equilibrium mixture as that produced from starch. E. M. J.

Colour changes in strawberry jellies. R. V. Decareau, G. E. Livingston and C. R. Fellers (*Food Technol.*, 1956, **10,** 125—128).—A high degree of correlation was found between loss in brightness, heat applied, pigment retention and u.v. absorption caused by sugar degradation; 140°F. was the highest temp. below which there was no appreciable loss in pigment content in cooking. Initial colour gains achieved through the use of sugared, frozen strawberries and/or vacuum concentration were lost when products were stored at 100°F.; storage at refrigerator temp., if economically feasible, would extend shelf life. (15 references.) E. M. J.

Rapid colorimetric methods for simultaneous determination of total reducing sugars and fructose in citrus juices. S. V. Ting (*J. agric. Food Chem.*, 1956, **4,** 263—266).—Ferricyanide is used in a carbonate–phosphate buffer as the oxidising agent for sugars and the ferrocyanide produced is measured colorimetrically, using the blue solution formed after the addition of Nelson's arsenomolybdate reagent. When heated at 100° for 10 min. glucose and fructose are oxidised in equal amounts; when heated at 55° for 30 min., all the fructose, but only $\frac{1}{3}$—$\frac{1}{5}$ of the glucose, is oxidised. The amounts of the two sugars in a mixture can then be calculated. Results obtained by this method for reducing and total sugar determinations, in orange juices are compared with duplicate determinations using the Shaffer–Hartmann volumetric method. Data on 24 samples are given. (12 references.) E. M. J.

I. Comparison of carotenoids of Valencia orange peel and pulp. II. Carotenoids of aged canned Valencia orange juice. A. L. Curl and G. F. Bailey (*J. agric. Food Chem.*, 1956, **4,** 156—162).—I. & II. A comparison of the peel and pulp content of early season Valencia oranges showed that the carotenoid mixtures are qual. similar to those found in late season juices. The peel contains >60% of the total carotenoids and a higher % of violaxanthin, but less free diols and polyols. One constituent not previously found in late-season juices was found in both peel and pulp and identified as cryptoxanthin furanoxide. Countercurrent distribution studies of the carotenoids of canned juice stored for three years at room temp. are reported. None of the carotenoid epoxides were detected but the corresponding isomeric furanoxides were all found in increased proportions. The diether diol fraction was much smaller than in fresh juice. N. M. WALLER.

Determination of water by nuclear magnetic absorption in potato and apple tissue. T. M. Shaw and R. H. Elsken (*J. agric. Food Chem.*, 1956, **4,** 162—164).—A method for the quant. measurement of hydrogen in liquids and suspension by nuclear magnetic resonance absorption (Shaw and Elsken, *Analyt. Chem.*; 1955, **27,** 1983) is applied to the determination of water content of potato and apple tissue. The results for potato agree with those obtained by the vacuum oven method but for apples the results are high because of the contribution of hydrogen nuclei in the soluble solids. N. M. WALLER.

Determination of water by nuclear magnetic resonance in hygroscopic materials containing soluble solids. K. J. Palmer and R. H. Elsken (*J. agric. Food Chem.*, 1956, **4,** 165—167).—The influence of soluble solids in the determination of moisture in food products by use of nuclear magnetic resonance is investigated. The H nuclei of solutes absorb in a manner indistinguishable from the H nuclei of water in the high moisture region under conditions of operation of the low-resolution spectrometer described by Shaw and Elsken (*Analyt. Chem.*, 1955, **27,** 1983). Data on sugar solutions, milk samples and apple juice concentrate illustrate this effect. N. M. WALLER.

New developments in the dehydration of fruits and vegetables. D. K. Tressler (*Food Technol.*, 1956, **10,** 119—124).—Recent research and development work is reviewed including: improvements in dehydrating fruits, dehydrating fruit juices, vegetable dehydration, special problems encountered in dehydrating (*a*) tomato juice, and (*b*) potatoes, retention of nutrients during dehydration and storage, vitamin losses during storage. (22 references.) E. M. J.

Experimental production of tomato powder by spray drying. M. E. Lazar, A. H. Brown, G. S. Smith, F. F. Wong and F. E. Lindquist (*Food Technol.*, 1956, **10,** 129—134).—Non-caking tomato powder of good quality was produced experimentally by spray drying. Advantages of this new product over vacuum-dried tomato powder include greater packing density and potentially lower production costs. (11 references.) E. M. J.

Methionine content of soya-beans as influenced by location and season. O. A. Krober (*J. agric. Food Chem.*, 1956, **4,** 254—257).—A three-year study was made of 14 soya-bean varieties, each grown at from 12 to 18 locations. Genetic differences indicated that it should be possible to increase methionine content in soya-bean protein by breeding. Strains from Dunfield crosses and the strain Clark were superior to other strains tested. The strain Clark was significantly higher in methionine content than either of its parents, indicating transgressive segregation within the Lincoln × Richland cross. The effect of location was not consistent enough to show significance at the 5% level except in six varieties; significant seasonal variations were found in four varieties, methionine content being higher in 1952 than in the two previous years. Results indicate that soya-bean protein is produced in the seed with variable proportions of amino-acids. (14 references.) E. M. J.

Taste panel method for detecting flavour changes in vegetables treated with pesticides. A. Kramer and L. P. Ditman (*Food Technol.*, 1956, **10,** 155—159).—A simplified variables taste panel method for detecting flavour changes in vegetables treated with pesticides is presented. (16 references.) E. M. J.

Carbohydrates of the roots of the parsnip, *Pastinaca sativa*. D. M. W. Anderson and C. T. Greenwood (*J. chem. Soc.*, 1956, 220—221).—Polysaccharides present in the roots of *Pastinaca sativa* are separated by successive extractions with cold and hot water, and cold and hot 5% NaOH, and each fraction is separately hydrolysed. Sugars obtained (quant. results are given) are galactose, glucose, mannose, arabinose, xylose, rhamnose and fructose. M. DAVIS.

Water and effluents in fermentation industry. I. B. Nietsch (*Mitt. VersSta. Gärungsgew.*, 1955, **9,** 192—196).—A review covering treatments of water supplies for the removal of corrosive and metallic constituents, and for softening and sterilisation. Reference is made to the advantages of plastic materials for the construction of water mains and pipes. (25 references.) P. S. ARUP.

Controlled fermentations. P. Dupuy (*Chim. et Industr.*, 1956, **75,** 65—74).—The progress in developing methods of control of the fermentation of grape must, by modifying the natural microflora in the process, control of temp. and of CO_2 pressure, is reviewed and the installation of a continuous wine-making plant is described. J. S. C.

History of technical microbiology in U.S.S.R. III. Microbiology of wine making. I. L. Rabotnowa (*Mitt. VersSta. Gärungsgew.*, 1955, **9,** 197—202; cf. J.S.F.A. Abstr., 1955, ii, 289).—A review with 86 references. P. S. ARUP.

Ferric ferrocyanide as heavy-cation exchanger. J. Bonastre (*Industr. agric. aliment.*, 1956, **73,** 21—23).—Ferric ferrocyanide is deposited in the interstices of glasswool or kieselguhr by reaction between $FeCl_3$ and $K_4Fe(CN)_6$ and is used to remove Cu from wines which have become contaminated by accident with Cu compounds, e.g., through Cu pipes. Analysis shows that no ferrocyanide is dissolved by the wine. J. S. C.

Treatment of wine with ion-exchange resins. B. C. Rankine (*Aust. J. appl. Sci.*, 1955, **6,** 529—540).—The treatment of wines with both cation- and anion-exchange resins is described. With cation-exchange resins used in the H form, acidity increased and concentrations of K, Na, Ca, Mg, Fe and Cu decreased correspondingly. The concentrations of ethanol, reducing sugar, volatile acid, and

tartaric acid were not altered. The treatment prevented the deposition of KH tartrate, but in some cases rendered the wine too acid to be palatable. The highly acidic sulphonic acid resins were found to be the most suitable because of their greater capacity. On treatment with anion-exchange resins, preferably weakly basic resins, the acidity and tartaric acid contents of the wines were decreased and the palatability of the wine was improved. Concentrations of other constituents were unaffected, but the precipitation of KH tartrate was not prevented. Study showed that much Cu and Fe was removed in most cases, the amounts remaining however exceeding a desirable max. The treated wines did not differ from the untreated samples in their relative resistance to subsequent refermentation. O. M. WHITTON.

Prevention of potassium bitartrate deposition in wine by cation-exchange resins. B. C. Rankine and R. D. Bond (*Aust. J. appl. Sci.*, 1955, **6**, 541—549).—The treatment of wines by cation-exchange resins in the Na or mixed Na and H form is described. The concentrations of K, Ca and Mg in the wine were decreased and those of Na, or of Na and H were correspondingly increased. As the vol. of wine treated increased, so the pH of the effluent wine rose slightly using resin in the Na form, but with the mixed Na and H form the pH decreased in approx. proportion to the ratio of H and Na in the solution used for regeneration. The treatment prevented the deposition of KH tartrate without altering the taste of the wine, in some cases taste improved. Off flavours were observed with new resin, but these disappeared after one or two regenerations. The significance of this treatment on wine stability, its low cost compared with the removal of tartrate by refrigeration, and the legality of its use are discussed. O. M. WHITTON.

Use of ion-exchangers in chemistry of wine. F. Engel and A. Komertzky (*Mitt. VersSta. Gärungsgew.*, 1955, **9**, 183—190).—The treatment of wine with ion-exchange resins for the removal of excessive acidity or of Fe can succeed only by bringing the wine into intimate contact with limited amounts of the resins during limited periods, under conditions allowing of the easy recovery of the resin. Laboratory apparatus and a large-scale installation are described in which definite proportions of resin are continuously injected into the wine (whilst flowing through a mixing chamber), and filtered off after passage through a tube of appropriate length. Before deacidification with an anion-exchanger, the wine must be freed from all but traces of Ca by treatment with a cation-exchanger. Wine of naturally low ash content may, after the above treatment, come under (wrongful) suspicion of having been watered. P. S. ARUP.

Corrosion and protection from corrosion in brewery practice. H. Bürkli (*Schweiz. Brauerei Rdsch.*, 1956, **67**, 18—24).—A review covering types of corrosion, protective coatings of various types, cathodic protection, and methods for removing O_2 and CO_2 from water. P. S. ARUP.

Cathode radiation as a means of sterilising distillers' barley malt. J. R. Stratton, C. J. Coulter, W. H. Day and C. S. Boruff (*J. agric. Food Chem.*, 1956, **4**, 260—262).—Irradiation doses varying from $0{\cdot}05$ to $2{\cdot}0 \times 10^6$ rep were used to sterilise barley malts which were subsequently tested for viable bacteria, residual α-amylase, and in laboratory scale alcohol fermentations. With doses of $0{\cdot}5 \times 10^6$ rep the bacterial counts were reduced to $<2\%$ of those present in the untreated malts; preliminary grinding of the samples and a rise of temp. accelerated the death rate. A dosage of $0{\cdot}2 \times 10^6$ rep did not appreciably affect α-amylase content or yield of alcohol, but greatly lowered the development of lactic-type flora in the mash during fermentation. Dosages of $1{\cdot}0 \times 10^6$ (and above) rep reduced alcohol yields. The cost of a cathode unit of the size needed to irradiate malt for a moderate sized to large distillery is not commercially practical at the present time. (15 references.) E. M. J.

Barley and malt. V. Determination of husk-content and mealiness of barley. R. E. Essery, B. H. Kirsop and J. R. A. Pollock (*J. Inst. Brew.*, 1956, **62**, [New Series **53**], 150—152; cf. J.S.F.A. Abstr., 1955, ii, 243).—Treatment of barley with 50% H_2SO_4 at room temp. allows the complete removal of husks. Comparison of the dry 1000-corn wt. before and after treatment for determination of husk content gave satisfactory results. The dehusked corns were classified by visual assessment. In the groups the ratio of vol. of steely to total endosperm was 0, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, and 1 and a coeff. of mealiness was obtained which represents accurately the % of mealy endosperm in the sample. E. M. J.

Comparative aspects of Proctor and Spratt-Archer barleys. B. H. Kirsop and J. R. A. Pollock (*J. Inst. Brew.*, 1956, **62**, [New Series **53**], 155—157).—Data are presented on Proctor and Spratt-Archer barleys, 23 and 16 samples respectively, including germination characteristics, N-contents, 1000-corn wt. and husk contents. The mean values of the germinative capacities for both varieties were high proving that they were equally resistant to the unsatisfactory ripening conditions during 1954. Marked water-sensitivity was observed in many samples of both varieties. The 1000-corn wt. of Proctor barley is lower than that of Spratt-Archer, but the varieties do not differ significantly in N-content. The mean husk-content for Proctor is $10{\cdot}02\%$, for Spratt-Archer, it is $10{\cdot}60\%$ the difference between these means, $0{\cdot}58\%$ being significant. The difference in the yield of extract between the two varieties may be ascribed to the differences in husk-contents. E. M. J.

Determination of brewing value of barley varieties. G. Aufhammer and K. Schuster (*Brauwelt*, 1956, **96,B**, 565—570).—A review covering advances due to barley genetics and improved methods of valuation, a comparison between the Weihenstephan and "pneumatic" systems for experimental maltings, comparisons between protein contents and extract yields of malts from continental and imported barleys, climatic and cultural effects on barley quality, and characteristics of barley varieties, including a discussion on the tendency to form opalescence in wort. P. S. ARUP.

Kaffircorn malting and brewing studies. I. The Kaffir beer brewing industry in South Africa. H. M. Schwartz. **II. Studies on the microbiology of Kaffir beer.** J. P. van der Walt (*J. Sci. Food Agric.*, 1956, **7**, 101—105, 105—113).—I. The brewing process for Kaffir beer, a traditional Bantu drink, is described. It is prepared from kaffircorn (*Sorghum caffrorum* Beauv.) or other cereals by a lactic acid fermentation, followed by alcoholic fermentation. The alcohol content ranges from 1 to 8 vol.-%. (14 references.)

II. The three main microbiological conversions involved were studied: the souring of the mash by lactic acid bacteria, alcoholic fermentation of the wort by a variety of indigenous yeasts, and spoilage due to volatile acid formation. In breweries which do not practise temp. control during souring, a very heterogeneous lactic acid microflora, with eight spp., was characterised. In the subsequent alcoholic fermentation, 20 different yeast spp. were isolated. *Saccharomyces cerevisiæ*, *Candida krusei* and *Kloeckera apiculata* predominated. Spoilage due to excessive acetic acid formation was due to the common *Acetobacter* spp. (16 references.) J. S. C.

Effect of composition of malt on biological quality of beer. E. Hess (*Mitt. VersSta. Gärungsgew.*, 1955, **9**, 190—192).—A sound malt correctly produced from barley of good quality yields a wort capable of supporting wholesome and vigorous fermentation, and is thus (in addition to cleanliness) an important factor in suppressing infections. Trial maltings, supplemented by chemical analyses (especially by the Lundin, Kolbach and Hartong methods) are advocated. P. S. ARUP.

Effect of hydrogen peroxide and of peracetic acid in malthouse steep liquor. J. W. Green and M. J. Sanger (*J. Inst. Brew.*, 1956, **62**, [New Series **53**], 170—179).—With English barleys of the 1954 harvest some of which exhibited pronounced dormancy, peracetic acid had a less stimulating effect on germination than had H_2O_2. In tests where dormant and water-sensitive barleys were wetted too quickly for optimum growth conditions to be reached, H_2O_2 in the steep improved the germination, whereas peracetic acid acted as a germination inhibitor; where the amount of water entering the grain was limited, neither H_2O_2 nor peracetic acid used in short steep affected the final count. In the third series of tests in normal steep time peracetic acid had no marked inhibiting effect at low concentration and H_2O_2 increased the germinative energy by stimulating germination and growth rates. E. M. J.

The balance of sulphur compounds [during the brewing process] from wort to beer. W. Kleber and P. Lampl (*Brauwissenschaft*, 1956, **9**, 66—69).—The S compounds contained in the raw materials of brewing, in the wort, and in beer were studied in connexion with the colloidal and organoleptic properties, in three pale and three dark brews. The total S contents (as sulphate or organically bound) in the wort, sediment, pitching wort and beer were: 6·2, 8·8, 53·5 and $43{\cdot}9\%$ respectively. S from sulphate in the brewing liquor was found in the same amount in the beer. E. M. J.

Photocatalysis in the darkening of wort colour. J. E. Buckingham and L. R. Bishop (*J. Inst. Brew.*, 1956, **62**, [New Series **53**], 160).—Direct tests were made by keeping portions of wort in darkness and the remainder in sunlight. Darkening in colour proceeded at a rate of ~1 unit (colour in E.B.C. units) an hour in strong sunlight. In methods for the measurement of wort colour, it is necessary to specify that mashes should be protected from strong light and that, subsequently the worts should be similarly protected up to the time of colour measurement. Ascorbic acid ($0{\cdot}1\%$) prevents darkening of colour in sunlight, and may result in decrease in colour. E. M. J.

Enzyme production by yeasts. J. White (*J. Inst. Brew.*, 1956, **62**, [New Series **53**], 161—169).—Yeasts exhibiting high fermentation activity in dough normally possess high "zymase" and "malto-

zymase" activities, ability to produce CO_2 from glucose and from maltose, respectively. Studies were made of these properties in yeast crops on various substrates (molasses worts, and solutions of glucose, sucrose and maltose). Enzyme adaptations have been found to take place; a high maltose-fermenting activity was developed in the yeast by growth and fermentation in maltose solution, indicating that maltose may be fermented directly rather than after a preliminary hydrolysis to glucose. The degree of ability to ferment maltose and glucose, and also the invertase content of the yeast, often proves to be a function of the stage of harvesting of the yeast crop as well as being influenced by the composition of the substrate. E. M. J.

Possibility of transformation of yeasts into bacteria. A. Stage (*Brauereitechniker*, 1956, **8**, 61—65).—Publications concerning the transformation theory are reviewed. P. S. Arup.

Methods for distinction between living and dead yeast cells. U. Kutscher (*Mschr. Brauerei wissen. Beil.*, 1956, **9**, 27—30).—Publications dealing with staining methods are reviewed and discussed. For routine work, methylene-blue (of max. purity, in freshly prepared 0·01% solution) is preferred to other stains. A phosphate buffer-solution of pH 4·6 is recommended for the prep. of the yeast suspension. Other factors affecting the accuracy of the test are mentioned. (18 references.) P. S. Arup.

Comparative studies of methods of hop analysis. I. Estimation of water content. L. R. Bishop (*Brauwelt*, 1956, **96, B,** 429—434; cf. J.S.F.A. Abstr., 1956, i, 41). E. M. J.

Influence of furanogenic constituents and proteins of hops on beer production. V. Salač, M. Kotrlá-Hapalová and M. Vančura (*Brauwelt*, 1956, **96,B,** 361—366).—The results of experimental brewings confirm the improvement of the quality of the resulting beer (in comparison with normally hopped beer) by the separate use in equivalent amounts of the isolated bitter + tannin hop-fraction. Undesirable effects on taste, retention in solution of bitters, colour and biological stability result from additions (together with the bitters + tannins) of aq. extracts of the hops obtained after removal of the bitters and tannins. These extracts contain the furanogenic and protein constituents (amounting to ~10%) of the hops; although >20% of their total amount passes into solution during hop-boiling, their presence has an undesirable effect on the outcome. The final hop-residue (left after the extraction with water) has a less undesirable effect on beer quality. These results explain the (previously observed) undesirability of exhaustive extraction of hops, and the advantages of the proposed stepwise addition of hops during boiling (cf. J.S.F.A. Abstr., 1955, ii, 181). P. S. Arup.

New approach to the analysis of hop oil. G. A. Howard (*J. Inst. Brew.*, 1956, **62,** [New Series **53**], 158—159).—Gas–liquid partition chromatography offered a new approach to routine appraisal of hop aroma and quant. analysis of hop oil. From the hop oil obtained from ripe, undried Fuggles hops, 18 components were separated; more volatile compounds may have escaped detection. The principal compounds present include myrcene, methyl nonyl ketone, β- or γ-caryophyllene and humulene, these together comprising more than 70% of the total oil of this hop sample. Geraniol was not found. E. M. J.

Development and present status of filtration methods for beer. F. G. Redlbacher (*Brauer u. Mälzer*, 1956, **9**, 2—8).—A review covering historical information, the construction and operation of various types of plant in use, economic aspects, and comparative operating data for mass-, layer- and kieselguhr filtration. P. S. Arup.

The problem of turbidity caused by protein in bottled beer. M. E. Barton-Wright (*Brass. et Malt. belge*, 1956, **6**, 2—8).—The nature of the protein fractions in barley, their behaviour on infusion in the wort, the danger of denaturation during boiling and of interference with filtration efficiency, and the various measures available for eliminating turbidity caused by proteins, by cooling the wort to 0° and filtering while cold, or by treatment of the beer with various types of proteolytic diastases, are reviewed. J. S. C.

Use of ultra-violet radiation in brewery. R. Pöhlmann (*Brauwelt*, 1956, **96,B,** 533—536).—The radiation can be usefully employed for air-sterilisation and the prevention of mould growth in brewery premises, but not for the sterilisation of bottles or brewing vessels. Yeasts or bacteria suspended in gelatin, wort or beer are not affected under conditions of radiation sufficing for their destruction in aq. suspension. (11 references.) P. S. Arup.

Estimation of oxygen sensitivity of beer. W. J. Klopper (*Brauwissenschaft*, 1956, **9**, 70—72).—A method for measuring the rapidly oxidisable substances in beer, based on the titration of a beer sample with 2 : 6-dichlorophenol-indophenol so that the indicator is decolorised in 20 sec. is described, the result being expressed in microvals (μg. equiv.) of reducing substances. The method is claimed to be less sensitive to temp. changes than the "indicator time test" method. E. M. J.

Detection of beer-spoilage organisms in water and in washed bottles. E. Wienke (*Brauwelt*, 1956, **96,B,** 496—499).—Examples are given of bacteriological counts on various media of water samples and of washings from cleaned bottles, the latter having been obtained by vigorously shaking 100 ml. of sterile water in the bottle during 5 min. Counts should be made both by the usual plating method and by the membrane-filter technique. Preference is given to yeast-water–agar for detecting spoilage bacteria (rods and cocci) and to wort–agar for detecting wild yeasts. P. S. Arup.

Paper-chromatographic analysis of worts and beers. A. Stöckli (*Schweiz. Brauerei Rdsch.*, 1956, **67**, 1—5).—The carbohydrate contents of eight worts and 20 beers are analysed by the methods described, which include colorimetric estimations by means of the anthrone reaction. In the worts are found fructose, glucose, sucrose, maltose, maltotriose, maltotetraose, carbohydrates of higher mol. wt., and (in small amounts) three unidentified sugars, in proportions depending on the mashing method, but no pentoses can be detected. In the fermentation cellar, the monosaccharides and sucrose are completely fermented, the maltose and (in less measure) the maltotriose are partly fermented, whilst the maltotetraose and the higher carbohydrates are not attacked. Further fermentation of the maltose and maltotriose occurs during storage. The carbohydrate composition of the beers varies considerably as between different types, and appreciably as between beers of the same type. Contents of fermentable sugars depend on the nature of the yeast and conditions of fermentation and storage, whilst contents of unfermentable carbohydrates depend on the nature of the malt and on mashing conditions. P. S. Arup.

Determination of original gravity of beer by means of the refractometer. R. E. Essery and R. D. Hall (*J. Inst. Brew.*, 1956, **62,** [New Series **53**], 153—155).—Equations were developed by means of which the original sp. gr. of beer can be calculated from its refractive index and present sp. gr. The precision of the refractometric method is less than that of the distillation procedure, but is satisfactory for routine control when applied to beers of similar composition. Replicate measurements on 12 samples of the same beer by three analysts agreed satisfactorily and there was considerable saving of time by use of the refractometric method. E. M. J.

Applying Hillig's rapid method for [determining] water-insoluble acids to fresh milk. T. R. Freeman and W. F. Lewallen (*J. Dairy Sci.*, 1956, **39**, 219—220).—In order to obtain the water-insol. fatty acid content of freshly drawn milk it is necessary to destroy the lipase present in skim milk by heating the whole milk at 160°F. for 30 min. before separating the cream. In the absence of this treatment, very rapid lipolysis occurs between collection and testing (<2 hr. when using Hillig's rapid method). S. C. Jolly.

Colloidal proteins of skim milk. II. Effect of heat and disodium phosphate on the composition of the casein complex. L. F. Edmondson and N. P. Tarassuk (*J. Dairy Sci.*, 1956, **39**, 123—128).—In raw milk the ratio Ca : P in the colloidal Ca phosphate of the casein complex is ~1·5, becoming 1·25 after heating at 88° for 15 min. with 0·15% of Na_2HPO_4, some $CaHPO_4$ probably being formed from $Ca_3(PO_4)_2$. The proportion of Ca, P and N in the sedimented complex was constant irrespective of the particle size of the complex. The amount of Ca in the serum phase was reduced by approx. two-thirds by the heating with Na_2HPO_4. S. C. Jolly.

Development of oxidised flavours in unhomogenised and homogenised milk. E. S. Guthrie (*J. Dairy Sci.*, 1956, **39**, 219).—The oxidised flavour scores of samples of unhomogenised and homogenised winter milk during February are recorded. S. C. Jolly.

Factors affecting the heat stability and viscosity of half-and-half homogenised milk. E. S. Humbert, J. R. Brunner and G. M. Trout (*Food Technol.*, 1956, **10**, 134—137).—The effects of homogenisation became more pronounced as the fat content of the sample was increased upward to that of light cream. Generally the heat-stability followed an inverse relationship to that of viscosity; other factors being approx. equal, the addition of non-fat dry milk resulted in a product of lower heat stability. None of the fresh experimental samples gave a "feathered" effect when the cream came in contact with hot coffee made with hard, tap water. The inclusion of various increments of Ca in distilled water coffee indicated that homogenisation, especially single stage processing, was conducive to the appearance of the "feathering" defect. (18 references.) E. M. J.

Sampling paper-packaged milk for determination of homogenisation efficiency. G. M. Trout and L. Jokay (*Mich. agric. Exp. Sta. Quart. Bull.*, 1955, **38**, 198—209).—Accuracy of sampling from paper con-

tainers was improved by pouring from a specially cut opening 0·25 in. above the level of the milk while gently compressing the bulged sides of the container. Greater % differences between the fat content of the upper 100 ml. and that of the remainder of the quart were found in glass-bottled than in paper-packaged milk. E. G. BRICKELL.

Use of hydrogen peroxide as a dairy preservative. H. Lück (*Dairy Sci. Abstr.* [Rev. Art. No. 48], 1956, **18,** 364—385).—A review. The use of H_2O_2 as a milk preservative, its effect on various milk constituents and bacteria, and on keeping quality are considered. (About 100 references.) A. G. POLLARD.

Seasonal factors in the cheese industry. S. Bakalor (*Fmg S. Afr.*, 1955, **30,** 472—476, 487).—A general survey illustrated by graphs. E. G. BRICKELL.

Italian cheese ripening. IV. Various free amino-acids and fatty acids in commercial Provolone cheese. W. J. Harper and J. E. Long. **V. Various free amino-acids and fatty acids in commercial Romano cheese.** J. E. Long and W. J. Harper (*J. Dairy Sci.*, 1956, **39,** 129—137, 138—145).—IV. The amounts of free NH_2-acids and free fatty acids in commercial Provolone cheeses of the same age varied widely, the former being related to the starter organism and the latter to the type of enzyme product used in the manufacture. Purified glandular lipases produced cheeses at least comparable in quality with that made with crude rennet pastes; the importance of the lipases in these enzyme products is related to butyric acid production. About 1 mg. of free butyric acid and 2 mg. of free glutamic acid per g. of cheese were found in cheeses with the characteristic flavour, and these concn. increased while maintaining approx. the same ratio as the flavour increased.

V. The results with commercial Romano cheeses were generally similar to those found for Provolone cheeses, the chief difference being that the amounts of free acids in cheeses of the same relative flavour intensity were much higher in Romano cheeses. S. C. JOLLY.

Manufacture of filled cheese. I. I. Peters (*Food Technol.*, 1956, **10,** 138—141).—Methods are described for making cheese of the Cheddar type in which the milk fat is replaced partially or wholly by other fats. A satisfactory cheese was obtained by homogenising at 500 p.s.i. a mixture of natural skim milk or reconstituted skim milk and vegetable fat at 55° before and after pasteurisation. The addition of sweet cream buttermilk powder to reconstituted filled milk resulted in improvements in flavour as well as body and texture in the ripened cheese. Increasing the proportion of filled milk added to natural milk progressively decreased the flavour score. E. M. J.

New method of microbiological determination of nicotinamide. J. Claveau (*Industr. agric. aliment.*, 1956, **73,** 3—10).—It is shown that the rate of growth of *Saccharomyces lactis β* is precisely related to the concn. of nicotinamide present in the medium (up to a concn. of 2·5 μg. per 50 c.c.) and methods are developed using this test organism to determine nicotinamide in cheeses and other milk products. One method is based on measuring turbidity electrophotometrically to give the rate of growth of the organism and a second method is based on measuring the rate by fermentation and amount of alcohol produced. In each case, comparisons are made with standards of known nicotinamide content. J. S. C.

Dehydrated pork studies: removal of glucose by yeast fermentation. C. L. Hendrickson, D. E. Brady, C. W. Gehrke and R. F. Brooks (*Missouri agric. Exp. Sta.*, 1955, *Res. Bull.* 586, 12 pp.).—Ground pork (<0·125 in. mesh) fermented with addition of 1·7—20·0% washed yeast cells (*Saccharomyces cerevisiæ*) yielded a more stable product than did untreated meat. Pork fermented with 5—10% of yeast for 4 hr. gave the most desirable dehydrated product. Off-flavours caused by high bacterial populations in raw pork were minimised by cooking the meat prior to fermentation. The dehydrated product obtained by fermenting cooked meat was more satisfactory than that obtained by fermenting raw meat. A. H. CORNFIELD.

Correlation between heat penetration and rate of bacterial growth during the roasting of turkeys. P. P. Iacono, C. O. Ball and E. F. Stier (*Food Technol.*, 1956, **10,** 159—161).—In the roasting time of turkeys, both from the frozen and thawed states, data indicate that the birds are in the optimum growth range for *Staphylococci* and *Salmonellæ* too short a time to permit extensive growth of the organisms. There is no apparent danger of food poisoning resulting from *Salmonella* organisms or enterotoxin produced during the roasting period under the conditions studied. E. M. J.

Factors affecting the rate of chicken meat dehydration under vacuum. Alden Yoo, A. I. Nelson and M. P. Steinberg (*Food Technol.*, 1956, **10,** 145—150).—Serving-size pieces of chicken muscle were dehydrated in a vacuum plate drier. Factors studied included drier plate temp., pressure, initial temp. and moisture content of the product, sample thickness, precooking and type of muscle. Freeze drying was used for comparison. Vacuum dehydration required one-fourth of the drying time without apparent impairment of the initial quality of the product. E. M. J.

Laboratory electrodialyser and desalter. T. Wood (*Biochem. J.*, 1956, **62,** 611—613).—A simple Perspex electrodialysis cell is described. It involves use of Permaplex ion-exchange membranes or collodion membranes. The apparatus, which can handle 10 g. of material in 2 hr., can be used for desalting or fractionation of complex mixtures by electrodialysis, and is suitable for use with commercial meat extracts. Collodion membranes are used for removal of smaller mol. species from protein or polysaccharide solution by electrodialysis. Permaplex ion-exchange membranes are permeable to inorg. ions and org. ions of approx. the same size as the amino-acids, and they are suitable for desalting and electrodialysing non-colloidal substances. J. N. ASHLEY.

Further studies on the red halophilic bacteria from solar salts and salted fish. R. Venkataraman and A. Sreenivasan (*Proc. Indian Acad. Sci.*, 1956, **43,B,** 197—206; cf. J.S.F.A. Abstr., 1954, ii, 48).—The media and methods suited for the study of red halophilic bacteria are discussed and the characteristics of the organisms are tabulated, including rod forms, cocci, packets of cocci. Rod forms do not produce acid from sugars; most of the cocci produced alkali from glucose, sucrose and lactose. Certain bacillary forms digested casein, liquefied gelatin or hydrolysed starch; growth was noted in presence of 10% of salt, but not in 3·5%. Most of the red halophilic bacteria fail to grow even in concn. of 10% of NaCl. The classification of the various forms is discussed. (14 references.) E. M. J.

Storage of frozen plaice fillets. W. J. Dyer and M. L. Morton (*J. Fish. Res. Bd Canada*, 1956, **13,** 129—134).—Organoleptic assessments indicated that frozen plaice fillets stored at −12° become unpalatable at 6—7 months, as compared with 2—3 months for cod and ~8 months for Atlantic halibut. The increase in toughness corresponded to the decrease in extractable actomyosin content, followed closely by off-odours and flavour which were similarly in correlation with lipin breakdown. Treatment with ascorbic acid was ineffective in preventing lipin deterioration. It is suggested that there is a relationship between protein denaturation and lipin deterioration in the fish. (13 references.) J. S. C.

Freezing, packaging and frozen storage of fish. O. E. Nikkilä and R. R. Linko (*Food Res.*, 1956, **21,** 42—46).—Important factors influencing the quality of frozen fish are: wrapping to protect against atm. influences, min. of air spaces within the package, quick freezing under pressure, constant temp. of frozen storage, fish should be fresh but should have passed the stage of *rigor mortis*. E. M. J.

Microbiological determination of the essential amino-acids in fish protein. N. L. Lahiry and B. E. Proctor (*Food Res.*, 1956, **21,** 87—90).—The results of microbiological determinations of ten essential amino-acids in three varieties of raw fish, viz., shad (*Alosa sapidissima*), haddock (*Melanogrammus æglefinus*) and pomfret (*Stromateus cinereus*) indicating that fish protein is of high nutritive value comparable with that of beef, are of special importance with reference to nutritional problems of nations like India suffering from a shortage of protein foods, but having access to deep-sea fishing. E. M. J.

Evaluation of amino-acids in fish processed by various methods. B. E. Proctor and N. L. Lahiry (*Food Res.*, 1956, **21,** 91—92).—In tests with shad and haddock, the amino-acid contents did not differ significantly whether the fish was raw, dehydrated or canned. E. M. J.

Literature review on oils and fats 1953. M. N. Krishnamurthi (*Counc. sci. industr. Res. New Dehli*, 1955, 42 pp.).—The review is arranged under five sections including as far as possible important developments in 1953, and also some of the non-edible oils and fats used as raw materials in the edible oil and vanaspati industry. The following are dealt with: statistical survey, world situation, India's position; oilseeds, cultivation, soils, crop-control agents etc.; oils and fats, processing, hydrogenation, fish oils, utilisation of waste etc.; analytical methods etc.; synthetic fats, fat production by moulds, from petroleum, chlorella culture; nutrition and metabolism and an appendix has been added. E. M. J.

Modified apparatus for estimation of volatile oils. R. N. Chakravarti, S. Dash and A. B. Datta (*J. Instn Chem. India*, 1955, **27,** 266—273).—The apparatus for the estimation of volatile oils recommended in the B.P. (1953, p. 762) is modified by the introduction of a wide cylindrical portion between the condenser and the graduated tube and a wide-bore stopcock is provided in the inclined arm joining the bottom of the graduated tube to the still head. For the estimation of volatile oils lighter than water the stopcock is kept open and the apparatus is used as described in the B.P. Oils heavier than water are collected in the wide cylindrical portion where a pellicle

is formed and excess water is removed by intermittently opening and closing the stopcock. Oils which tend to solidify are distilled together with a small quantity of xylene to maintain the distillate liquid. J. M. JACOBS.

Sterols of olive oil. I. J. Aracián and J. Martel (*Grasas y Aceites*, 1955, **6**, 269—275).—Of various methods of isolation of the sterols which were examined, the most simple and rapid procedure was found to be pptn. of the digitonides in the fatty acids obtained from the oil. In this way 0·13% of sterols were obtained, of m.p. 136° and $[\alpha]_D^{25}$ −29·4°. L. A. O'NEILL.

Digestion and absorption of fat. F. H. Mattson (*Food Res.*, 1956, **21**, 34—41).—The passage of fat through the mouth and stomach, the digestion in the intestine, the respective rôles of bile and pancreatic juice, transport of lipins from the intestinal wall and absorption from the intestinal tract are discussed. (24 references.) E. M. J.

Direct estimation of saturated acids in small amounts of fats or mixed fatty acids. A. S. Sethi and A. R. S. Kartha (*J. sci. industr. Res.*, 1956, **15,B**, 103—105).—Using 5 g. and 1 g. samples, each of seven fats were oxidised by the acetic acid–acetone permanganate procedure and the products of oxidation isolated and hydrolysed with KOH. Excess KOH was neutralised with dil. H_2SO_4 and the pptd. acids were redissolved in aq. NH_3 before applying the Bertram separation. Results indicated very little difference in the yield of saturated acids employing 1 g. and 5 g. under the experimental conditions specified. The loss of saturated acids of higher mol. wt. during estimation by the Bertram method is therefore negligible. Data are presented and techniques are discussed. E. M. J.

Rapid estimation of the oil content of oilseeds. A. R. S. Kartha and A. S. Sethi (*J. sci. industr. Res.*, 1956, **15,B**, 102—103).—The sample of oilseed (0·1—0·3 g.) is ground with ~2 g. each of anhyd. Na_2SO_4 and glass powder, packed into a small glass percolator over a ¼-in. layer of anhyd. Na_2SO_4, and light petroleum (b.p. 70—90°) is percolated through. Most of the oil from the seed sample is contained in the first 7—8 ml., a negligible quantity only being contained in the next 3—4 ml. The light petroleum is driven off by heating the percolate in an air-oven at 100°. By placing four filter paper strips (1 sq. in.) in the dish the time of evaporating off the light petroleum was shortened from 2—3 hr. to 20—30 min. When the I val. or free tocopherol content of the oil is to be determined, the extraction is made with 25—30 ml. of ether, and no filter-paper strips are used. E. M. J.

Oil distribution in different parts of the coconut kernel. A. S. Sethi and A. R. S. Kartha (*J. sci. industr. Res.*, 1956, **15,B**, 105—106).—Six horizontal sections were cut from a mature coconut, when fresh, the wt. being 45, 56, 59, 49, 52 and 52 g. respectively. The sections were then cut into thin slices, the brown testa was removed from each slice and the remaining portion cut into three approx. equal parts transversely, and dried to constant wt. at 60°. The testa contained ~25% of oil. There was a rapid decrease in oil content from the outermost to the innermost layers. The outer layer (top section of the nut containing germ) contained 63 to about 66·7% of oil in the last four sections; middle layer contained ~51% in section I to ~64% in section VI; and the innermost layer 37—41%. All the cells in the kernel synthesise oil of the same composition. E. M. J.

Some seed fats of the *Santalaceæ* family. H. H. Hatt and R. Schoenfeld (*J. Sci. Food Agric.*, 1956, **7**, 130—133).—Examination of the seed fats of *Santalum acuminatum* (sweet quandong), *S. Murrayanum* (bitter quandong) and *S. spicatum* (Australian sandalwood) showed that substantial amounts of the octadecenynoic acid, ximenynic acid (**I**), were present. The composition of the fatty acids from seed fat of *S. acuminatum* was calculated to be: palmitic, 3%; stearic, 1%; oleic, 50%; linolenic, 2%; and **I**, 44%. The higher ethenoid acids which accompany **I** in *Ximenia* seed fats were either absent or present only in small amounts in *Santalum* seed fats. J. S. C.

Paper chromatographic separation of phospholipids. G. V. Marinetti and E. Stotz (*J. Amer. chem. Soc.*, 1955, **77**, 6668—6670).—Unmodified phospholipins are firmly bound to filter paper because of the positive charge on the N atom. An acylation technique is described which produces *N*-acyl phospholipin derivatives of greater mobilities which can be separated more readily by paper chromatography. (15 references.) J. S. C.

Free amino-acids and sugars in some food materials. M. V. Lakshminarayan Rao, N. Subramanian and M. Srinivasan (*J. sci. industr. Res.*, 1956, **15C**, 39—47).—Chromatographic methods were used to test for amino-acids and sugars in fruits, vegetables, spices and coconut water. Methionine and trytophan were not found, the varying contents of other amino-acids and sugars are tabulated. The no. and nature of free amino-acids in citrus fruits varied in different varieties. In spices in addition to sucrose, glucose and fructose, sugars with R_F values approximating to that of raffinose are present. In coconut water sucrose appeared only after the layer of meat or kernel was formed inside the nut. E. M. J.

Isolation of arginine and lysine from protein hydrolysates by means of ion-exchange resins. II. Column treatment. P. M. Strocchi and P. Drago (*Ann. Chim., Roma*, 1955, **45**, 1159—1173).—A procedure is described for isolating from a hydrolysed protein previously treated to eliminate inorg. ions, dicarboxylic and neutral amino-acids, a mixture of arginine and lysine in a state of high purity and with a yield of ~60%. A cationic resin of carboxylic type is employed, with a H-cycle and frontal exchange procedure. Control is effected by paper electrophoresis. The cause of the operation can be followed not only by the pH of the effluent, but also by the expansion of the bed of resin. The rate of flow through the column should be sufficiently slow such that equilibrium is approached. L. A. O'NEILL.

Nutrition and "civilisation diseases." H. D. Cremer (*Angew. Chem.*, 1956, **68**, 30—40).—Tumours, arteriosclerosis, vascular diseases, obesity and dental caries, described as "civilisation diseases," are caused by unnatural nutrition. An investigation is made of the effect of nutritional factors in promoting or causing a particular clinical picture. In many cases such an influence exists, but in other such a connexion cannot be shown. Primitive manners of nutrition can cause more damage than a normally mixed diet. (46 references.) C. A. FINCH.

Study of new degradation products of ascorbic acid in solutions of different pH values. A. F. Damanski and M. Milosavljević-Jovanović (*Bull. Soc. chim. Belgrade*, 1955, **20**, 111—117).—Investigations into the relation between pH values, in the range 1·8—3·9, and the equilibrium of ascorbic acid solutions comprise tests on L-ascorbic acid buffered, according to Britton and Robinson, with a solution of 2·40 g. of H_2SO_4, 3·92 g. of H_3PO_4 and 2·48 g. of H_3BO_3 in 1000 ml. of water, and adjusted by addition of 5N-NaOH, which method corresponds with the buffering system in plants. Oxidation with 0·1N-$KMnO_4$ at 0° and at 20° for 24 and 48 hr. and subsequent titration with 0·001N-Tillman's reagent revealed that the pH influence was directed towards the formation of new compounds, i.e., de-tetrahydroascorbic acid and a dehydrated form of ascorbic acid. Results suggest that the $[\alpha]_D^{20}$ value for ascorbic acid should be accepted as +32·5° and not 20—22°, as quoted in the literature. (16 references.) L. S.

Photochemical oxidation of ascorbic acid in solutions containing oxalic acid. II. Mechanism of the reaction. L. H. Lampitt, L. C. Baker and E. Wittenberg (*J. Sci. Food Agric.*, 1956, **7**, 120—124).—A solution at pH 1·7, containing 0·25% oxalic acid, 0·02% ascorbic acid and $Fe(NH_4)(SO_4)_2$ (≡11·6 μg. Fe^{III}/ml.) was irradiated with the Hg vapour lamp and a stream of N_2 passing through. It was found to develop additional reducing power as measured by the dye solution. This is consistent with the view that the initial step in the photochemical oxidation of ascorbic acid is a photochemical reduction of ferri-oxalate to Fe^{II} salt. It is shown that Fe^{III} salts do not oxidise ascorbic acid in oxalic acid solution although they do so in H_2SO_4 solution, and it is suggested that the oxidation depends on OH and HO_2 radicals produced by autoxidation of the Fe^{II} ions. It was shown that irradiation of 0·25% oxalic acid solution with varying proportions of Fe^{III} gives a reducing power consistent with complete reduction to Fe^{II}. Irradiation of the same solution in air gives a degree of oxidation dependent on the Fe : C_2O_4'' ratio. When this ratio is >1 : 50, Fe^{II} ions are actually formed from Fe^{III} ions. (13 references.) J. S. C.

Use of *p*-chloromercuribenzoic acid in determination of ascorbic acid with 2 : 6-dichlorophenolindophenol. J. A. Owen and B. Iggo (*Biochem. J.*, 1956, **62**, 675—680).—Colorimetric determination of indophenol reducing activity is unreliable below pH 3·5 because of the extent of spontaneous fading of indophenol that occurs. *p*-Chloromercuribenzoic acid does not affect the reaction between indophenol and ascorbic acid, but it inhibits almost entirely the decolorisation of indophenol by many interfering substances in biological fluids and extracts. Its use is recommended to increase the specificity of the indophenol method for determination of ascorbic acid in biological material. J. N. ASHLEY.

Black pepper. I. Analysis of bite principles. F. Tausig, J. I. Suzuki and R. E. Morse (*Food Technol.*, 1956, **10**, 151—154).—The effect of light on piperine (a principle contributing to "bite" in black pepper) dissolved in benzene or chloroform was studied. The rate of piperine loss, based on transmittance studies at 342 mμ. was less for benzene than for chloroform solutions. One fraction of an oleoresin of Malabar black pepper was further fractionated by molecular distillation, crystallisation and crude counter-current distribution. Each fraction was analysed for heat and for piperine by

Kjeldahl and spectrophotometric methods. Large variations between the two methods of piperine determination were found in the fractions. Heat values were in good agreement with spectrophotometric piperine in each fraction. (16 references.) E. M. J.

Is it possible, without risk, to improve the natural appearance, odour and flavour of foodstuffs? L. de Saint-Rate (*Industr. aliment. agric.*, 1955, **72**, 795—796).—An account of discussions of the subject at (i) the conference of the International Commission of Agricultural Industries and the International Bureau of Analytical Chemistry for human and animal foodstuffs, July 6th—9th, 1955, Vienna, and (ii) The World Health Organisation and the Food and Agriculture Organisation, September 19th—24th, 1955, Geneva. J. S. C.

Simultaneous vs. successive presentation in a paired comparison situation. N. Schwartz and C. H. Pratt (*Food Res.*, 1956, **21**, 103—108).—The nature and extent of the differences between the two approaches are measured. The simultaneous presentation yields stronger preferences than does the successive. E. M. J.

Airtight package bags made from aluminium complexes. P. Prévot (*Rev. Alumin.*, 1955, **32**, 1025—1031).—The manufacture, testing procedures and various uses are described in respect of a packaging material composed of Al foil as a barrier fixed on a plastic or cellulosic backing. The material constitutes an efficient vapour seal of high strength. Bags made from it are closed and hermetically sealed by automatic machines. J. S. C.

Drying of cereal flakes etc. W. S. Barron & Son, Ltd. (Inventor: A. H. Rowles) (B.P. 735,480).—Cereal flakes etc. are carried in a stream of hot air int.o a cylindrical vessel which contains two co-axial perforated cylinders The flakes pass down the space between the cylinders and are dried by the hot air which passes down the inner cylinder, through the perforations and then upwards through the outer jacket. At the base of the cylinder, the flakes are cooled in a stream of cold air and discharged. The hot air passing up the outer jacket acts as a heat-insulating jacket to maintain a high temp. in the vessel. J. S. C.

Manufacture of dough and baked products. Wallace and Tiernan Process Co. (B.P. 735,184, 3.8.51. U.S., 3.8.50).—The materials for production of an undeveloped dough are fed to a mixer and intimately mixed. The rate of removal of this dough by a pump is metered in relation to the input. The undeveloped dough is then worked by two rotating paddles which intermesh with one another but do not aid the flow through the mixing and developing chamber. The development rate is thus adjusted by altering the speed of the paddles. This rate corresponds to the feed rate, with the result that a continuous production of correctly-developed dough is possible. K. Ridgway.

Production of bakers' yeast. Backhefe G.m.b.H. (B.P. 739,702, 14.5.53).—Cultured yeast is grown in aërated sugar-containing medium at pH 2·5—4 (in presence of material capable of adversely affecting growth at pH 3·5—4, e.g., formaldehyde or acetaldehyde), to give seeding yeast. This is then subjected (after separation of the wort) to a hindering fermentation at 12—21° (optionally in presence of a small amount of yeast nutrient medium, with weak aëration), followed by final multiplication fermentation without alcohol formation to give a high yield of bakers' yeast of improved quality. F. R. Basford.

Preparation of coffee and other beverages. P. Gregorelli (B.P. 736,106, 20.12.52).—A percolating device suitable for attachment to the spout of an ordinary kettle is described. J. A. Barnard.

Treatment of palm oil. Standard Oil Co. (B.P. 736,134, 8.10.53 U.S., 31.10.52).—Carotene in palm oil is concentrated by a thermal diffusion method. A thin film of palm oil is formed on surfaces of heat-conductive inert material (glass, stainless steel, aluminium) which are uniformly spaced apart 0·01—0·15 in. A temp. gradient is maintained across the film of oil. A carotene-enriched fraction is withdrawn from the cooler surface and a lighter-coloured, carotene-impoverished, fatty acid-enriched fraction from the hotter surface. The palm oil may be dehydrated and inert viscosity-reducing agents and/or antioxidants may be added before being subjected to thermal diffusion. I. Jones.

Production of small pieces of chocolate. A/S Toms Laboratium (Inventor: A. N. Neergaard) (B.P. 736,010, 22.9.52).—Small regular pieces of chocolate can be produced by allowing the melted chocolate to form a plate-like layer on a conveyor band moving through a cooling channel. After 25—35% of the total time of cooling has elapsed the chocolate is cut into the required pieces, or marked with depressions corresponding to the desired final shapes by means of a drum rotating just above the conveyor; the chocolate then breaks into pieces on removal from the support. J. A. Barnard.

3.—SANITATION

Reports of co-operative research on insect control in farm-stored grain, July 1941 to June 1946. H. H. Walkden (*J. econ. Ent.*, 1955, **48**, 766—767).—These reports are briefly listed under subject matter and 14 American agricultural libraries are named in which sets of the reports may be seen. A. A. Marsden.

Effect of streptomycin on some stored grain insects. R. K. De (*J. econ. Ent.*, 1955, **48**, 774—775).—Addition of streptomycin to rice or wheat grain at the rate of 1 : 1000 or 1 : 5000 had no effect on the mortality of adults of *Sitophilus oryzæ* and *Tribolium castaneum*. However, 100% mortality of the insects was obtained much earlier in the controls than in the treated grain. A. A. Marsden.

Insecticides for control of pests in groundnut warehouses. Anon. (*Agric. Chemicals*, 1955, **10**, 63, 131).—Two types of mixed insecticides (one suitable for mechanical generators and the other for thermal type generators) for the control of pests in stored groundnuts are described. A 2·5% DDT spray is recommended before the warehouse is filled. A. H. Cornfield.

Toxicity and interaction of stereo-isomers of BHC in cockroaches. K. van Asperen (*Bull. ent. Res.*, 1956, **46**, 837—843).—Tabular data of the joint action of the γ- and δ-, and the γ- and α-isomers on *Periplaneta americana*, (L.) are presented and discussed statistically. Antagonistic effects of the two poisons are reported. E. G. Brickell.

Experimental compounds for control of chlordane-resistant German cockroaches. E. W. Laake (*J. econ. Ent.*, 1955, **48**, 753).—When used in houses, grocery or meat stores, Malrin (a proprietary material containing malathion 1·8 and Perthane 3·6%) was the safest insecticide tested for controlling resistant German cockroaches. Mixtures of malathion and dieldrin were the most effective toxicants for controlling resistant German, American and Oriental cockroaches where these species were found together. A. A. Marsden.

Diaryl-trifluoromethylcarbinols as synergists for DDT against DDT-resistant house flies. A. S. Tahori (*J. econ. Ent.*, 1955, **48**, 638—642).—Insecticidal and synergistic activities of 29 materials structurally related to DDT were compared with DDT against a moderately resistant strain of house flies (*Musca vicina* Macq.). Bis-(*p*-chlorophenyl)-trifluoromethylcarbinol was the most active synergist tested and considerably extended the residual effect of DDT. The carbinols were more effective synergists than were the corresponding esters or H compounds. Relationship between structure and synergistic effect is discussed. A. A. Marsden.

Granular baits for the control of house flies. J. C. Keller and H. G. Wilson (*J. econ. Ent.*, 1955, **48**, 642—643).—On garbage and trash heaps heavily infested with house flies, a maize meal bait containing malathion (2) and sugar (10%) gave good control after one distribution, whilst daily applications gave excellent control. Five daily treatments of a similar Diazinon (1%) bait were required for satisfactory fly control during 24 hrs. All malathion baits tested gave excellent results against flies in dairy barns. A 2% Chlorthion–sand bait was equally effective but Chlorthion (1%) in maize meal was less satisfactory. A. A. Marsden.

Relative toxicity to house flies of Pirazinon, Am. cyanamid 4124, and malathion in comparison with parathion and pyrethrins. W. A. Gersdorff, P. G. Piquett and R. H. Nelson (*J. econ. Ent.*, 1955, **48**, 680—681).—The three compounds, Pirazinon, Am. cyanamid 4124 (the *o*-chloro derivative of methyl parathion) and malathion were respectively 0·36, 0·60 and 0·062 as toxic as parathion, and 12, 20 and 2 times as toxic as pyrethrins. At the concn. used these materials caused no appreciable knockdown of flies in 25 min. The relationship of the chemical structure of these materials to their toxicity is discussed. A. A. Marsden.

Larvicides for control of the house fly. L. N. Standifer (*J. econ. Ent.*, 1955, **48**, 731—733).—Of 25 formulations of chlorinated hydrocarbon and phosphate insecticides, an aldrin emulsion with an LD_{50} of 0·9 p.p.m. had the highest toxicity to third-instar larvæ of house flies. EPN-300 wettable powder and Diazinon emulsion were almost as toxic as was aldrin emulsion. $C_6H_6Cl_6$ as an emulsion or wettable powder had the least toxicity of the chemicals tested. A. A. Marsden.

Excretion of a radioactive metabolite by house flies treated with carbon-14-labelled DDT. L. C. Terriere and R. D. Schonbrod (*J. econ. Ent.*, 1955, **48**, 736—739).—Susceptible and resistant flies when given sub-lethal doses of DDT excreted up to 88 and 63%, respectively, of the toxicant in the form of a water-sol. conjugate. This excretion began the first day after topical application and apparently continued until all of the absorbed dose was metabolised. Attempts to identify this metabolite failed. A. A. Marsden.

Relative toxicity of topically applied allethrin and pyrethrins to house flies and cockroaches. N. Mitlin and F. H. Babers (*J. econ. Ent.*, 1955, **48**, 747—748).—Allethrin was equally as toxic as pyrethrins to both susceptible and resistant female house flies, 0·03 as toxic to the susceptible and resistant adult males of *Blattella germanica*, and 0·15 as toxic to last-instar nymphs of *Periplaneta americana*. A. A. MARSDEN.

Resistance to ovicides by eggs of the body louse. M. M. Cole (*J. econ. Ent.*, 1955, **48**, 746—765).—Eggs of the body louse, *Pediculus humanus humanus*, from a Korean DDT-resistant strain showed high resistance to a chlorinated hydrocarbon, lauseto neu (a chlorinated hydrocarbon), but little or none to lindane and dinitroanisole. Lindane (1%) and dinitroanisole (10%) gave 92 and 84% kill, respectively, of resistant eggs, and 95 and 72% kill, respectively, of non-resistant eggs. A. A. MARSDEN.

Aërosol deodorant insecticides. L. Trademan (*Soap, N.Y.*, 1956, **33**, No. 1, 149, 171).—A combined insecticidal and deodorant formulation composed of chlordane and knock-down agent, solvent, co-solent, quaternary ammonium compound and propellant, is described and details are briefly reported of trials carried out. J. S. C.

Some anticoagulant properties of 2-acyl-1 : 3-indanediones and warfarin in rabbits. S. R. Heisey, J. P. Saunders and K. C. Olson (*J. agric. Food Chem.*, 1956, **4**, 144—147).—The ability of warfarin, [3-(α-acetonylbenzyl)-4-hydroxycoumarin] and a series of acylindanediones to produce increased plasma coagulation time after single injections of 40 mg./kg. in rabbits is compared. Diphenylacetylindanedione is the most powerful of those tested, warfarin being reliatively ineffective. Less active indanediones and compounds of related structure are tested at low diurnal and semi-diurnal doses, *iso*butyrylindanedione being most active in this group. Vitamin K_1 is effective in reversing the anti-coagulant effects. (16 references.) N. M. WALLER.

Drinking water taste and odour. Correlation with organic chemical content. F. M. Middleton, W. Grant and A. A. Rosen (*Industr. Engng Chem.*, 1956, **47**, 268—274).—Organic wastes in the water source cause serious tastes and odours in the drinking water of a municipal supply. Threshold odours from 100 to several thousand were observed in the raw water. Organic materials recovered from this raw and finished water showed direct variation with the odour thresholds and an inverse relationship to river flow. Aromatic hydrocarbons and oxygenated neutral materials were the most odorous classes of substances isolated from the water. The results illustrate some of the techniques available for the study of organic contaminants affecting water quality. O. M. WHITTON.

Detection of traces of nitrites and nitrates, by means of phenolphthalein, hydrogen peroxide and sulphuric acid. T. Garbuliński (*Roczn. Chem.*, 1955, **29**, 1109—1111).—A drop of reagent (to 50 ml. of 0·05% phenolphthalein in ethanol is added one drop of 30% H_2O_2) is added to 4 ml. of H_2SO_4, when a pink coloration develops. A few drops of test solution are added, when the coloration is discharged if the HNO_3 or HNO_2 content exceeds 0·7 p.p.m. R. TRUSCOE.

Rodenticidal preparations. Schering A.-G. (B.P. 735,694, 21.8.51. Ger., 23.8.50).—A pulverulent rodenticidal prep., essentially non-toxic to other animals, comprises a systemic rodenticide [a thiourea, e.g., naphth-1-ylthiourea (**I**), or a coumarin derivative, e.g., 4-hydroxy-3-α-acetonylbenzylcoumarin] and optionally dry filler, coated with non-repellent, water-insol., non-toxic adhering agent, viz., natural or artificial resin (coumarone resin), metal salt of a high-mol. fatty acid (of >8 C), e.g., Ca oleate. Thus, a mixture of **I** 20 and slate meal 80 pt. is well wetted with a 5% benzene solution of coumarone resin, then the solvent is evaporated, to give an adherent powder. F. R. BASFORD.

4.—UNCLASSIFIED

Glass polymerisation vessel for small-scale studies. J. D. Sutherland and J. P. McKenzie (*Industr. Engng Chem.*, 1956, **48**, 17—19).—The ½-gal. size glass polymerisation vessel described and illustrated is suitable for studying any type of polymerisation, such as high-solid latex studies, requiring turbulent agitation combined with good heat transfer over a wide rage of temp. and pressure. The vessel is made of ½-in. thick impact-resistant borosilicate glass and can withstand pressures up to 300 lb./sq. in. O. M. WHITTON.

Liquid air level indicator. J. Rohleder (*Roczn. Chem.*, 1955, **29**, 1129—1133).—One junction of a differential constantan–Cu thermocouple is at room temp., whilst the other, surrounded by an insulated heating coil, is immersed in liquid air. The heat supplied by the coil is used to vaporise the liquid, so that this junction is at the temp. of liquid air until the level of the latter falls to about 1 mm. lower, when it warms up rapidly. This is indicated by return of the milliammeter needle to zero deflection. R. TRUSCOE.

Separation of substances and determination of their relative molecular sizes by use of columns of starch in water. G. H. Lathe and C. R. J. Ruthven (*Biochem. J.*, 1956, **62**, 665—674).—Neutral mol. are eluted from potato starch columns in buffer in the descending order of their mol. wt., and there is good resolution of substances in the mol. wt. range of 100—1000. Large mol., remain outside untreated starch grains, but starch, swollen by warming in water, is permeable to larger mol., and in columns separates substances in the mol wt. range of 1300—150,000. The possibility of determination of mol. wt. with such columns is discussed. Values of 6000 and 35,000 are obtained for insulin and myoglobin, respectively. J. N. ASHLEY.

Separation and determination of microquantities of lower aliphatic acids, including fluoroacetic acid. F. Bergmann and R. Segal (*Biochem. J.*, 1956, **62**, 542—546).—Fluoroacetic acid is separated from formic, acetic, and other straight chain fatty acids by paper chromatography of the hydroxamic acids in NH_3–ethanol–pyridine–water. Hydroxamic acids are determined by direct oxidation with I to NO_2', which is used to diazotise sulphanilic acid, and the resulting diazo compound is coupled with α-naphthylamine. The azo dye is then determined colorimetrically. This new method which involves two-dimensional paper chromatography of the hydroxamic acids, extraction and formation of the dye, has a lower limit of 0·5 μg./ml. of final solution. J. N. ASHLEY.

Collimated windowless Geiger counter for scanning chromatograms. D. R. Bangham (*Biochem. J.*, 1956, **62**, 550—551).—A simple windowless He–ethanol counter is described; it operates in the Geiger region with a standard rate meter. It is designed to give narrow collimation and low background suitable for scanning paper chromatograms or electrophoresis strips. J. N. ASHLEY.

Simple helium–ethanol flow counter for monitoring chromatograph-column effluents containing weak β-emitting isotopes. D. R. Bangham (*Biochem. J.*, 1956, **62**, 552—553).—A simple He–ethanol flow counter is described. It is suitable for indicating radioactivity due to weak β-emitting isotopes such as ^{14}C or ^{35}S in the effluent from a chromatographic column. It provides a continuous, immediately available record of radioactivity coming off a column and obviates several tedious sampling operations. It is not intended as an accurate measuring instrument. J. N. ASHLEY.

Determining traces of octamethylpyrophosphoramide (schradan) in crops. D. F. Heath, J. Cleugh, I. K. H. Otter and P. O. Park (*J. agric. Food Chem.*, 1956, **4**, 230—233).—The schradan-treated crop samples (50 g.) are macerated with water, (50 ml.) followed by filtration or centrifugation, and extraction of the aq. macerate at pH 8—10 (0·1N-NaOH) with two equal vol. of chloroform. The solution is clarified and concentrated to ~25 ml. and transferred to a micro-distillation apparatus which is described. The chloroform is evaporated, the residue is heated and a vac. (1 mm. of Hg) is applied. The convective air-stream conveys the more volatile part of the residue to the cold finger and schradan (v.p. $2·46 \times 10^{-4}$ mm. at 25°) can be separated. The schradan residues are transferred to a Kjeldahl flask, treated with 5 ml. of 10N-H_2SO_4 + 0·3 g. of NH_4 persulphate and finally a dilute solution of colourless phosphate is assayed by a method based on that of Berenblum and Chain which is described. The photo-absorption is measured at 7350 A. on a Spekker absorptiometer. Recoveries and blanks are satisfactory. (14 references.) E. M. J.

Determining traces of tetramethylphosphorodiamidic fluoride (dimefox) in crops. L. F. Dupée, D. F. Heath and I. K. H. Otter (*J. agric Food Chem.*, 1956, **4**, 233—236).—The most general method consists of macerating a sample of the crop (50 g.) with water, filtering, extracting with chloroform, evaporating to low bulk and transferring to a micro-distillation apparatus, and distilling in the presence of a few drops of glycerol–glycol mixture. The dimefox in the distillate is estimated as phosphate by the method of Berenblum and Chain. A second method, used for oily crops, e.g., cocoa beans, consists of distilling a macerate in oil and separating the dimefox from interfering compounds in the oily distillate. The methods described give satisfactory and reproducible recoveries (10 crops tested) and satisfactory low blanks on 15 crops. E. M. J.

Trace elements from feed to food. G. K. Davis (*J. agric. Food Chem.*, 1956, **4**, 124—127).—The importance of trace elements, Cu, Fe, Mn, Zn and Co in plants and animals is reviewed. (23 references.) N. M. WALLER.

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