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## An Army routed

Kweheli is a peace loving man. His tribe no longer goes to war—in the ways his forefathers knew. Yet, throughout his life, regularly year by year (and sometimes several times a year) Kweheli has had to fight—and just as regularly lose—a battle which has threatened his very livelihood. Today, that battle has been won. For Kweheli. By Shell.

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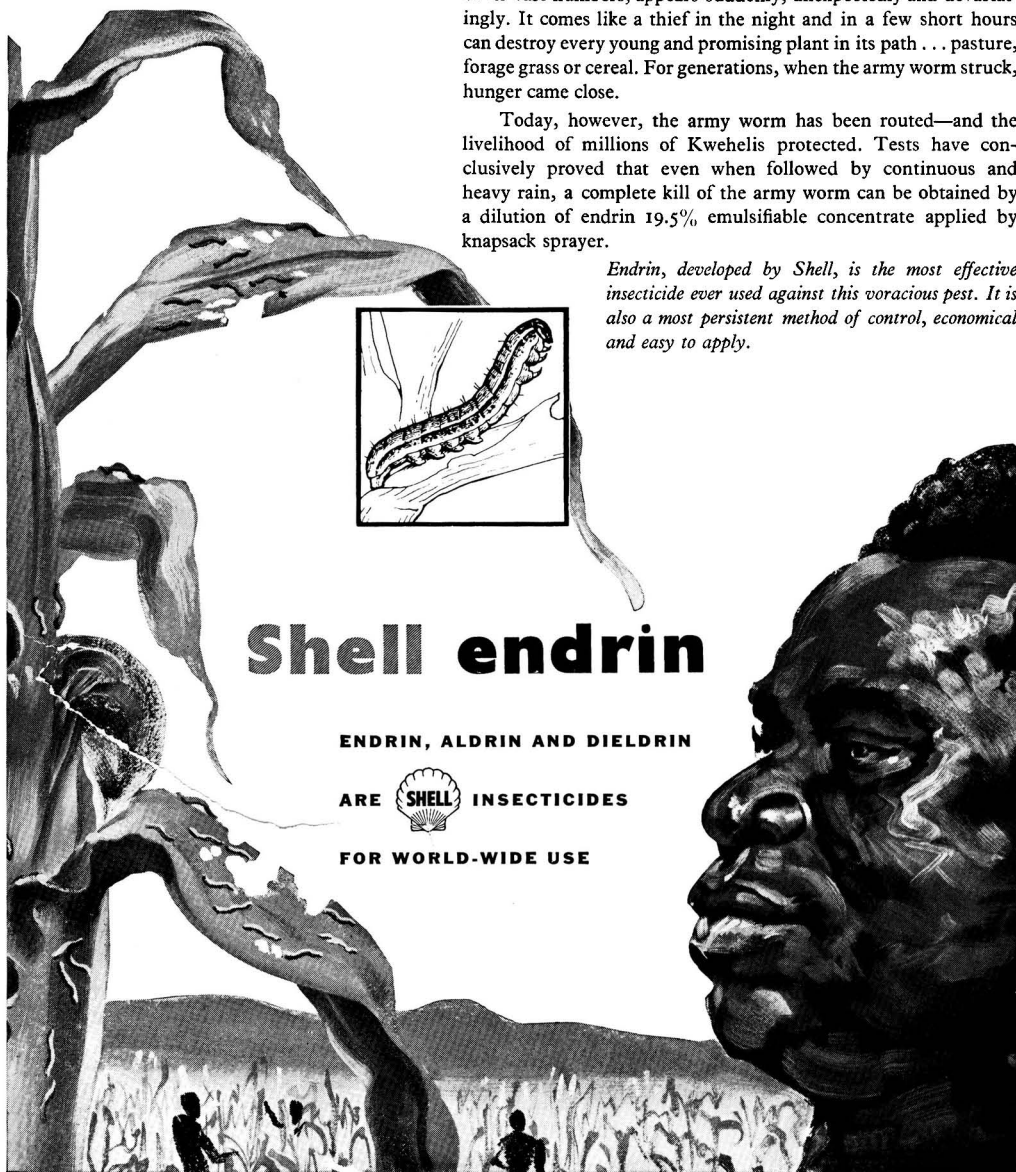


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## EXPERIMENTS ON COCOA FERMENTATION IN WEST AFRICA

By G. R. HOWAT, B. D. POWELL and G. A. R. WOOD

A series of experiments on cocoa fermentation using heaps, boxes and steel vessels has been carried out in West Africa. Different methods of fermentation were investigated and scientific data relating to temperature, pH and moisture changes collected. A vessel designed to ferment the beans from 150 to 500 pods was tested and found to be satisfactory. Attention was also given to answering the questions which planters ask about fermentation—the effect of the degree of ripeness of the pods, of the length of time elapsing between harvesting and the beginning of fermentation, and whether the transportation of wet beans is detrimental.

### Introduction

This paper gives an account of research work on cocoa fermentation carried out in the Gold Coast, partly at Bekwai, Ashanti, during October, November and December 1954, but mainly at Tafo (Fig. 1) during the corresponding period in 1955. Laboratory facilities were available at Tafo (W.A.C.R.I.) but not at Bekwai, where all the experiments took place in the compound of the Cadbury & Fry agent's bungalow.

It is well-established custom to use the term 'cocoa fermentation'—and it will be used throughout this paper—but it should be noted that scientifically it is unsatisfactory, as many reactions occurring during the process are not true fermentations in the normally accepted sense of the term.

The fermentation of cocoa beans, usually begun within one or two days of harvesting the pods, is one of the most important processes involved in the production of cocoa and chocolate of high quality. Isolated investigations have been made from time to time, but considering the importance of the process, it is surprising that so little is known about it. The work at Bekwai and Tafo was therefore a contribution to the attempts now being made by various research institutes to remedy this serious lack of knowledge.

In the winter of 1953-4, experiments at Bournville<sup>1</sup> with beans from pods flown from West Africa, showed that it was possible to ferment beans as few in number as 30. They were kept for 3 days at 35°, followed by 3 days at 50°—either untreated or with the addition of an odourless and tasteless compound\* which diminished the activity of the micro-organisms in the pulp—and steps were taken to remove the carbon dioxide evolved. It was desired to repeat these experiments on a larger scale and with this end in view a large fermentation vessel (thermostatically controlled) was designed.

In addition, as hitherto there has been no reliable method of fermenting a quantity of beans of the order of 30 to 100 lb. wet weight (a problem which faces planters and others working with new varieties), a small vessel was constructed at Bournville for this purpose (described in detail below) which it was necessary to test in a cocoa-growing area.



FIG. 1.—Fermenting house at Tafo

\* Vantoc B, a 12.5% aqueous solution of an alkylpyridinium halide (essentially tetradecylpyridinium bromide), manufactured by Imperial Chemical Industries, Ltd.

Fermentations are usually carried out in heaps by peasant farmers, and in boxes on estates and plantations, and for each method there is a recommended procedure. From the results of the small-scale laboratory experiments it was thought that it would be advantageous in both heap and box fermentations if the temperature could be kept at about 35° during the first 3 days, a lower figure than is usually observed. Modifications were introduced in an attempt to attain this condition.

Investigations were also made in an attempt to find answers to some of the questions which planters ask, or, looking to the future, which they are likely to ask, about fermentation, e.g., the effects of the degree of ripeness, and the length of time elapsing between harvesting and the beginning of fermentation. Central fermentaries are being set up in various producing areas—in the Far East, for example—and it may be asked whether the transportation of wet beans before fermentation has any adverse effects. It would, clearly, be more economical for the wet beans themselves to be delivered to a fermentary, rather than the pods.

Considering that the annual world production of cocoa has increased from 70,000 tons to 700,000 tons over the past 60 years, it is remarkable that our knowledge of the chemical processes involved in fermentation is so scanty. Interest has grown, however, during recent years and both the chocolate and cocoa industry and tropical research stations are bringing more resources to bear on the problem.

At Bekwai and Tafo, three main aspects were studied :

- (a) Changes in the moisture content of the beans during fermentation and drying,
- (b) Changes in the pH of the cotyledons and pulp during fermentation and drying,
- (c) Changes in the composition of the gas surrounding the beans during fermentation.

An indication of the results is given below, but their full consideration will be made the subject of a separate paper.

## Experimental

### Equipment

Fermentations were carried out in heaps and in three ways requiring specially constructed apparatus : (a) Large fermentation vessel, (b) small fermentation vessel, (c) boxes.

(a) *Large fermentation vessel (thermostatically controlled).*—This apparatus was designed to ferment quantities of beans of the order of 500 lb. wet weight, under selected conditions of temperature and aeration. A stainless steel tank, depth 3 ft., diameter 2 ft. 6 in., was fitted with a jacket through which water could circulate from a kerosene-fired heater (Fig. 2). The temperature of the water could be thermostatically controlled. A perforated false bottom was fitted into the tank so that sweatings could run off from the fermenting beans and air could be supplied beneath it from a compressor.\* The tank lid, false bottom, and all fittings with which the beans come into contact were constructed from stainless steel. Several thicknesses of plantain leaves were put on the top of the beans to retain heat and moisture.

(b) *Small fermentation vessel.*—With this compact piece of apparatus—overall height 3 ft. 6 in.—it was possible to ferment quantities of beans from 30 to 100 lb. wet weight (Fig. 3). It comprised a stainless steel tank, depth 24 in., diameter 14 in., complete with lid and fitted with a water jacket heated directly by two kerosene burners arranged for manual control. The tank had a false, perforated, stainless steel bottom, below which was a vent with an adjustable throat through which air could circulate by natural convection, and sweatings could escape. No provision was made for aeration by the compressor and plantain leaves were not placed on the top of the beans. The design, though simple, was such that contamination of the beans by any fumes from the burners was not possible.

(c) *Boxes.*—The fermentation boxes 3 ft. square  $\times$  2 ft. deep were constructed of wood (*Chlorophora excelsa*) 1 in. thick, mounted 1 ft. above the ground on a wooden framework. About 50 holes,  $\frac{3}{8}$  in. diameter, were bored through the bottom at regularly spaced intervals to allow sweatings to drain away. A charge of 400 lb. of wet beans half-filled a box, and wooden lids were provided for use with certain experiments. The sides of the boxes were lined with

\* Oil-free diaphragm pattern—working pressure 22 lb. per sq. in. (Hymatic Engineering Co. Ltd., Redditch)





FIG. 2.—Large fermentation vessel at Bekwai

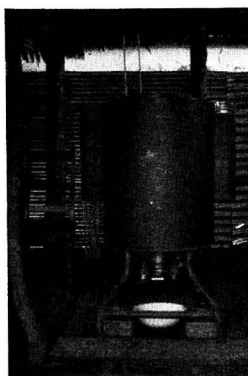


FIG. 3.—Small fermentation vessel

plantain leaves and a thick layer placed on the top of the beans as well. No leaves were put on the floor of the boxes and care was taken to keep draining holes clear.

#### Materials and methods

*Supply of cocoa pods.*—Cocoa pods for the Bekwai experiments were obtained from local farmers and from the Bunso station of the Department of Agriculture, whilst at Tafo, supplies were also drawn from Bunso and mainly from W.A.C.R.I.'s own plantation. Amelonado and both 'approved' and 'unapproved' \* Amazonian beans were available, but the latter varieties were only used in three small-vessel fermentations and one box fermentation. Altogether, the beans from 40 tons of pods were used. Usually, 2 days elapsed between harvesting and breaking. All records of weight were made to the nearest lb.

*Drying the beans.*—All the beans were sun-dried on trays which could be quickly covered in the event of rain. During the period of drying, the beans were frequently raked and picked over, but not all were dried in the same manner. In some experiments the thickness of the drying layer was kept at about 2 in., and in others, to secure slower drying initially, at about 8 in. for the first 2 days. These two methods are referred to below as the 'flat' and the 'heaped', respectively.

*Treating the beans to diminish the activity of micro-organisms in the pulp.*—A 0.1% solution of Vantoc B (calculated on the aqueous solution as supplied) was used in the proportion of one gallon per 100 lb. of wet beans. The solution was added from a watering can to about 30 lb. of wet beans at a time, which were well mixed by hand.

*Mixing the fermented beans.*—A mass of 500 lb. of wet beans in the large fermentation vessel cannot be readily mixed by stirring. All the beans were removed into some 8 baskets, the contents of which were then replaced in the order necessary to secure the most thorough mixing. It was found convenient and desirable to use a similar procedure for mixing the beans in the small fermentation vessel. In box fermentations the most effective way of mixing was to transfer the beans quickly to another box, with the aid of a scoop or basket, so with two concurrent fermentations it is useful to have a set of three boxes.

*pH measurements.*—(a) *Pulp.* A sample of beans was taken from the fermenting mass and 10 of these picked at random were covered in a 250-ml. beaker with 85 ml. of water at room temperature (about 30°). After 15 minutes, with occasional gentle stirring, the liquid was poured off and the pH of this liquid, measured by means of a glass electrode, was taken to be that of the pulp. The method used by Rombouts<sup>3</sup> was slightly different in some respects—chiefly that the testas were removed and then blended with water at 50°.

\* 'Approved' Amazonian varieties are those which come within the West African flavour range, as decided by the Cocoa Scientific Advisory Committee. [Report of Cocoa Conference, (London, 1953), p. 68.]

(b) *Cotyledons*. A further 20 beans from the same sample used above were wiped with filter paper and after removing the testas, 10 g. of broken cotyledons were weighed and transferred to a mortar. Grinding was continued with the occasional addition of water (up to 85 ml. at room temperature) until the particles were fine. The pH of the suspension was taken to be that of the cotyledons.

*Moisture determinations* were made on the cotyledon only—about 20 beans were weighed, and after removal of pulp and testas, the cotyledons were quickly broken up in a small mortar, 6 to 7 g. being taken and dried at 100° for 2 hours.

*Gas analysis*.—Samples of the gas surrounding the beans were withdrawn into a gas burette and analysed for carbon dioxide and oxygen content by the use of solutions of potassium hydroxide and alkaline pyrogallol in the usual way.

*Temperature measurement*.—Mercury-in-glass thermometers and two mercury-in-steel thermographs were used to determine the temperatures during fermentations. The former included a number—30 in. overall length, calibrated 20° to 60°, with the scale at the top 6 in. of the length—found to be indispensable for measuring the temperature near the bottom of a deep fermenting mass. In each fermentation, six or seven thermometers were employed to determine both the temperature distribution throughout the mass of beans at a given time, and the variation in temperature during the course of a fermentation. They occupied the same positions in the mass throughout a particular experiment, being removed only when the beans were mixed, and were read every 4 hours from 6.0 a.m. to 10.0 p.m.

*Chocolate for tasting*.—Chocolate, made up at Bournville from each sample of beans by a carefully standardized factory process, was tasted by a panel composed of experienced persons, against chocolate made by the same process from good quality West African beans. The identity of the chocolate samples was not revealed to the panel until the tasting test was complete.

## Results and discussion

### *Confirmation of laboratory fermentation results on a large scale*

Twelve experiments were carried out with Amelonado beans in the large fermentation vessel. The temperature/time curve in Fig. 4 is typical of those from all the large- and small-vessel

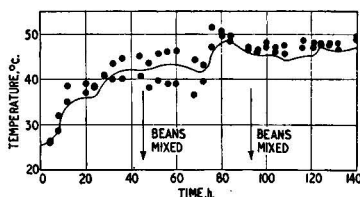


FIG. 4.—Temperature cycle during a vessel fermentation

fermentations. The line represents the temperature cycle through which 80% of the beans in a vessel pass (determined by several thermometers within the fermenting mass) and each dot, the temperature as measured by a thermometer placed in some position in the outermost 2-in. layers of the mass—side, top and bottom. A temperature of about 48° is gradually approached during the first 3 to 3.5 days, followed by a slight fall, and at a given time there is generally little temperature variation throughout the fermenting mass. It had been hoped to control the temperature of the fermenting beans for the first 3 days at 35°, followed by 3 days at 50°, but in practice this was found to be impossible. During the early stages of fermentation a large quantity of carbon dioxide is evolved and laboratory experiments\* had shown that to obtain well-fermented beans it was necessary to remove it. Two methods were adopted—either putting

\* Three small-scale fermentations were carried out—(1) in an atmosphere of nitrogen, (2) in an atmosphere of carbon dioxide, (3) taking steps to remove the carbon dioxide evolved. Only the last gave beans making up into good chocolate.



concentrated potassium hydroxide solution in the bottom of the vessel or aerating at frequent intervals with the compressor. The latter was the more successful and was used exclusively in the later experiments. In some fermentations the beans were sprayed with Vantoc B solution to reduce the microbiological activity in the pulp (see below).

The beans from all the experiments were satisfactory in appearance, showing the commercially desirable characteristics of well-fermented cocoa—predominantly brown, open cotyledons, loose shells and a complete absence of slaty beans. Chocolate flavour was good.

#### *Testing of the small fermentation vessel*

In the small fermentation vessel 8 experiments were carried out, with various weights of Amelonado and 'unapproved' Amazonian beans. Fermentation was allowed to proceed for 6 days with mixing after 2 days and after 4 days and the throat slide was closed after 3 days, as evolution of carbon dioxide is much diminished by this time. It was possible to observe the flow of sweatings (some 3 litres per 100 lb. wet beans) which ceased after about 20 hours from the beginning of fermentation. The typical temperature/time curve of Fig. 4 was obtained by keeping the water in the jacket as nearly as possible at 35° for the first 3 days and at 50° for the last 3, by manual adjustment of the burners.

By external and internal appearance the beans were well fermented—brown, open cotyledons, loose shells with a complete absence of slaty beans—and made up into good chocolate.

It is therefore considered that the small vessel will satisfactorily ferment from 30 lb. to 100 lb. of wet beans and its use is recommended for this purpose.

#### *Improvement of fermentation methods*

(a) *Fermentation in boxes.*—In 6 experiments, charges of  $450 \pm 250$  lb. of wet beans were fermented for periods of 6 days, with 'mixing by transfer' after 2 days and after 4 days. Fig. 5

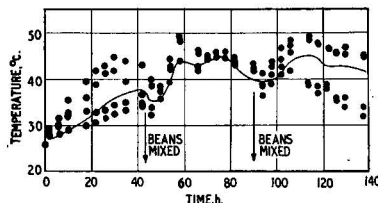


FIG. 5.—Temperature cycle during a box fermentation

depicts a typical temperature cycle for a box fermentation where the line and dots have the same significance as in Fig. 4. In comparison with a vessel fermentation it is noticed that the initial rate of temperature rise is less, and that the maximum bulk temperature reached after 3 days is 45°. There is also a greater fluctuation thereafter, and a wider variation in temperature from one point to another in the fermenting mass at a given time.

The method gave well-fermented beans, making up into good chocolate.

(b) *Fermentation in heaps.*—Heap fermentations were carried out using about 500 lb. of beans in each, (i) following the method recommended by the Gold Coast Department of Agriculture, namely 6 days, with mixing after 2 days and after 4 days; (ii) following a method thought to be typical of Gold Coast farmers' practice, namely 4 days, with one mixing after 2 days; (iii) placing the beans in a 4-in. layer for the first 3 days then heaping for the last 3 days of a 6-day fermentation. In this way it was hoped to keep the temperature down to 35° for the first 3 days before allowing it to rise for the last 3 days.

In each case the beans were placed on, and well covered with, plantain leaves, and drying was in 2-in. layers.

The method (i) recommended by the Department gave the best beans (judged on the basis both of appearance and of subsequent chocolate flavour) followed by those from the 'farmers' method (ii) which were superior in appearance to the usual beans of commerce. This suggests

that the majority of farmers may shorten fermentation time further and not mix at all. The modified method (iii) was not a success: it did not yield the temperature/time curve desired, and although the beans had the appearance of being well fermented they made up into poor chocolate. Temperature variation throughout a heap is considerable and much greater than in a box.

#### *Effect of ripeness and treatment of beans*

A number of box fermentations were carried out to provide information to answer enquiries which are made by planters. The three main points were as follows:

(a) *Effect of transportation of beans.*—Beans were taken from the pods in the morning, loaded in baskets on to a lorry where they remained for the next 24 hours, during which time they were driven some 50 miles, before being put into a fermentation box. In this period the sweatings had run and the beans were very wet at the beginning of fermentation. Three experiments were carried out—fermenting for 6 days in two of these, and for 5 days in the third, mixing after 2 days and 4 days in each. The temperature/time curve observed was very similar to that for a 'normal' box fermentation (Fig. 5).

(b) *Effect of ripeness.*—A thousand pods, judged to be unripe by their greenish-yellow colour and the absence of the characteristic dull rattle of a ripe pod when shaken, provided 225 lb. of beans for a box fermentation.

(c) *Effect of delay in breaking pods.*—Some 3000 pods were kept for a week after harvesting. About 400 became unusable and were discarded, and beans from the remainder were fermented in a box for 6 days.

In each of these three cases, the beans were, in appearance and subsequent chocolate flavour, satisfactory, and indistinguishable from those fermented by the 'standard' box method. Temperature, pH and moisture measurements were virtually identical.

#### *Chemical analyses*

(a) *Measurement of pH.*—Changes in the pH of both pulp and cotyledons were followed during fermentations in the two vessels and in boxes. The pH/time curves of all the vessel fermentations were similar (see Fig. 6). The pH of the cotyledons, originally about 6.6, decreases slowly and then falls rapidly in the second and third days to about 4.5 at which it remains approximately constant until the end of fermentation (6 days), rising to about 5.0 during drying. The pH of the pulp, after a slight fall in the first day, rises gradually until it is equal to the pH of the cotyledon, 4.5, at the sixth day. In the drying period there is a rapid rise at first until a steady value is reached (not shown).

The pH/time curves for the box fermentations were again similar amongst themselves (see Fig. 7), with the difference in comparison with the vessel fermentations, that the pH of the pulp and cotyledon became equal during the fourth day, and at a slightly higher value, 4.75. On drying the pH of the cotyledon rises steadily to about 5.4.

Rombouts<sup>2</sup> only considered the pH of the pulp of beans taken from the centre of a large fermentation box, and found that its value rose rather more rapidly than in the Tafo experiments, and even reached 6.4 at the end of the sixth day.

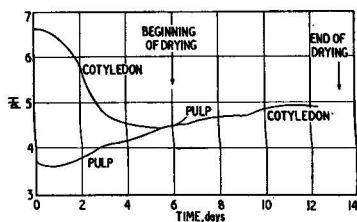


FIG. 6.—Change in pH of pulp and cotyledon during vessel fermentation, and drying

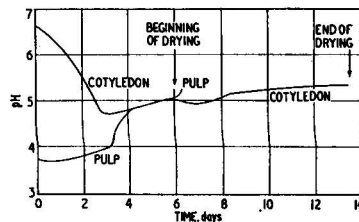


FIG. 7.—Change in pH of pulp and cotyledon during box fermentation, and drying



Neither the addition of Vantoc B to the pulp, nor the alternative drying procedures (see below), made any detectable difference in the pH/time pattern.

(b) *Moisture determinations.*—During fermentation the moisture content of the cotyledon (see Fig. 8) rises from 35% to almost 40%, at first rapidly and then more slowly, over a period of 6 days, and throughout 30 fermentations in boxes and vessels the same behaviour was observed. Sun drying until the moisture content was between 6 and 8% lasted about 7 days but varied according to the weather conditions.

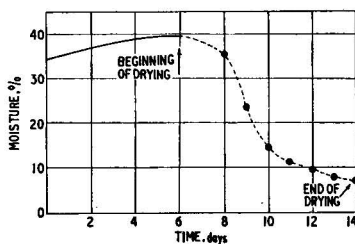


FIG. 8.—Change in moisture content of cotyledon during all fermentations (solid line) and during the drying period of one experiment (broken line)

It was found that when half of the beans from any particular fermentation were dried more slowly by the heaped (or thick) layer method and the other half more rapidly by the flat (or thin) layer method, the former procedure led to beans of definitely better appearance, as judged by the number which were partially purple, than the flat layer. On the basis of the flavour of the subsequent chocolate, however, there was no difference between the two methods.

(c) *Gas analysis.*—During the first two or three days of fermentation there was always a high percentage of carbon dioxide (about 95%) in the atmosphere surrounding the beans and very little oxygen. Thereafter it fell rapidly and at 80 hours there was little carbon dioxide and nearly 20% oxygen. These observations agree with the results of laboratory experiments made with beans in a respirometer—at 35° a single bean evolves carbon dioxide copiously, but not at all at 50°.

*Use of Vantoc B.*—Many fermentations were repeated with Vantoc B solution sprayed on to the beans (at the beginning, as well as during, the fermentation) which would have the effect of curtailing the activity of micro-organisms. In no case was any significant change observed in any of the properties studied, or in the chocolate flavour, which could be attributed to this cause.

#### *Recovery of Amazonian and Amelonado beans*

Recovery, as applied to the fermentation of cocoa beans, is defined as the ratio—

$$\frac{\text{weight of dried fermented beans}}{\text{weight of unfermented beans}} \times 100$$

Comparing Amelonado and Amazonian beans which have been fermented by the same method and dried to approximately the same moisture content, it was found that the recovery with the former was greater, a typical comparison being—

Amelonado	44%
Amazonian	38%

#### **Conclusions**

1. Successful 6-day fermentations can be carried out in a box (3 ft. square  $\times$  2 ft. deep) using charges of from 200 to 700 lb. of wet beans and turning after 2 days and after 4 days.

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2. Fermentations by the heap method can, of course, yield well-fermented cocoa, always provided that sufficient care is exercised. The box method, however, can be more closely and simply defined, and in new cocoa-growing areas its introduction is to be preferred.

3. A small fermentation vessel, designed to ferment the beans from 150 to 500 pods, for the assistance of the planter and plant breeder, has been tested and found to be satisfactory. Its use is recommended.

4. Beans from pods which have been harvested a week before breaking, can be fermented without modification of the 'standard' box method. Beans from unripe pods can also be fermented without modification of the method.

5. Transportation of wet beans, and delays of up to 24 hours before the beginning of fermentation, produced no adverse effects on the fermented beans—as judged by appearance and chocolate flavour.

6. Scientific data relating to pH changes in pulp and cotyledon, and moisture content of cotyledon, during fermentation and drying, and the temperatures of fermenting masses, have been collected and will prove of value in eventually giving an explanation of cocoa fermentation.

7. The recovery of Amazonian beans is significantly less than that of Amelonado.

#### Acknowledgments

The authors thank most sincerely the West African Cocoa Research Institute for very kindly supplying laboratory and other facilities.

We also thank the Board of Cadbury Bros., Ltd. for permission to publish this paper.

Cadbury Bros., Ltd.  
Bournville

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## THE PHENOLIC SUBSTANCES OF MANUFACTURED TEA. I.—Fractionation and Paper Chromatography of Water-soluble Substances\*

By E. A. H. ROBERTS, R. A. CARTWRIGHT and (MISS) M. OLDSCHOOL

Methods are described for the fractionation of the complex mixture of phenolic substances and their oxidation products occurring in manufactured tea. Products of oxidation detected include two fractions, S I and S II, responsible for the greater part of the colour intensity of a tea infusion, and nine unidentified substances, A, B, C, D, P, Q, X, Y and Z. S I and S II have been obtained almost free from other contaminants. They have acidic properties, and mean molecular weights of the order 600. They are probably mixtures of dimers, each dimer consisting of two oxidized flavanol units. X and Y have no acidic properties and are also distinguished from S I and S II by several characteristic colour reactions. P may be an anthocyanidin.

\* Paper presented at the XIVth International Congress of Pure & Applied Chemistry, Zurich, 1955



### Introduction

As a result of the pioneering work of Bradfield and his co-workers,<sup>1-3</sup> and subsequent paper chromatographic studies,<sup>4, 5</sup> it is established that unprocessed tea-leaf contains substantial quantities of (–)-*epigallocatechin gallate*, rather smaller amounts of (–)-*epigallocatechin* and (–)-*epicatechin gallate*, and relatively small amounts of (+)-*gallocatechin*, (–)-*epicatechin* and (+)-*catechin*. In plucked tea-leaf from North-East India these flavanols account for about 20% of the total dry weight of the tissue.

The only other major phenolic component of unprocessed tea-leaf is theogallin,<sup>6, 7</sup> although leucoanthocyanins,<sup>8</sup> flavonols,<sup>9</sup> chlorogenic acids,<sup>5</sup> and *p*-coumarylquinic acids<sup>10</sup> have also been detected.

The enzyme chemistry of tea fermentation has been extensively studied in the past and the results summarized in two review articles.<sup>11, 12</sup> It was concluded that the initial products of fermentation were *o*-quinones, obtained by enzymic oxidation of catechol and pyrogallol groups, and it was suggested that the brown pigments in an infusion of manufactured tea were derived from these *o*-quinones by polymerization. So far comparatively little work has been done relating to the chemical composition of these presumed polymerization products.

Bradfield & Penney separated the phenolic substances of a black tea infusion into two fractions by continuous extraction with ethyl acetate.<sup>13</sup> Another method of fractionation, due to Kursanov *et al.*,<sup>14</sup> employed successive Soxhlet extractions of dry tea with a variety of solvents. More recent work from Japan made use of counter-current distribution and also employed paper chromatography in analysing the fractions obtained.<sup>15-17</sup>

The work to be reported below describes several methods of fractionating the phenolic substances of black tea, manufactured in North-East India, and paper chromatographic analyses of these fractions leading to the recognition of new substances produced as a result of the fermentation process. More detailed examinations of these fractions will be reported in subsequent communications.

### Experimental

#### *Paper chromatographic methods*

Two-way paper chromatograms were run, first with butanol–acetic acid–water (4 : 1 : 2.2), and then with 2% aqueous acetic acid. If the chromatograms were run first with 2% acetic acid, separation of the phenolic oxidation products was poor. The chromatograms were first examined in visible light and then under ultra-violet light, with subsequent exposure to ammonia vapour. They were then sprayed with 1% ethanolic aluminium chloride and finally dipped into a mixture of ferric chloride and potassium ferricyanide.<sup>6, 18</sup> When necessary, separate papers were sprayed with the vanillin reagent<sup>6</sup> and others with 0.5N-aqueous sodium hydroxide.

#### *Examination of fractions obtained by the method of Bradfield & Penney<sup>13</sup>*

A black tea infusion was prepared by refluxing the tea (15 g.) with boiling distilled water (375 c.c.) for five minutes, and filtering hot through a cotton-wool plug. The infusion (250 c.c.) was extracted continuously for six hours with ethyl acetate, and the extract evaporated to dryness under reduced pressure (Fraction 1A; yield 1.0 g.). Polyphenolic substances in the residual aqueous layer were precipitated with saturated lead acetate (5 c.c.). The precipitate was suspended in methanol and decomposed by hydrogen sulphide, and the filtrate from the lead sulphide evaporated to dryness under reduced pressure (Fraction 1B; yield 1.0 g.).

A typical paper chromatogram for Fraction 1A is illustrated in Fig. 1. Such chromatograms indicate the presence in Fraction 1A of several uncharacterized substances not found in the original green tea-leaf. The most abundant of these are associated with an orange-brown streak, S I, and two semicircular patches, X and Y. Other uncharacterized substances detected include A, B, P, Q and Z, although it is not always possible to detect Q and Z unless Fraction 1A is further fractionated. The fluorescences and colour reactions of these substances are described in Table I.

As previously shown,<sup>4</sup> tea fermentation results in the production of free gallic acid, and the chromatograms of Fraction 1A show a strong spot for this substance. The chromatograms also

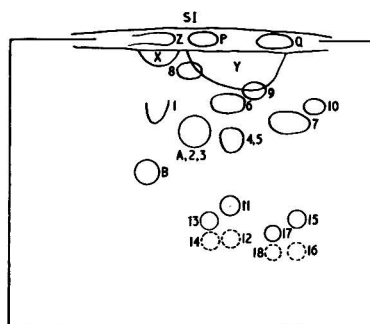


FIG. 1.—Paper chromatogram of Fraction 1A

The chromatogram was run first from left to right with butanol-acetic acid-water (4:1:2.2), and then downwards with 2% aqueous acetic acid.

Key to spots:

- (1) (-)-*epi*Gallocatechin
- (2) (+)-Gallocatechin
- (3) (-)-*epi*Catechin
- (4) (+)-Catechin
- (5) Gallic acid
- (6) (-)-*epi*Gallocatechin gallate
- (7) (-)-*epi*Catechin gallate
- (8) Myricetin-3-glucoside
- (9) Quercetin-3-glucoside (*iso*quercitrin)
- (10) Kaempferol-3-glucoside (*astragal*in)
- (11-14) Chlorogenic acids
- (15-18) *p*-Coumarylquinic acids

A, B, P, Q, SI, X, Y and Z represent uncharacterized substances.

Table I

Substance	Colour	Fluorescence and colour reactions of phenolic substances extracted from an infusion of black tea		Dip and spray reagents			
		Ultra-violet fluorescence Untreated	+ NH <sub>3</sub> vapour	FeCl <sub>3</sub> + ferricyanide	1% ethanolic AlCl <sub>3</sub>	Vanillin	0.5N-NaOH
SI & S Ia	Orange-brown	Dark	Dark	Blue	Little change	Colour modified	Darkens
S II	Brown	Dark	Dark	Brown	Little change	Colour modified	Darkens
X	Orange	Chocolate	Chocolate	Blue	Pink	Orange-pink	Mauve-purple, fading
Y	Orange	Chocolate	Chocolate + violet	Blue	Pink	Orange-pink	Mauve-purple, fading
Z	Orange	Blue	Pale yellow	Blue	Little change	—	—
P	Pink	—	—	Blue	Little change	Little change	Blue
Q	Yellow-orange	Dark brown	Dark brown	Blue	Deeper orange	Colour modified	Mauve-purple, variable
A	Colourless	Violet	More intense violet	Blue	nil	Pink	Pink-faint brown
B	Colourless	Violet	More intense violet	Blue	nil	Pink	Pink-faint brown
C	Colourless	nil	nil	Blue	nil	Pink	Pink-faint brown
D	Colourless	nil	nil	Blue	nil	—	—

indicate the presence of (-)-*epigallocatechin*, (-)-*epigallocatechin gallate*, (-)-*epicatechin gallate*, myricetin-3-glucoside, *iso*quercitrin, kaempferol-3-glucoside, chlorogenic acids and *p*-coumarylquinic acids, all of which occur in the original green tea-leaf. (+)-Catechin is obscured by the very much stronger spot for gallic acid, but its presence can be inferred from the weak, but positive, reaction given with vanillin. (+)-Gallocatechin and (-)-*epicatechin* are obscured by the rather diffuse spot for A. The presence of these flavanols in Fraction 1A was established chromatographically after further fractionation by the Craig procedure.

A typical paper chromatogram for Fraction 1B is illustrated in Fig. 2. The main feature is a heavy brown streak of zero  $R_F$  in 2% acetic acid. In addition to substance B and traces of A, also found in Fraction 1A, this fraction contains the uncharacterized products C and, presumably, D, although the latter is only detected after further fractionation. Known substances detected include gallic acid, theogallin, chlorogenic acids, *p*-coumarylquinic acids, rutin, kaempferol-3-rhamnoglucoside, quercetin-3-rhamnoglucoside, kaempferol-3-rhamnoglucoside and the aglycones, quercetin and kaempferol. The last-named must be artefacts, produced by hydrolysis of the glycosides during the working up of the fraction, as they should have been completely extracted from the original tea infusion by ethyl acetate. Apart from gallic acid and the flavonol aglycones these known substances were present in the green leaf before manufacture.

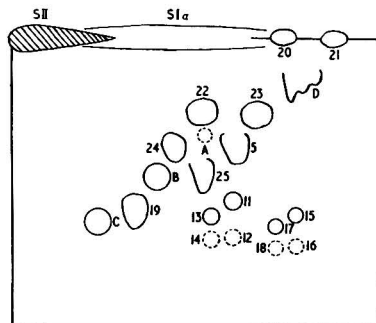
FIG. 2.—Paper chromatogram of Fraction 1B

Conditions of chromatography as in Fig. 1.

Key to spots as in Fig. 1 with the following additions:

- (19) Theogallin
- (20) Quercetin
- (21) Kaempferol
- (22) Quercetin-3-rhamnoglucoside (rutin)
- (23) Kaempferol-3-rhamnoglucoside
- (24) Quercetin-3-rhamnoglucoside
- (25) Kaempferol-3-rhamnoglucoside

C, D and S II represent uncharacterized substances. S II is shaded to distinguish it from the S I $\alpha$ . The spot for A is very weak, and its outline is indicated by a dotted line.



Separation of S I from Fraction 1A

Fraction 1A (1 g.) was dissolved in a small volume of acetone and precipitated with excess chloroform. The precipitate was washed first with chloroform and then with light petroleum (yield 0.65 g.). The filtrate consisted largely of caffeine together with a little gallic acid and flavanols.

The precipitate was dissolved in a small volume of acetone and precipitated with a large excess of ether. The ether precipitate was washed with light petroleum, and air-dried. It was then redissolved in acetone and again precipitated with excess ether. This procedure was repeated at least four times, until paper chromatograms of the final precipitate showed it to be free from X, Y, gallic acid and flavanols. The final product (yield 0.3 g.) consisted almost entirely of S I together with traces of A, B, flavonol glycosides and chlorogenic acids. The combined filtrates from the ether precipitations were shown by paper chromatography to contain all the substances present in Fraction 1A, but traces only of S I.

Craig fractionations

Fraction 1A, after being freed from caffeine by chloroform precipitation (3 g.), was further fractionated in a Craig counter-current apparatus. The solvent system used was ether-ethyl acetate-water (9 : 1 : 10) and the phase volume 60 c.c. In one typical separation, after ten fundamental distributions, the contents of the tubes were analysed by two-way paper chromatography with the results recorded in Table II.

As confirmed in separations with a higher number of transfers, S I, X and Y are separable from each other by this procedure, but the separation of Y from Q and (-)-epicatechin gallate is poor. This fractionation effected a considerable concentration of substances Q and Z, and

Table II

Substances detected after a ten-tube Craig fractionation

Substance	Detected in tubes	Tube with highest concentration
S I	0-1	0
B	0-1	0
A	0-3	1
(-)-epiGallocatechin	0-2	1
P	1-4	2
X	1-5	2
(+)-Gallocatechin	4-5	4, 5
(-)-epiCatechin	4-6	4
(-)-epiGallocatechin gallate	2-6	4
Gallic acid	2-7	4
Z	3-8	5
(-)-epiCatechin gallate	3-9	6
Y	3-9	6, 7
Q	5-9	7, 8

the colour reactions and fluorescences recorded in Table I were observed with these concentrates. As a result of the Craig fractionation it was also possible to demonstrate the presence of (–)-*epi*-catechin and of a trace of (+)-gallocatechin in Fraction 1A.

*Treatment of Fraction 1A with saturated aqueous sodium bicarbonate*

The ethyl acetate solution of Fraction 1A, obtained by continuous extraction of an infusion of black tea (250 c.c.), was shaken with an equal volume of saturated aqueous sodium bicarbonate. The ethyl acetate layer, which retained only about half of its original colour density, was evaporated to dryness under reduced pressure (yield 0.3 g.). Paper chromatograms showed the product to contain X, Y, (–)-*epi*gallocatechin gallate and (–)-*epi*catechin gallate as the principal components together with smaller amounts of A and Q.

S I, gallic acid, Z, the chlorogenic acids, the *p*-coumarylquinic acids and the greater part of the flavonols were completely removed from the ethyl acetate solution by this treatment with sodium bicarbonate.

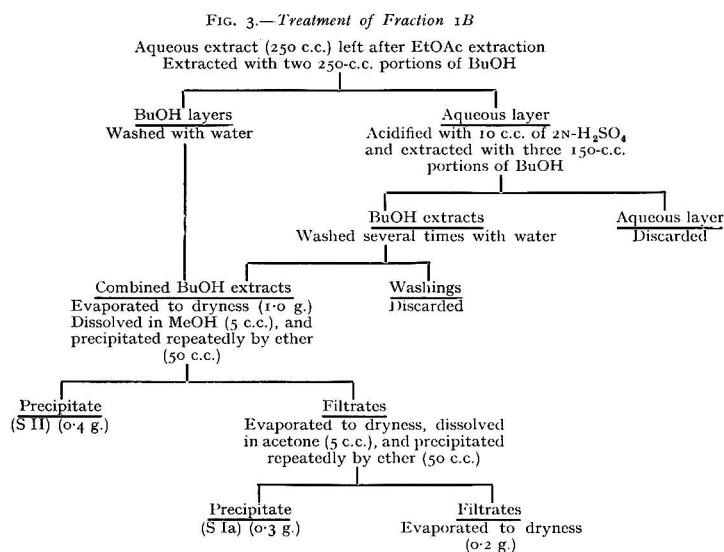
The brown sodium bicarbonate layer darkened rapidly, and this darkening was not reversed completely on acidification. This appears to be due to an irreversible change in the S I dissolved by the sodium bicarbonate solution.

Extraction of the sodium bicarbonate layer by ether, after acidification with sulphuric acid, yielded a few mg. of material which was shown by paper chromatography to contain gallic acid, Z, Q and small amounts of the flavanols.

*Separation of S Ia and S II from Fraction 1B*

The method used is indicated diagrammatically in Fig. 3.

*Fraction S II.*—Paper chromatograms of this fraction had a short, dark-brown, pennant-like streak as their most characteristic feature. During the run with butanol-acetic acid-water, the spot at the origin lengthened to about 8 cm. while the solvent front advanced 10 cm., after which the resultant streak remained unaffected by a further advance of 25 cm. of the solvent front. The streak was almost completely unaffected in the subsequent run with 2% acetic acid. Its dark brown colour persisted after dipping the paper in the ferric chloride-potassium ferricyanide reagent.



After four successive precipitations with ether, paper chromatograms of this fraction showed only this streak, and traces of flavonols and chlorogenic acids. No other phenolic substances could be detected, and the fraction was also found to contain no ash or amino-acids. It was possible to resolve the material further, by fractional precipitation with ether from methanol solution, into fractions differing somewhat in their behaviour on paper chromatograms, so that it cannot be considered a homogeneous substance.

The S II fraction was readily soluble in methanol, ethanol and hot water, but was insoluble in dry acetone and ethyl acetate. Nothing was extracted when an aqueous solution was shaken with ethyl acetate, but butanol effected a complete extraction of the material. The aqueous solution was acidic (pH 3.2) and the material dissolved readily in cold aqueous solutions of sodium bicarbonate. This solution was unaffected when extracted with butanol.

*Fraction S Ia.*—Paper chromatograms of this fraction showed a long orange-brown streak, turning blue with the ferric chloride-potassium ferricyanide reagent. The streak extended from approximately  $R_F$  0.1 to 0.8 in the butanol-acetic acid-water run, and broadened slightly during the run with 2% acetic acid. Unlike S II the length of the streak was proportional to the length of run of the first solvent. There was nothing to distinguish this streak from the S I streak obtained on chromatograms of the ethyl-acetate-extractable Fraction 1A.

After four precipitations with ether, paper chromatograms of this fraction also showed the presence of small amounts of theogallin, B and C, and traces of flavonols, chlorogenic acids and *p*-coumarylquinic acids. From intensities of colour reactions on the chromatograms it was estimated that these impurities accounted for less than 5% of the total material present. The fraction contained no ash or amino-acids.

The fraction was soluble in methanol, ethanol, acetone and hot water, and rather less soluble in ethyl acetate. When an aqueous solution was shaken with ethyl acetate, partial extraction of the S Ia was effected. This extraction was complete with butanol. The aqueous solution was acidic (pH 3.2) and the material dissolved freely in aqueous sodium bicarbonate. Neither ethyl acetate nor butanol extracted any material from the sodium bicarbonate solution.

*Filtrates.*—The filtrates represent what is left of the 1B Fraction after elimination of the greater part of the substances responsible for the streak in Fig. 2. Paper chromatograms of these filtrates showed that some S Ia was still left, and that the other components of 1B were relatively much more abundant. A new substance, D, was also detected, with a very characteristically shaped spot. This presumably was not sufficiently concentrated in Fraction 1B to give positive reactions with the reagents applied.

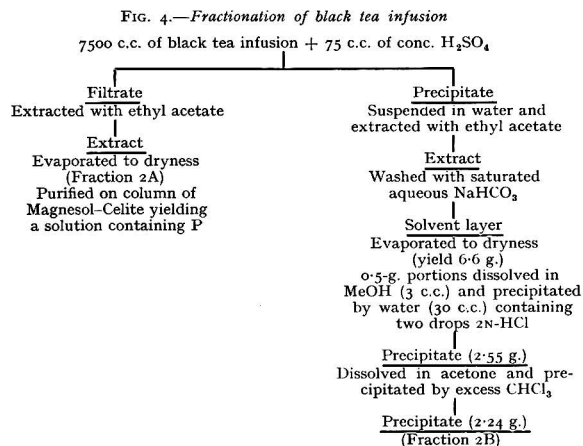
#### *Fractionation based upon precipitation by 1% sulphuric acid*

Acidification of a black tea infusion by sulphuric acid (final concentration 1% v/v) results in partial precipitation of the coloured oxidation products.<sup>19</sup> The flavonols are not precipitated, although they are adsorbed to some extent by the substances precipitated. Precipitation by acid is therefore a means of effecting a considerable degree of separation of the oxidation products from unchanged flavanols, and a scheme of fractionation, based upon this, is outlined in Fig. 4.

Each of the stages leading to the production of Fraction 2B was attended by a concentration of Y, although much Y was lost in the portions discarded. Paper chromatograms showed Fraction 2B to contain Y as its main constituent, together with rather less X, and smaller amounts of A, Q, (–)-*epicatechin* gallate and (–)-*epigallocatechin* gallate. Further purification was achieved in a Craig counter-current apparatus with 20 fundamental transfers, using the solvent system ethyl acetate-light petroleum (60–80°)-water (5 : 1 : 6). The purest Y was found in the two leading tubes, and paper chromatograms showed it to be associated with quite small amounts of Q and (–)-*epicatechin* gallate, and to contain no detectable amounts of the other substances in the 2B Fraction.

Paper chromatograms showed Fraction 2A to resemble 1A generally, but with much less S I, X and Y, and with an unusually strong spot for P. The fraction was dissolved in the minimum quantity of acetone and applied to a column (13 × 2 cm.) of Magnesol-Celite, made up exactly as described by Pearl & Dickey.<sup>20</sup> The column was eluted with a mixture of ether and ethyl acetate (5 : 1), saturated with water. A broad orange band (X and Y) ran rapidly down the column, followed by a more slowly moving purple band, while a yellow band remained at





the top. When elution of the orange band was complete, the solvent was changed to a 1 : 1 mixture of ether and ethyl acetate, saturated with water. The purple band ran faster in this solvent and was collected separately. This fraction was mixed with an equal volume of 2% hydrochloric acid, and sufficient chloroform added to transfer the colour to the aqueous phase. The aqueous layer was then extracted with a small volume of *isoamyl* alcohol.

This solution was examined on one-way paper chromatograms with the Forestal solvent.<sup>21</sup> It gave a single spot of approximate  $R_F$  value 0.47 (cyanidin  $R_F = 0.50$ , delphinidin  $R_F = 0.30$ ). No other substance could be detected by paper chromatography in the *isoamyl* alcohol extract.

#### Successive Soxhlet extractions

In the initial stages of this fractionation, the method of Kursanov *et al.*<sup>14</sup> was followed exactly. Powdered tea (10 g.) was extracted in a Soxhlet, first with benzene, then with ethyl acetate, and finally with ethanol. Extractions were continued until the solvents siphoning over were colourless. Ethyl acetate extracted a few mg. only, in contrast with the yield of 1.58% reported by the Russians. On the other hand, ethanol extracted 1.0 g. of phenolic substances (10.0%), considerably more than the 1.81% reported in the Russian work. Paper chromatograms showed this ethanol extract to resemble Fraction 1A in chemical composition, but to have a considerably higher content of S I, and also to contain C.

After exhaustive extraction with ethanol, the residual tea was air-dried and infused with six successive lots of boiling distilled water. Instead of following the Russian procedure, the aqueous extract was acidified with sulphuric acid, and extracted three times with butanol. The united butanol extracts were washed free from acid and evaporated to dryness under reduced pressure. A paper chromatogram of the product showed a strong streak, of zero  $R_F$  in 2% acetic acid, corresponding with a mixture of S I and S II, and very weak spots corresponding with gallic acid and several flavonols.

#### Molecular weight determinations

S I was methylated by diazomethane (OMe = 28.0%). The molecular weight, determined ebullioscopically in acetone, was found to be 800, equivalent to 677 for the unmethylated S I. Molecular weights for S Ia and S II were also determined cryoscopically in dioxan, values of 596 and 638, respectively, being obtained. Slightly lower values were obtained for these fractions by the ebullioscopic method, after methylation, and correcting for the content of methoxyl groups.

### Discussion and conclusions

A paper chromatographic study of the chemical composition of aqueous infusions of black tea indicates that the uncharacterized substances S I, S II, A, B, C, D, P, Q, X, Y and Z have been produced during the manufacture of the tea. None of these substances has ever been detected in the freshly plucked tea-leaf.

The same six flavanols have been detected in both black tea and fresh leaf. However, in black tea extracts these flavanols are confined to one fraction, amounting to 3% of the total weight of the tea, which remains after the elimination of the caffeine from Fraction 1A and the subsequent precipitation of the S I. As the original flavanol content of the green leaf is about 20%, on a dry weight basis, it is evident that the flavanols have decreased in amount very considerably, and it is apparent that the new substances, characteristic of manufactured tea, must largely have originated from the flavanols which have undergone chemical change.

*The S I and S II fractions.*—The yields of S I, S Ia and S II together amount to more than 10% of the total weight of the tea extracted, without taking into account any losses brought about by the washings and other manipulations. The total content of phenolic substances in the black tea infusion (Fractions 1A + 1B, less the caffeine) is about 16%, so that these fractions together account for much more than half of the products of manufacture. They also account for at least 75% of the total depth of colour of the infusion.

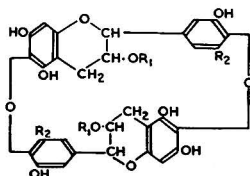
Differences in solubility in organic solvents, in the ease of precipitation by ether from methanol solution, and in chromatographic behaviour, justify the distinction drawn between S II and the S I fractions, although it is not claimed that this distinction is necessarily a sharp one.

The three fractions are acidic, and as the pH of a black tea infusion is about 5.0 it follows that these acids are partially neutralized. S I is extractable from aqueous solution by ethyl acetate, but not after neutralization. Continuous extraction with ethyl acetate therefore removes only part of the S I, Fraction S Ia representing the neutralized portion. There is probably little difference chemically between Fractions S I and S Ia. As S II is insoluble in ethyl acetate, the S II in excess of the neutralized portion is not extracted.

Partial neutralization of the free acids also probably accounts for the incomplete extraction of gallic acid, the chlorogenic acids and the *p*-coumarylquinic acids by ethyl acetate from the infusion.

Molecular weight determinations for S I, S Ia and S II have varied between 596 and 677. These average molecular weights may be a little low, as the fractions contained traces of other substances, but it is not thought that this contamination was sufficient to affect the values seriously. The values are of the same order as those found by Kursanov *et al.*<sup>14</sup> for fractions isolated from an aqueous extract of tea after previous exhaustive Soxhlet extractions with ethyl acetate and ethanol, which the present results show to consist essentially of a mixture of S Ia and S II. These values are consistent with the view that the main products in manufactured tea are dimers, produced by the linking together of two oxidized flavanol units. A dimeric structure was suggested by Kursanov *et al.*<sup>14</sup> and a generalized form of this proposed structure is represented below.

With six parent flavanols, no less than 21 dimers of this general structure are possible, and this complexity accounts satisfactorily for the heterogeneity of the S I and S Ia fractions. The proposed structure, however, fails to account either for the colour or for the acidity of the fractions, nor does it account for the existence of S I and S II fractions. It is believed that one of the alternative dimeric structures would account better for these facts.



$R_1 = \text{H or galloyl}, R_2 = \text{H or OH}$

*Substances X and Y.*—Apart from the S I, S Ia and S II fractions, the bulk of the remaining phenolic matter in a tea infusion is accounted for by unoxidized flavanols, theogallin, gallic acid and Y. From the yields reported in the various methods of fractionation it is estimated that Y accounts for about 1% of the total dry weight of the tea. The amount of X is substantially less.

Although fractions have been obtained very rich in Y it has not yet proved possible to obtain it free from other phenolic impurities.

Y and X are distinguished from S I, S Ia and S II in having no appreciable acidity, and they are not extracted from ethyl acetate solution on shaking with aqueous sodium bicarbonate. The Craig separations also demonstrate an essential difference between Y and S I, the former having a very much higher solubility in the non-aqueous solvent. Colour reactions with ethanolic aluminium chloride and dilute sodium hydroxide also further distinguish X and Y from S I.

*Substances A, B, C, D, P, Q and Z.*—The first four of these substances are colourless. There seems to be some justification in classing A, B and C together, because of the regular intervals in their  $R_F$  values in the solvents used (*vide* Fig. 2), and in the similar colour reactions given by all three, particularly with dilute sodium hydroxide and with vanillin. P has properties of an anthocyanidin, but is clearly not to be identified with cyanidin or delphinidin. Q cannot be separated from purpurogallincarboxylic acid<sup>18</sup> on a paper chromatogram, but is not necessarily to be identified as such as it fails to give the very characteristic blue colour, fading to green and yellow, obtained when purpurogallincarboxylic acid is treated with dilute alkali. The characteristic change in the fluorescence of Z in ultra-violet light, on exposure to ammonia, is also given by ellagic acid. Z and ellagic acid, however, do not have the same  $R_F$  values in butanol-acetic acid-water, although both have zero  $R_F$  in water. Although Z is not to be identified with ellagic acid, some relationship is possible. Z is probably an acid.

#### Acknowledgments

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## DETERMINATION OF THE VAPOUR PRESSURE OF ETHYLENE DIBROMIDE\*

By F. CALL

The vapour pressure of ethylene dibromide has been determined over the temperature range 0–25° by both static and dynamic methods.

A study of the literature disclosed considerable disagreement between the values obtained by different workers for the vapour pressure of ethylene dibromide and it was therefore decided to redetermine the vapour pressure curve by both static and dynamic methods.

### Experimental

#### *Purification of material*

The starting material was technical ethylene dibromide supplied by Messrs. British Drug Houses Ltd. One kg. of this material was shaken with 10% NaHCO<sub>3</sub> solution, washed twice with water and dried with CaCl<sub>2</sub>. The liquid was distilled twice slowly at atmospheric pressure using a 10-in. Vigreux column. Each time the first 30 or 40 ml. were rejected and the middle fraction, boiling between 131 and 131.5° at 756 mm., collected. A dark residue remained after each distillation. The distillate was re-dried with anhydrous MgSO<sub>4</sub> and fractionated under reduced pressure, the middle fraction only being collected. The air entering through the capillary leak was dried (by passing through a CaCl<sub>2</sub> tube) to reduce any possibility of catalytic 'cracking' induced by water. After 3 successive distillations the middle fraction boiled sharply at 45.5° under a pressure of 28 mm. Hg. This material had a density of 2.179 at 20°, melted at 9.35° and boiled at 131.3°/760 mm. It was clearly far from pure since the most reliable values, obtained by Timmermans & Martin<sup>1</sup> for the International Bureau of Physical and Chemical Standards, are a density of 2.1804 at 20°, melting point 10.00° and boiling point 131.70°. Fractional crystallization was therefore effected by freezing slowly in a stoppered test-tube immersed in a bath of ice and water. After a small amount of solid had formed round the walls of the tube, the liquid core was poured off into another tube which was in turn frozen. Forty-four g. of distillate yielded, after 5 crystallizations, 18 g. of material melting at 10.05° (uncorr.), the melting point being unchanged by further recrystallization. From the intermediate fractions 15 g. of material melting at 9.7–9.8° and 1 g. melting below 7.6° were obtained. The following figures were obtained for the fraction melting at 10.05°:

$$d_4^{13} \ 2.1970 \quad d_4^{20} \ 2.1805 \quad n_D^{20} \ 1.5387$$

Timmermans quotes values of 2.1950, 2.1804 and 1.53870, respectively.

It is known that pure ethylene dibromide exists mainly in the *trans* form, the *cis* form being eliminated by steric hindrance (Hassel & Viervoll<sup>2</sup>), but de Hemptinnes<sup>3</sup> believes that an unknown form may also be present.

#### *Vapour pressure by static method*

The apparatus (Fig. 1) consisted of a mercury manometer connected to a reservoir by means of standard ground joints. The manometer had limbs 8 cm. long and 3 cm. diam., the wide diameter serving to eliminate meniscus effects. The reservoir consisted of a tube 15 cm. long and 2.5 cm. diam. Both manometer and reservoir were fitted with good quality taps lubricated with Apiezon grease. The apparatus was baked for 24 hours in an oven at 120° and cooled, and the manometer filled with freshly distilled mercury.

The following precautions were taken to exclude both air and water vapour. By means of a hypodermic syringe about 0.2 ml. of recrystallized ethylene dibromide (m.p. 10.0°) was introduced into a small glass ampoule consisting of a thin-walled bulb of 1 cm. diam. blown on to the end of 2-mm. bore glass tubing which was drawn out to form a neck. The open end of the ampoule was at once attached to a vacuum pump through a drying tube containing magnesium

\* Part of a thesis approved for the degree of Ph.D. in the University of London

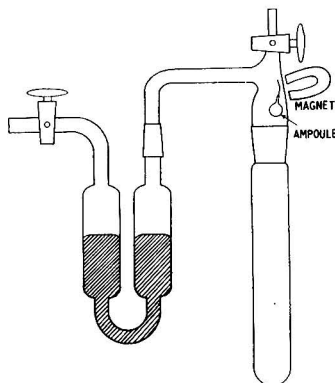


FIG. 1.—Apparatus for determination of vapour pressure by static method

perchlorate. The bulb of the ampoule was immersed in a mixture of acetone–solid  $\text{CO}_2$  and by carefully warming the glass neck any ethylene dibromide was driven down into the bulb and frozen. The pump was then started and run for some minutes until a pressure of  $0.01$  mm. was obtained on a vacuostat gauge. The neck of the ampoule was then sealed off after gently warming to drive off any ethylene dibromide remaining on the tube.

A small steel washer was attached to the ampoule by a short length of steel wire. The ampoule was then inserted into the reservoir and held near the top by means of a permanent magnet fastened outside the tube with Sellotape.

Both taps were then connected to a high-vacuum pump through traps of solid carbon dioxide–acetone and magnesium perchlorate and evacuated for 48 hours to a pressure of  $10^{-4}$  mm. Hg. The surface of the reservoir was lightly flamed to drive off adsorbed gases and water vapour. The apparatus was tested by turning off the taps for 2–3 hours and observing the mercury levels by means of a vernier microscope reading to  $0.01$  mm. No difference in level was detectable.

The apparatus was now removed from the pumping system and placed in a room whose temperature was constant to  $0.05^\circ$ . The permanent magnet was removed thus allowing the ampoule to fall to the bottom of the reservoir and break.

The levels of the mercury were read at intervals by means of the vernier microscope until readings had become constant. The mercury meniscus was illuminated from behind by a diffuse light, and a horizontal shutter, mounted on a rack and pinion so as to be adjustable in height, was moved until only a narrow beam of light passed between the shutter and the surface of the meniscus at grazing incidence to the latter. By this means the readings were reproducible to  $0.01$  mm., the limits of the vernier. No readings were taken until the air temperature had remained constant for at least one hour. The apparatus was then moved to another constant temperature room at a different temperature. The results appear in Table I.

Table I

<i>Vapour pressure—static method</i>			
Temp. °C	Vapour pressure mm. Hg	Temp. °C	Vapour pressure mm. Hg
25.10	10.83	11.10	4.24
20.00	7.69	9.60	4.18
18.20	6.66	8.80	3.97
17.50	6.45	6.35	3.29
14.45	5.07	5.80	3.21
12.80	4.62	2.95	2.61
12.55	4.52	0.00	1.98



*Vapour pressure by dynamic method*

The apparatus was set up as shown in Fig. 2. Clean air at a rate of 50 ml./min. entered through a tower filled with coarse lumps of  $\text{CaCl}_2$  and was further dried by passage over the surface of phosphorus pentoxide. The dry air was then passed first through a spiral lead tube and then through a saturator consisting of five tubes each 12 cm. long by 2 cm. diam. and loosely packed with glass wool well moistened with recrystallized ethylene dibromide. The spiral tubing and the saturator tubes were immersed in a large Dewar vessel containing water at the required temperature which was arranged always to be below room temperature in order to prevent subsequent condensation of ethylene dibromide vapour.

Before each determination was started the temperature of the water in the Dewar vessel was adjusted to the desired value and an empty bubbler was connected to the furnace which was not heated. The aspirator was replaced by an air pump which was run for 3 or 4 hours to ensure thermal equilibrium. The furnace was then switched on, the bubbler replaced by one containing 5 ml. 0.1N-NaOH containing 4% v/v of 100-vol.  $\text{H}_2\text{O}_2$  and the aspirator started. Usually three determinations were carried out at each temperature. The efficiency of the saturator was tested at two temperatures by connecting a preliminary saturator containing ethylene dibromide and maintained at a higher temperature so as to supersaturate the air at the working temperature. No significant difference was observed between concentrations thus approached from above and below the saturation concentration. The results are believed to be accurate to within 0.25%. Vapour pressures were calculated from the saturation concentrations assuming the Gas Law to be obeyed.

*Determination of ethylene dibromide.*—The air saturated with ethylene dibromide vapour at the temperature of the water-bath was passed through a silica combustion tube described below. The products of combustion passed first through a bubbler, then through a flow-limiting valve (Call<sup>4</sup>) to an aspirator. The contents of the bubblers were analysed for bromide by the gold chloride method described by Wade.<sup>5</sup>

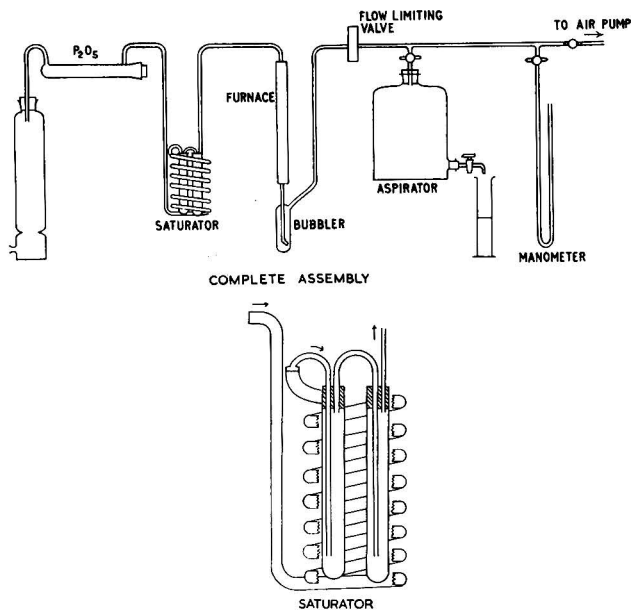


FIG. 2.—Apparatus for determination of vapour pressure by dynamic method

The combustion tube consists of a 40-cm. length of 2-mm.-bore silica tubing bent at right angles some 10 cm. from one end. The ends of the tube are ground flat as are the inlet tubes of the bubblers so that good butt joints can be made by rubber sleeving. For a distance of 25 cm. along the longer vertical portion of the tube there is an external winding of No. 34 s.w.g. 80/20 Nichrome resistance wire, the winding pitch being 2 mm. This heater winding is lagged by asbestos wool enclosed in a 1.5-cm.-bore Pyrex tube which also serves to support the furnace. The ends of the heater winding are brought out and connected to copper clips screwed tightly to the Pyrex tube. Since there is some evidence that the combustion of halogenated hydrocarbons is more efficient in the presence of platinum, there is a snugly fitting spiral of No. 39 s.w.g. platinum wire inside the silica tube, retained by friction. The silica tube can be raised to a bright red heat by passing a current of 1.2 amp. under a potential difference of 90 volts through the heater winding.

When the silica tube was at a bright cherry-red heat, a bubbler containing 5 ml. of 0.1N-NaOH and 0.2 ml. of 100-vol.  $H_2O_2$  was attached to the lower part of the tube by a short length of rubber tubing. An aspirator was connected to the outlet of the bubbler and a known volume of the air to be analysed was thus drawn through the furnace and bubbler. The exact volume of air was obtained by collecting the water run out of the aspirator in a graduated cylinder and measuring its volume accurately. The final pressure inside the aspirator, which may differ from atmospheric pressure owing to the height of the liquid column in the bubbler, was measured with a water manometer and the appropriate correction applied. The maximum rate of air flow was restricted to 50 ml. per minute by means of the flow-limiting valve described by Call.<sup>4</sup> This flow rate ensures that the mean time of passage of the gas through the furnace is about 1 sec. Flow rates of 100 ml. per minute have also proved satisfactory but there is a danger that if the time of passage is too short the combustion may not be complete. When a sufficient volume of air had been driven through the apparatus the bubbler was disconnected and the contents analysed for bromide. The contents of the bubbler were blown into a test-tube graduated at 10 ml. and the bubbler rinsed twice with 1 ml. of distilled water, the washings being added to the sample. The tube was then immersed in a boiling water bath for 15 minutes and cooled, and 1 ml. of 6N- $H_2SO_4$  was added followed by 1 ml. of gold chloride solution. The contents of the tube were then made up to the mark and thoroughly mixed by shaking. The colour developed almost immediately and reached a maximum in 3-5 minutes. The extinction of the solution was then measured in the Spekker absorptiometer against a blank treated in exactly the same way, using Ilford Spectrum Blue Filters. The concentration of bromide could be read off directly from a calibration curve prepared from solutions of potassium bromide of known strength. Eight or ten tubes could be treated and measured at one time.

The above technique differs in minor details from that of Wade.<sup>5</sup> The volume of solution is 10 ml. instead of 8 ml. thus permitting 2 ml. to be used for rinsing the Spekker cells between readings. The volume of gold chloride has been increased from 0.6 ml. to 1 ml. as being easier and quicker to add by pipette, and a new calibration curve has been prepared. The method enables 0.3 mg. of bromide ion to be estimated rapidly with an accuracy over most of the range of about 0.25%. Duplicates were easily reproducible to this degree of accuracy. In some cases the bromide content was estimated by potentiometric titration with silver nitrate (Lubatti & Blackith<sup>6</sup>). Excellent agreement was obtained between replicates by both methods. Collected results appear in Table II.

The overall efficiency of the combustion tube was compared directly with Lubatti & Blackith's combustion tube using potentiometric titration. Lubatti has measured the efficiency of his technique by using known weights of methyl bromide in ampoules and found it to be better than 99.5%. The two tubes were therefore connected in turn to a saturator containing ethylene dibromide and the saturation concentration measured at 20° and 0°. This dynamic technique avoids errors due to sorption and the weighing of small quantities of fumigant.

The results were :

	0°	20°
Concn. in mg./l. by Lubatti's technique	22.58	83.20
Present technique	22.70	83.21

Table II

Vapour pressure—dynamic method			
Temp. ° C	Saturated vapour concn. mg./l.	Molar volume ml.	Vapour pressure mm.
19.95	83.24	21,220	8.09
17.85	70.70	21,300	6.96
13.85	55.33	21,100	5.17
10.18	46.12	21,600	4.33
9.82	45.13	21,850	4.23
9.60	44.74	21,900	4.20
7.00	38.26	21,750	3.55
6.78	37.20	21,800	3.45
4.85	34.16	21,700	3.15
0.02	22.70	21,800	2.05

The results are in excellent agreement with those obtained by Radelescu & Alexa<sup>7</sup> over most of the range but are slightly lower at temperatures above the melting point.

### Discussion

The static and dynamic vapour pressures are also plotted in Fig. 3 from which it will be seen that the values from the dynamic method are higher than the corresponding static values, the differences increasing with increasing temperature. It will be noted that there is a well-defined triple point at about 9.7° or just below the melting point. This triple point does not appear to have been previously observed. Molar volumes, calculated on the assumption that the static vapour pressures are correct, increase steadily as the temperature decreases from 20° to the triple point and then remain reasonably constant at about 21,800 ml.

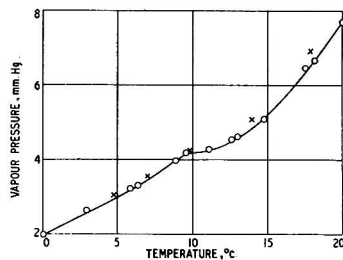


FIG. 3.—Vapour pressure curve of ethylene dibromide  
O by static method      X by dynamic method

### Acknowledgments

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Imperial College Field Station  
Sunninghill  
Bérks.

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## THE DIFFUSION OF ETHYLENE DIBROMIDE VAPOUR IN AIR\*

By F. CALL

The diffusion coefficient of ethylene dibromide vapour in air has been measured at different temperatures. The effect on the coefficient of varying the relative humidity and carbon dioxide content of the air has been investigated also.

A knowledge of the diffusion coefficient of ethylene dibromide vapour in air was required for an investigation into the physico-chemical processes of soil fumigation, but no reliable value could be found in the literature. It was therefore decided to measure the coefficient over the range of temperatures normally found in soil fumigation. In natural soils the vapour will diffuse through an atmosphere which is almost saturated with water vapour and which in addition contains a considerable percentage of carbon dioxide. It was therefore thought desirable to investigate the influence of these two factors on the diffusion coefficient.

### Experimental

A simple and versatile technique for measuring the diffusion coefficient of vapours is that originated by Stefan.<sup>1</sup> A small quantity of liquid is placed at the bottom of a narrow tube, the length of tube from liquid surface to mouth constituting the diffusion path. The concentration of vapour surrounding the mouth of the tube is kept at a very small value by means of a current of air. The mass of vapour which has diffused out of the tube can be determined by weighing, measuring the rate of fall of the meniscus or by analysing the vapour in the air stream. Since the last method is the most precise, it was adopted for the present work.

### Apparatus

The apparatus is as shown in Fig. 1. The flat-bottomed diffusion tube was 4.18 cm. long and had a diameter of 0.068 cm. The outer vessel of the diffusion cell consisted of a standard A.40 joint whose female socket had inlet and outlet tubes while the male cone carried a well into which the diffusion tube fitted fairly closely. A small drop of mercury in this well facilitated thermal conductivity between inner and outer tubes. The air stream, after being dried by  $\text{CaCl}_2$  and  $\text{P}_2\text{O}_5$ , entered a spiral of  $\frac{3}{16}$ -in.-bore copper tube consisting of about 15 turns of  $2\frac{1}{2}$  in. diameter and surrounding the diffusion cell. This part of the assembly fitted into a Dewar flask containing water at the desired temperature. From the copper spiral the air stream passed into the diffusion cell jacket.

Air leaving the diffusion cell and containing diffused vapour passed through a silica combustion tube as described by Call,<sup>2</sup> glass and silica tubes being connected by ground butt-joints and a rubber sleeve. A small rotary air pump and a flow-limiting valve (Call<sup>3</sup>) maintained the air stream at a constant flow rate, usually about 100 ml. per minute.

### Technique

The diffusion tube was mounted in a vertical position in a clamp and about 0.5 ml. of pure ethylene dibromide was introduced by means of a drawn-out glass tube, great care being taken to avoid wetting or splashing the walls of the tube. The tube was closed by a rubber stopper to prevent ingress of water vapour. The distance from the bottom of the meniscus to the ground end of the tube was then accurately measured with a vernier microscope. In order to transfer the tube to the diffusion cell without splashing the walls of the tube, the ethylene dibromide was frozen solid by immersion of the tube in a mixture of acetone and solid carbon dioxide. The tube was then dried outside, the stopper removed and the tube dropped into the well of the diffusion cell. The male cone was fitted, the joint being made watertight by a little silicone grease, and the Dewar vessel containing water at the required temperature was raised into position so as to immerse completely the diffusion cell and copper spiral. The air stream was now

\* Part of a thesis approved for the degree of Ph.D. in the University of London

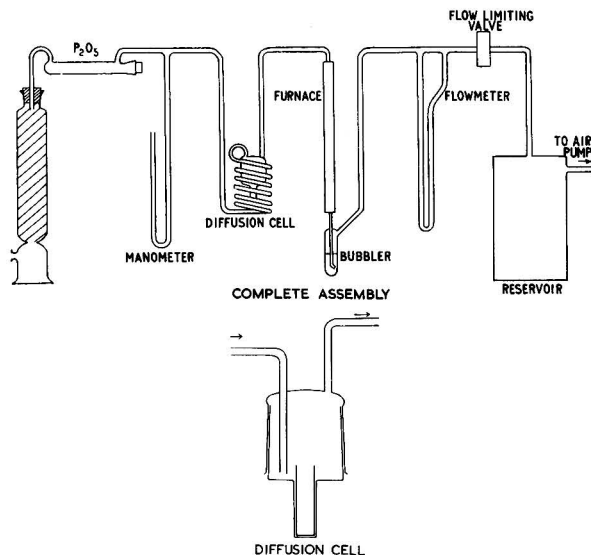


FIG. 1.—Apparatus for measuring diffusion coefficients in air

started and run for at least 20 hours with the silica tube off to allow thermal equilibrium to be established in the diffusion cell and for a steady state to be achieved. The combustion tube was then switched on and when it had reached the operating temperature the air stream was stopped and the empty bubbler was replaced by one provided with 5 ml. of 0.2N-NaOH containing 4% of 100-vol.  $H_2O_2$ . The air flow was immediately restarted. After a little practice it was found possible to replace the bubbler in 5-6 seconds. After 30-60 minutes the bubbler was replaced by a fresh one, the time of bubbling being carefully noted. When three or four replicates had been obtained, the contents of the bubblers were rinsed into test tubes or beakers and analysed for bromide content by the gold chloride method (Call<sup>2</sup>). From the results the mass of ethylene dibromide which had diffused out of the tube in a known time could be calculated.

Distortion of the meniscus at temperatures below the freezing point of ethylene dibromide was prevented by adding small quantities of liquid after the main bulk had been frozen, each addition being frozen before adding the next. The length of the diffusion path in this case was measured by using a fine bristle mounted on a rack and pinion with vertical movement. The bristle was brought into contact with the solid surface and the microscope focused on a mark on the bristle above the mouth of the tube.

To determine the effect of water vapour the same apparatus as formerly was used but the incoming air was first led through water saturators maintained at a temperature slightly below the operating temperature to avoid supersaturation and subsequent condensation of water. The saturator consisted of 4 test-tubes in series each loosely packed with glass wool moistened with water. When working at 20° the saturators were kept at 19.8° and the relative humidity of the air stream was thus 98.8%.

Some difficulty was met in maintaining a steady concentration of  $CO_2$  in the air stream, but this was solved by using a Dewar vessel fitted with a rubber bung with an inlet and outlet tube and containing lumps of solid  $CO_2$ . A slow stream of air passing through the Dewar vessel became enriched with  $CO_2$  and this air was drawn into the main stream by means of a jet similar to a Pitot tube. By varying the relative flow rates of air through the Dewar vessel and in the main air stream, the final concentration of  $CO_2$  could be accurately adjusted. An additional

metal cooling coil immersed in water at 20° was placed in circuit to neutralize the cooling effect of the CO<sub>2</sub>. The concentrations thus produced were very steady over long periods of time. Thus in one experiment the measured concentrations of CO<sub>2</sub> were initially 7.87%, after 18 hours 7.95% and after 24 hours 7.82% by volume.

The concentration of CO<sub>2</sub> in the air stream was determined by connecting to it an evacuated flask of about 150 ml. capacity containing 10 ml. of 0.1N-NaOH. After shaking the flask to complete the absorption of the CO<sub>2</sub> by the NaOH, the carbonate was estimated by Winkler's method by adding 3 ml. of saturated BaCl<sub>2</sub> solution and titrating with standard hydrochloric acid.

### Results

Diffusion coefficients were calculated by means of the expression derived by Stefan<sup>1</sup>

$$\frac{dm}{dt} = C_0 \frac{D_0 A}{L} \log \frac{C_0 - C'}{C_0 - C_s}$$

where  $\frac{dm}{dt}$  is the mass of vapour diffusing out of the tube per second,  $C_0$  is the concentration of inert gas (air) in  $\mu\text{g. per ml.}$  from  $P = cRT$ ,  $C_s$  is the saturation concentration of vapour in  $\mu\text{g. per ml.}$ ,  $C'$  is the mean concentration of vapour in  $\mu\text{g. per ml.}$  at the mouth of the diffusion tube calculated by dividing  $\frac{dm}{dt}$  by the flow rate of air,  $L$  is the length of the diffusion path corrected for meniscus error and  $A$  is the cross-sectional area of the tube.

The meniscus correction was obtained by calculating the mean height of the meniscus from its volume found by plotting the meniscus curve from measurements made by means of a microscope fitted with a graticule eyepiece. The correction amounted to 0.15 mm. and was rarely greater than 0.5% of the diffusion path.

The overall error of the method was estimated to be about 0.35%.

The value of the diffusion coefficient should be independent of the length of the diffusion path, but in practice the value often falls as the path is decreased. This effect has been shown by Le Blanc & Wupperman<sup>4</sup> to be due to a slight temperature fall at the surface of the liquid. Although this effect might be expected to be small with such a high-boiling liquid as ethylene dibromide, a few determinations were made with different lengths of diffusion path and the following results (means of three) were obtained:

Diffusion path (cm.)	4.399	3.376	2.498	1.698
Diffusion coeff. at 15° in dry air	0.07774	0.07778	0.07760	0.07656

It will be seen that there is a slight tendency towards a lower value of diffusion coefficient as the path length becomes very short, but that in general the results are within the range of experimental error.

Collected results for dry air are shown in Table I, and for moist air in Table II. Since the diffusion coefficient varies inversely as the barometric pressure, all results have been corrected to 760 mm. pressure.

The results for dry air when plotted against temperature lie on a smooth curve. The relationship between the diffusion coefficient of a vapour and temperature is given by the expression

$$D_t^p = \left[ \frac{T}{273} \right]^n D_0^p$$

where  $D_0^p$  is the value of the coefficient at 273° K and  $n$  is an integer whose value has been variously given as between 1.78 and 2.00. For ethylene dibromide  $n$  is here found to be 1.96. The diffusion coefficient is not, however, a constant and its value varies with concentration, an extreme variation of about 10% being usual. This factor will tend to make the value of  $n$  too high as found by the present method. For most practical purposes, however, the value of the coefficient in dry air at any temperature and pressure can be found from the relationship

$$D_t^p = \left[ \frac{T}{273} \right]^2 \cdot \frac{760}{P} \cdot D_0^{760}$$

where  $D_0^{760}$  is 0.708.



The diffusion coefficient in air saturated with water vapour thus appears to be about 0.5–1.0% lower than in dry air.

The diffusion coefficients measured in presence of CO<sub>2</sub> are shown in Table III.

These results lie on parallel straight lines of slope 0.0019 per unit-%. The diffusion coefficient is thus reduced by about 0.24% by 1% of CO<sub>2</sub>.

**Table I**

*Diffusion coefficient of ethylene dibromide in dry air*

Temperature, ° C	Diffusion coefficient $D_t^{760}$	
	Mean	
20.0	0.08132	0.08131
	0.08120	
	0.08141	
15.0	0.07792	0.07778
	0.07760	
	0.07782	
7.1	0.07360	0.07360
	0.07361	
0.0	0.07065	0.07078
	0.07091	

**Table II**

*Diffusion coefficients of ethylene dibromide in moist air*

Temperature, ° C	$D_t^{760}$ at 100% R.H.	$D_0$ 100% R.H.	
		D <sub>0</sub> 0% R.H.	
20.00	0.08077	0.994	
15.00	0.07690	0.989	

**Table III**

*Diffusion coefficients in presence of carbon dioxide*

Temperature, ° C	CO <sub>2</sub> % by vol.	$D_t^{760}$
20.00	7.80	0.07985
	12.90	0.07894
	2.78	0.07718
15.00	0.35	0.07642
	39.20	0.07098

### Discussion

Sufficient data have been accumulated to enable the diffusion coefficient of ethylene dibromide vapour in air to be calculated at any temperature between 0° and 20°. The effects of moisture and carbon dioxide are small but definite. It would seem that the diffusion coefficient in the upper 12 to 15 inches of normal field soils will be reduced by between 1 and 2% since the carbon dioxide content is normally between 1 and 4% in this region.

### Acknowledgments

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## ABSORPTION AND METABOLISM OF BHC IN SUSCEPTIBLE AND RESISTANT HOUSEFLIES\*

By F. R. BRADBURY

Quantitative and qualitative work on the resistance of houseflies to  $\gamma$ -BHC is described, in which BHC isomers labelled with  $^{36}\text{Cl}$  or  $^{14}\text{C}$  were used.  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -BHC are converted to water-soluble compounds by houseflies, the  $\alpha$ - and  $\gamma$ -isomers being more susceptible to the conversion than the  $\beta$ - and  $\delta$ -compounds. The reaction in the cases of  $\alpha$ -,  $\gamma$ - and  $\delta$ -BHC is accompanied by production of chloride ions corresponding to the removal of 4 to 6 atoms of chlorine from each molecule of BHC metabolized. It is therefore considered unlikely that the metabolites are trichlorobenzene derivatives. Chromatographic evidence indicates the ultimate production of eleven water-soluble compounds from both  $\alpha$ - and  $\gamma$ -BHC. It is deduced from two-dimensional chromatograms and from paper electrophoresis that the two isomers produce an identical or very similar series of compounds and that these are acidic. The role of metabolism in defending the insect against poisoning is discussed in terms of concentration  $\times$  times effects and it is concluded that detoxication by metabolism is essential for the complete recovery of the insect following absorption of insecticide. Measurement of the capacity of a number of susceptible insect species to metabolize BHC isomers shows that the susceptible strain of housefly exhibits exceptional ability to eliminate  $\gamma$ -BHC as water-soluble products.

### Introduction

This discussion is concerned specifically with resistance to  $\gamma$ -BHC. Comparative experiments at Widnes are described in which were used the toxic  $\gamma$ -isomer and its non-toxic  $\alpha$ -,  $\beta$ - and  $\delta$ -isomers as chemicals with susceptible and resistant strains of *Musca domestica*. Some comparative work has been done also on non-resistant strains of other insects.

In contrast with the large amount of experimental work on DDT resistance in flies there is very little published work on BHC resistance. The Dutch workers Oppenoorth & Van Asperen<sup>1-3</sup> have reported experiments on the detoxication of BHC isomers by susceptible and resistant flies and by mice. They found that both strains of flies possess the ability to metabolize  $\gamma$ -BHC, the resistant strains being able to destroy virtually the whole of an injected dose of 0.3  $\mu\text{g}$ /fly in 24 hours. They also found that  $\delta$ -BHC is less readily destroyed by flies than the  $\alpha$ - and  $\gamma$ -isomers. On the other hand mice decomposed the  $\alpha$ -isomer less rapidly than  $\gamma$ - or  $\delta$ -BHC.

Although it is considered that detoxication to DDE is one of the factors contributing to DDT resistance, reviews of the subject by Chadwick and others<sup>4-8</sup> indicate that reduced absorption and different distribution of DDT within the insect body are also involved. It is concluded from the experiments made here that resistance to  $\gamma$ -BHC may involve similar factors although the detoxication process must be different.

### Experimental

#### *Insects*

The susceptible flies used were the normal Jealotts Hill stock *Musca domestica*. The resistant strain originated in Uruguay and has been maintained at Jealotts Hill on a breeding medium containing 0.5%  $\gamma$ -BHC. The susceptibility of this strain was measured in 1954 by Busvine<sup>9</sup> who found the  $\gamma$ -BHC M.L.C. (minimum lethal concentration) of 0.8% compared with 0.008% for a normal laboratory strain.

All other insects used in our experiments have come from Jealotts Hill cultures.

#### *Exposure of insects*

In all the experiments described insects have been exposed to the chemicals as vapours at 25°. On account of the relative high vapour pressure of  $\gamma$ -BHC and its isomers, this has been conveniently done by confining the insects over treated filter paper in Petri dishes or on a glass surface in Erlenmeyer flasks, so avoiding injection techniques.

\* Read before Pesticides Group, 9 January, 1956

*Analytical*

In early experiments a colorimetric method of BHC analysis was employed,<sup>10</sup> but when supplies of radioactive benzene became available <sup>14</sup>C-labelled BHC was used. This gave greatly enhanced sensitivity making it possible to determine as little as 0.04  $\mu$ g. of BHC. For determining <sup>14</sup>C compounds they were converted to CO<sub>2</sub> by combustion and precipitated as barium carbonate for counting in a flow-type proportional counter. Details of <sup>14</sup>C estimation have been given in an earlier paper.<sup>11</sup> Some experiments were done with <sup>36</sup>Cl-labelled BHC; in this case chloride, produced from BHC by alkaline hydrolysis, was precipitated as mercurous chloride for counting. Experimental details are given in the following section.

*Determination of radioactivity of samples labelled with chlorine-36*

The <sup>36</sup>Cl-labelled benzene hexachloride had an activity of approximately 10  $\mu$ c/g.

The chloride was precipitated as mercurous chloride, using as precipitating reagent 0.1N-mercurous nitrate prepared by triturating 2 g. of mercurous nitrate with 50 ml. of distilled water and filtering before use.

For determination of chloride in fly tissues the bodies, freed from benzene hexachloride by extraction with boiling carbon tetrachloride and from carbon tetrachloride by air blowing, were transferred to 30 ml. of distilled water containing 2 ml. of 8N-nitric acid. This solution was boiled under reflux for two hours after which the flies were removed by filtration. Four ml. of 0.1N-hydrochloric acid were added and the chloride precipitated by the addition of 4 ml. of approximately 0.1N-mercurous nitrate. The solution was set aside overnight before filtering through a filter paper disc in the apparatus used for barium carbonate in carbon-14 estimations. The residue was washed with water and then with acetone, dried and mounted on a brass block for counting in the flow-type proportional counter.

After counting, the pad of mercurous chloride was weighed and a correction for self-absorption read off from the curve obtained by counting a series of mercurous chloride pads containing a constant amount of <sup>36</sup>Cl but of varying weight.

For determination of benzene hexachloride, the carbon tetrachloride solution of benzene hexachloride was reduced to small bulk (less than 0.5 ml.) by air blowing and hydrolysed by refluxing with 10 ml. of 5% aqueous caustic soda for one hour. The solution was acidified with 4 ml. of 25% nitric acid; 4 ml. of 0.1N-hydrochloric acid was added followed by 5 ml. of 0.1N-mercurous nitrate and the mixture kept overnight before filtering.

The possibility that chloride ions were produced from benzene hexachloride during the analysis for chloride was investigated. 100  $\mu$ g. of <sup>36</sup>Cl- $\gamma$ -BHC was boiled under reflux for two hours with 30 ml. of water containing 2 ml. of 8N-nitric acid. The chloride precipitated from the solution in the usual way was inactive. To check the possibility that radioactive chloride would be produced from the  $\gamma$ -benzene hexachloride in the presence of water during the exposure of the flies, 1 mg. of <sup>36</sup>Cl-labelled  $\gamma$ -benzene hexachloride was stored for six hours at 25° in the presence of 4 ml. of 0.1N-hydrochloric acid. The chloride precipitated from the aqueous solution at the end of the storage period was inactive.

As it appeared possible that mercury might form insoluble salts with the benzene hexachloride metabolites and thereby become radioactive with organically-combined <sup>36</sup>Cl, an experiment was made in which fifty resistant female flies were exposed to 200  $\mu$ g. of <sup>14</sup>C-labelled  $\gamma$ -benzene hexachloride in a 250-ml. conical flask for six hours. During the period approximately 100  $\mu$ g. of water-soluble metabolites are produced. The extraction process previously described was used and the precipitate of mercurous chloride formed in the usual way from the dilute nitric acid extract. This had a relative count per minute equivalent to 1  $\mu$ g. of <sup>14</sup>C-labelled  $\gamma$ -benzene hexachloride. This shows that the contamination of chloride precipitates with organic <sup>36</sup>Cl compounds is of the order of 1% and can therefore be ignored.

To investigate the possibility that a benzene hexachloride metabolite might produce chloride ions during the process for precipitation of mercurous chloride, three lots of 50 flies were separately exposed to 200  $\mu$ g. of <sup>36</sup>Cl- $\gamma$ -BHC for two hours in conical flasks. The 150 resistant flies were then extracted for half an hour with boiling carbon tetrachloride, and after filtering, were allowed to dry in air. They were then subjected to extraction with boiling water for two hours,

after which time the aqueous washings from the three exposure flasks and filter papers were added. The combined extracts were divided into three equal volumes; in (1) chloride ion was precipitated by addition of hydrochloric acid and mercurous nitrate after acidification with nitric acid; (2) was acidified with nitric acid and extracted with ether before chloride precipitation; to (3) 2 ml. of 8N-nitric acid were added and the solution boiled for two hours under reflux before chloride precipitation. The activity found in the mercurous chloride pads, expressed in terms of  $\mu\text{g.}$  of benzene hexachloride per fly was as follows: (1) 0.50; (2) 0.54; (3) 0.60. Extraction with ether, which removed about one-third of the metabolites, did not seriously affect the chloride recovery.

#### Extraction

Radioactive material was recovered from treated insects by extraction with carbon tetrachloride and with water. In some experiments the carbon tetrachloride-soluble matter was collected as two fractions, 'outside' and 'inside'. In these cases a preliminary rinse of the whole insect with carbon tetrachloride was taken for the 'outside' extract. The insects were then crushed with anhydrous sodium sulphate or kieselguhr and the extraction repeated for 'inside' determination.

In one experiment the respired air was collected and examined for radioactive carbon dioxide. None was found.

#### Chromatography

BHC was identified by reversed-phase chromatography on vaselined paper using 80% ethyl alcohol/water mixture as mobile phase, as described by Winteringham *et al.*<sup>12</sup>

The water-soluble materials were chromatographed with a variety of solvent systems including butanol/ammonia, butanol/benzene/ammonia, butanol/pyridine/saturated brine, methyl ethyl ketone/water, and butanol/acetic acid. In the earlier experiments the chromatographs were analysed by cutting the paper into strips and counting them directly in the proportional flow counter. More recently X-ray film has been used. 'Kodirex' paper kept in contact with a chromatogram for four days would clearly define spots containing the equivalent of 0.02  $\mu\text{g.}$  of BHC/cm.<sup>2</sup> The specific activity of the BHC used was approximately 3 mc/g. so that the activity of the spot detected was approximately  $0.6 \times 10^{-4} \mu\text{c/cm.}^2$  (Herz<sup>13</sup> gives a figure for <sup>14</sup>C of  $3.6 \times 10^{-4} \mu\text{c/cm.}^2$  to give a density of 0.6 with an exposure of 15 days on Eastman no-screen film.) This technique is very useful for two-dimensional chromatograms where cutting and counting are tedious.

### Results

#### Quantitative

The quantitative effects of BHC absorption and metabolism have been described in detail in an earlier paper<sup>14</sup> and will not be repeated here. They indicated that all four common isomers,  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -BHC are metabolized both by susceptible and resistant flies, but that the resistant flies metabolize a greater proportion of the absorbed dose.  $\alpha$ - and  $\gamma$ -BHC are more readily converted to water-soluble products,  $\beta$ - and  $\delta$ -less readily. The quantitative experiments also indicated that resistant flies absorbed less BHC than the susceptible strain.

#### Qualitative

It is of the greatest importance to identify the metabolic products of BHC. So far all that has been done in this direction is to reveal the complexity of the problem.

It is of interest first to check the identity of the radioactive material in the carbon tetrachloride extracts of treated flies. By reversed-phase chromatography of such extracts we obtained evidence that, in the case of  $\gamma$ - and  $\delta$ -BHC treated flies, the carbon tetrachloride extract consisted mainly of unchanged BHC.<sup>11</sup> This has been checked for both  $\gamma$ - and  $\delta$ -BHC by autoradiography of reversed-phase chromatograms with the pure isomers run parallel with the extracts. Only one spot was obtained, the  $R_F$  value being in each case that of the unchanged BHC isomer.

Two possible dehydrohalogenation products of BHC soluble in carbon tetrachloride are pentachlorocyclohexene and trichlorobenzene. The presence of trichlorobenzene in carbon tetrachloride extracts of  $\gamma$ -treated flies has not been demonstrated.<sup>11</sup> Both pentachlorocyclohexene and trichlorobenzene are volatile substances, however, and small amounts may have been produced by the fly and have been lost during concentration of the carbon tetrachloride extracts.\*

There is, however, evidence which shows that metabolism is accompanied by dechlorination of the BHC molecule. Table I shows mass-balance experiments on flies exposed to mixed  $^{36}\text{Cl}$ - and  $^{14}\text{C}$ - $\gamma$ -BHC, an arrangement which permitted parallel determinations of chloride, by converting to mercurous chloride, and water-soluble metabolites. BHC was determined by both methods, as  $^{14}\text{C}$  carbon dioxide by combustion and  $^{36}\text{Cl}$  mercurous chloride by hydrolysis and precipitation. The figures for chloride are compared with those for water-soluble matter. The radioactive chloride found corresponds to four to six chlorine atoms being released from the BHC molecule in metabolism.

Table I

Exposure of normal female flies to mixed  $^{36}\text{Cl}$ - and  $^{14}\text{C}$ -labelled BHC isomers

	$\alpha$ -Isomer		$\gamma$ -Isomer	
	$^{36}\text{Cl}$	$^{14}\text{C}$	$^{36}\text{Cl}$	$^{14}\text{C}$
Outside + inside BHC	29	28	60	61
BHC remaining in flask	29	26	55	45
Water-soluble products	89*	99	55*	66
Residue (unextracted)	n.d.	33	n.d.	15
Total		186		187
Started with	206	206	205	205

All figures have been converted to  $\mu\text{g.}$  of  $\text{C}_6\text{H}_6\text{Cl}_6$   
n.d. = not determined

\* Assuming  $6\text{Cl}^- \equiv \text{C}_6\text{H}_6\text{Cl}_6$

Table II shows other results including the  $\delta$ -isomer. The figures for  $\delta$ - correspond with liberation of five or six chlorine atoms from the  $\delta$ -isomer on metabolism; for  $\alpha$ - and  $\gamma$ - the figures again indicate 4-6. There are considerable difficulties in the way of calculating stoichiometric figures from results of the kind shown. The figures quoted assume that the water-soluble compounds contain all six carbon atoms of the BHC molecule. They also depend on an assumption concerning the nature of the 'residue'. If this is assumed to be BHC, the figures give a loss

Table II

Exposure of female resistant flies to  $^{36}\text{Cl}$ - and  $^{14}\text{C}$ -labelled BHC in separate parallel tests

	$\alpha$ -Isomer		$\gamma$ -Isomer		$\delta$ -Isomer	
	$^{36}\text{Cl}$	$^{14}\text{C}$	$^{36}\text{Cl}$	$^{14}\text{C}$	$^{36}\text{Cl}$	$^{14}\text{C}$
Outside + inside BHC	40	17	56	42	97	87
BHC remaining in flask		40	17	48	22	60
Water-soluble products		97*	112	90*	112	50
Residue	n.d.	11	n.d.	18	n.d.	8
Total		180		220		205
Started with	200	200	200	213	200	200

All figures have been converted to  $\mu\text{g.}$  of  $\text{C}_6\text{H}_6\text{Cl}_6$   
n.d. = not determined

\* Assuming  $6\text{Cl}^- \equiv \text{C}_6\text{H}_6\text{Cl}_6$

\* (Note added since the lecture was given.) Sternberg & Kearns<sup>18</sup> have claimed that pentachlorocyclohexene is formed by resistant flies as the major initial metabolic product of  $\gamma$ -BHC, and that chromatography fails to distinguish between  $\gamma$ -BHC and its monodehydrohalogenation product. Isotopic dilution experiments (Bradbury & Standen, unpublished results) indicate that the radioactivity in carbon tetrachloride extracts of resistant flies which have been exposed to  $\gamma$ -BHC for 24 hours is largely  $\gamma$ -BHC with little, if any, pentachlorocyclohexene. The bulk of the absorbed insecticide (>90%) is however converted to water-soluble products in this period.

of five chlorine atoms, by the  $\alpha$ - and  $\gamma$ -BHC molecules, and six by  $\delta$ -BHC. If the 'residue' is a metabolite the figures come nearer to the loss of four from  $\alpha$ - and  $\gamma$ - and five from  $\delta$ -BHC. At the best the figures are an average over a series of metabolic products of which there are a great number (see below). The results may be regarded as evidence that a proportion of the BHC metabolized by the fly to water-soluble products loses more than the three chlorine atoms required if the process were entirely based on dehydrohalogenation to trichlorobenzene.

In an earlier paper<sup>14</sup> it was shown that flies possess the power to metabolize all four common isomers of BHC. This led to the examination of the nature of the pattern of compounds produced from the different isomers.

Fig. 1 shows a diagrammatic representation of a two-dimensional chromatogram of the water-soluble radioactive products extracted from flies treated with radioactive  $\gamma$ -BHC. The two solvent systems used were butanol/benzene/ammonia (3N) and butanol/acetic acid/water. It will be observed that the compounds fall into two groups, those of relatively low  $R_f$  values in butanol/benzene/ammonia and those of higher  $R_f$  values in this solvent system. There are eleven well-defined compounds present.

Fig. 2 shows the results of two-way chromatography with the same solvent systems using the water-soluble metabolites from  $\alpha$ -BHC-treated flies. Again there are eleven compounds and from their position on the chromatogram it is probable that most of the compounds are common to the metabolism of  $\alpha$ - and  $\gamma$ -BHC. In the case of eight compounds, further evidence suggesting identity has been obtained by paper electrophoresis of corresponding spots in 0.02N-caustic soda solution. It should be noted that the aqueous extracts used for these chromatograms were collected over periods of up to seven days' exposure to BHC and that amongst the compounds present are therefore likely to be secondary products formed by further breakdown of the initial metabolite or metabolites.

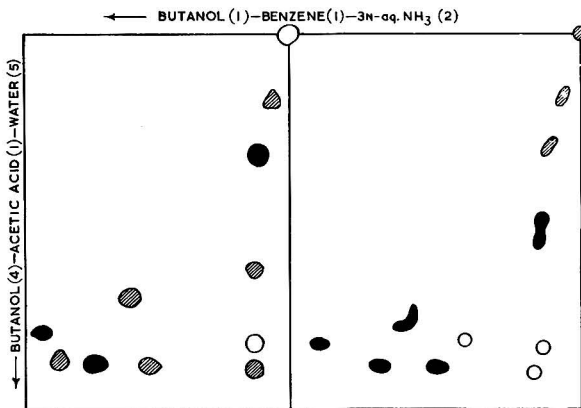


FIG. 1.—Water-soluble metabolites from  $\gamma$ -BHC

FIG. 2.—Water-soluble metabolites from  $\alpha$ -BHC

(Diagrams of chromatograms shaded to indicate relative intensity of spots)

Structurally identical derivatives from two stereo-isomers implies that the stereo-isomerism is removed in the process; this could be achieved by conversion from cycloaliphatic to aromatic and would result either from dehydrohalogenation to trichlorobenzene or dechlorination to benzene. Since more than three chlorine atoms were set free in the process by each of the three isomers ( $\alpha$ ,  $\gamma$ ,  $\delta$ ), it is unlikely that all the metabolites are trichlorobenzene derivatives and it is possible that benzene is an intermediate stage in the production of some of these.



Perry *et al.*<sup>15</sup> have recently shown that the metabolism of DDT by flies begins and ends with dehydrohalogenation to DDE; other compounds amount to less than 10% of the DDT metabolized. Our results show that detoxication of BHC involves other mechanisms—dehydrohalogenation products can at most be intermediates.

### Discussion

The general picture of insecticide resistance to  $\gamma$ -BHC on the basis of the facts as they are known to us at present is broadly parallel to that currently held for DDT. We can say that two factors contribute to resistance to poisoning. The first is reduced pick-up of poison, the second a more rapid conversion to excretable metabolites. It is probable, although we have as yet no direct evidence of this for BHC, that there is a third factor, distribution within the insect body. The work of Munson and others on cockroaches<sup>8</sup> suggests that variation in the nature of the lipoids between susceptible and resistant strains may be important in controlling distribution of DDT. The work of Langenbuch<sup>16</sup> also emphasizes the importance of lipoids in conferring resistance to fat-soluble insecticides.

The extent of the difference in BHC content between normal and resistant flies due to the combined effects of reduced absorption and increased metabolism appears from this work to be at the most a six-fold effect compared with a resistance factor of up to a hundred-fold. As indicated above, it is likely that metabolism is only a contributing factor in resistance. It is hoped to study the distribution aspect of resistance to  $\gamma$ -BHC in the immediate future, but if there is a distribution factor of the type described by Munson a detoxication process is an essential corollary to it.

In this connexion it is convenient to look upon the insect as a series of phases between which the insecticide is distributed. When an insect is placed in the vapour of BHC, we know that the first step in pick-up is the entry of the organic compound into the waxy layer of the epicuticle.<sup>17</sup> This then acts as a reservoir to supply other tissues, aqueous, fatty, proteinaceous, etc. It is probable that only one, or part of one, of these phases is critical for disturbing the physiological processes of the insect. In this critical phase a high concentration for a short time or a low concentration for a long time, that is to say, an effect proportional to the product of concentration and time (CT), may bring about the death of the insect.

If in defending itself against the poisonous action, the insect adopts a redistribution pattern which reduces the concentration of poison in the critical phase, a supplementary line of defence to reduce the time of exposure to the poison in the critical tissue—that is to say T of the CT mentioned above—is required. This would appear to be essential for effective protection against a chemical whose physico-chemical properties dispose it to storage in fatty tissues for indefinite periods. Obviously here metabolism can play an important role. It has been shown that the resistant fly is able to metabolize BHC very rapidly to water-soluble products which are excreted and so removed from the system. The more rapidly this is done the greater the protection to the insect on account of the reduction in CT.

If there is a CT effect in the action of the poison on a critical site in the fly, reduced absorption and changed distribution, unsupported by a detoxication process, would at the best only prolong the period of survival of the insect following absorption of a chlorinated hydrocarbon insecticide. To this extent it is misleading to speak of detoxication mechanisms of resistance and distribution mechanisms as though they were alternatives.

It is of interest to enquire how far the two factors, penetration and metabolism, operate in other species of insects. Some comparative experiments with a variety of insect species are now in progress with the object of comparing the extent of absorption and metabolism of benzene hexachloride. Table III shows some of the results.

Cockroaches, grain weevils, locusts, mosquitoes and khapra beetles were compared with susceptible and resistant flies for absorption and metabolism. The figures of interest are those showing 'inside'-BHC and 'water-soluble metabolite'. It is immediately obvious that the houseflies, both susceptible and resistant, are in a class by themselves as regards the ability to metabolize BHC, even though, in the case of the  $\gamma$ -isomer, resistant flies metabolize almost twice as much as susceptible ones. It is tempting to speculate that the widespread ability of flies

Table III

Absorption and metabolism of BHC by various insects

(All results calculated to  $\mu\text{g.}$  of BHC per g. of insect)

	Cock- roach	Bean Weevil	Grain Weevil	Locust	Mosquito	Khapra beetle	Housefly Sus- ceptible	Housefly Resistant
<i><math>\gamma</math>-Isomer</i>								
Picked up	42	111	34	100	213	166	193	230
'Outside' BHC	10	7	14	16	17	81	22	13
'Inside' BHC	19	94	13	83	141	45	54	21
Water-soluble metabolite	7	8	1	4	10	11	99	174
Residue	8	7	5	9	3	5	17	16
Total found	44	116	33	112	171	142	192	224
<i><math>\alpha</math>-Isomer</i>								
Picked up	81	103	31	58	204	140	214	199
'Outside' BHC	8	5	6	18	22	67	11	5
'Inside' BHC	47	79	13	32	182	17	27	7
Water-soluble metabolite	5	4	1	2	5	13	140	166
Residue	9	12	3	4	3	3	20	7
Total found	69	100	23	56	212	100	198	185

quickly to become resistant to poisoning by  $\gamma$ -BHC is related to this exceptional ability to decompose the insecticide to the excretable metabolites. Although cases of resistance to chlorinated hydrocarbon insecticides have been encountered with other insects, cockroaches and mosquitoes, for example, in neither case is the resistance widespread. Our view is that the existence in the normal fly of a metabolic pathway for dealing with BHC very much predisposes this insect to the acquisition of insecticide resistance.

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## THE DESTRUCTION OF VITAMIN E IN FLOUR BY CHLORINE DIOXIDE

By T. MOORE, I. M. SHARMAN and R. J. WARD

Chemical estimations of the tocopherols present in wheaten flours treated with chlorine dioxide, or untreated, have been made by a method which allows the separation of the different forms of the vitamin. The chlorine dioxide caused almost complete destruction of each of the tocopherols. Biological tests demonstrated that untreated flour, when included as the main component of the diet of rats, contains enough tocopherol to satisfy their requirements. Rats developed various signs of avitaminosis E, however, when they were given a similar diet containing flour which had been treated with chlorine dioxide. Enough tocopherol survived during the baking of bread from untreated flour to suffice the requirements of rats. Improvement with potassium bromate or ascorbic acid did not appear to decrease the tocopherol content of bread, but some destruction was caused by the aeration process of breadmaking.

### Introduction

Partial destruction of vitamin E in wheat flour during bleaching or 'improvement' was first reported by Engel.<sup>1</sup> The unbleached flour, of 70% extraction rate, contained 1.6 mg. of tocopherol per 100 g., which was reduced to 1.1 mg. by weak treatment with benzoyl peroxide or to 1.0 mg. by strong treatment. Weak treatment with nitrogen trichloride caused a reduction to 1.2 mg., and strong treatment to 0.5 mg. per 100 g. Similar decreases were observed in rye and barley flours of 60% extraction. The estimations of tocopherol, which did not distinguish  $\alpha$ -tocopherol from other forms, were carried out by the method of Emmerie & Engel<sup>2</sup> with ferric chloride and  $\alpha\alpha'$ -dipyridyl. Carotenoids and other interfering reducing substances were removed by adsorption on Floridin earth.

The effect of chlorine dioxide on the tocopherols of wheat flour was studied by Moran, Pace & McDermott.<sup>3</sup> Estimations of total tocopherols (method of Emmerie & Engel<sup>2</sup>) in a flour, rich in germ, before and after treatment with chlorine dioxide at the ordinary commercial level (30 p.p.m.), indicated a destruction of about 70%. In another experiment<sup>4</sup> it was found that as much as 95% of the total reducing value of the unsaponifiable fraction was destroyed by the same treatment.

The purpose of the present investigation was to confirm and extend the observations of Moran and his colleagues.

### Experimental

#### *The effect of chlorine dioxide on vitamin E in unbaked flour*

The experiments were made either on untreated flour or flour treated with chlorine dioxide. A large batch of flour, derived from a mixed grist and with an extraction rate of about 80%, was prepared every two months at a commercial mill. The material was sent to the Cereals Research Station, St. Albans, and was thence passed on in weekly supplies to Cambridge. Immediately before transmission to Cambridge half of the flour was treated with chlorine dioxide in amounts corresponding to the commercial level then current of 3.75 g. per 280-lb. sack (approx. 30 p.p.m.). Weekly routine checks were made by extracting the flour with light petroleum, and measuring the intensity of the yellow colour in the extract. In this way information was obtained about the degree of uniformity of the bleaching effect caused by the chlorine dioxide.

In addition to the main batch of flour, other batches sometimes were used in subsidiary experiments.

*Chemical assays.*—Chemical measurements of  $\alpha$ -,  $\beta$ -,  $\epsilon$ - and  $\zeta$ -tocopherols were made on specimens of flour taken for examination at about the half-way point in the feeding experiments. The oil containing the vitamin was extracted by the percolation of 150-g. portions of the flour, in duplicate, with light petroleum, b.p. 40–60°, in a column of 5 cm. diameter. A modification of the procedure of Green, Marcinkiewicz & Watt<sup>5</sup> was then employed. The fat was saponified in the presence of pyrogallol<sup>6</sup> and carotenoids and sterols were removed from the unsaponifiable

fraction by adsorption on Floridin earth. The individual tocopherols were then separated on two-dimensional paper chromatograms, which were run in duplicate for each extract, and were estimated by the method of Emmerie & Engel.<sup>2</sup> For assessing the total vitamin-E activity, it was assumed that the relative activities are  $\alpha = 100$ ,  $\beta = 30$ ,  $\epsilon = 10$  and  $\zeta = 90$ . From the results in Table I, it will be seen that the activity of the untreated flour was equivalent to about 0.6 mg. of  $\alpha$ -tocopherol per 100 g., as compared with only 0.04 for the flour treated with chlorine dioxide. On a dry-weight basis these figures must be increased to 0.69 and 0.046 mg., respectively.

Table I

*Tocopherol in untreated flour and in the same flour after treatment with chlorine dioxide, as estimated by paper chromatography*

(The values for each extract are averages for two chromatograms)

Results as mg./100 g.

Tocopherols	Untreated flour			ClO <sub>2</sub> -treated flour			Total vitamin-E activity
	Extract No. 1	Extract No. 2	Average	Extract No. 1	Extract No. 2	Average	
$\alpha$	0.32	0.38	0.35	0.02	0.02	0.02	0.02
$\zeta$	0.08	0.09	0.09	0	0	0	0
$\beta$	0.22	0.27	0.25	0.03	0.03	0.03	0.01
$\epsilon$	0.88	0.88	0.88	0.14	0.14	0.14	0.01
Total			<u>1.57</u>			<u>0.19</u>	<u>0.04</u>

*Minimum requirements of rats for vitamin E.*—The biological tests on flour were preceded by exploratory trials in which groups of weanling rats were reared on an ordinary basal diet deficient in vitamin E, which was supplemented by graded doses of DL- $\alpha$ -tocopheryl acetate. The diet consisted of casein (vitamin-free) 25 parts, sugar 50, lard 10, dried yeast 10, and minerals 5 parts, with adequate supplements of vitamins A, D and K. In males it was found that doses of 0.25 mg. weekly were adequate, but that doses of 0.18 mg. were inadequate, for the normal development and maintenance of the testes. For prevention of brown discoloration of the uterus, doses of 0.25 mg. were usually adequate, but doses of 0.125 mg. were inadequate.

#### *Biological tests on flour*

From the preceding paragraphs it would appear that a diet containing substantial amounts of untreated flour should provide enough vitamin E to meet the requirements of rats, whereas a diet containing flour treated with chlorine dioxide would be inadequate. The amount of the undried, untreated flour necessary to supply the minimum requirement of 0.23 mg. of  $\alpha$ -tocopherol alcohol would presumably be about 40 g. per week. The main object of the biological tests, however, was to confirm the superiority of the untreated flour when used as the principal ingredient and sole source of vitamin E in the diet of the rat, rather than to attempt an accurate biological estimation of the vitamin in comparison with the results of the chemical method. The flour was therefore incorporated as the main constituent of a diet, which had the following composition: Flour (untreated or ClO<sub>2</sub>-treated) 85.5 parts, casein 5, lard 8, dried yeast 8 and minerals 4. Adequate supplements of vitamins A, D and K were supplied.

Each diet was given to groups of 6 male and 6 female weanling albino rats, housed separately. Tests for the biological activity of the flour were made by five methods, which were based on the following criteria (1) the haemolysis test with dialuric acid, (2) pigmentation of the incisor teeth, (3) size and condition of the testes, (4) reproduction in the female, (5) colour of the uterus, (6) kidney degeneration.

*Haemolysis test.*—The degree of haemolysis was estimated, by a modification of the method of György & Rose,<sup>7</sup> after the rats had been receiving their diets for 98–103 days. Blood was collected from the tail into a citrate-saline solution (0.6% sodium citrate, 0.9% sodium chloride). After centrifugation the erythrocytes were washed once with normal saline and then made up to a 5% suspension in normal saline. Portions of this suspension (0.25 ml.) were then mixed in small test-tubes with 0.1 mg. of dialuric acid freshly dissolved in 0.25 ml. of phosphate buffer at

pH 7.4, and were incubated for 15 minutes at 37°. After keeping for 45 minutes at room temperature the tubes were centrifuged. A portion of the supernatant fluid was removed, suitably diluted, and the intensity of its red colour measured in a photoelectric absorptiometer. The percentage of haemolysis was estimated by comparison of the red colour with that produced by another portion of the original suspension which had been completely haemolysed with distilled water.

From Table II it will be seen that the degree of haemolysis in the twelve rats given untreated flour ranged from 0 to 1.9%. In the groups given flour treated with chlorine dioxide the range was from 70.5 to 100%. The difference between the groups was significant according to statistical standards, with  $P \ll 0.01$ .

Table II

Percentages of haemolysis in the dialuric acid test of erythrocytes taken from rats given untreated or  $\text{ClO}_2$ -treated flour

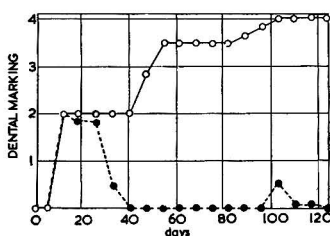
Rat No. in group	Untreated flour		$\text{ClO}_2$ -treated flour	
	Males %	Females %	Males %	Females %
1	0.7	0	70.5	91.5
2	1.6	0	83.8	92.8
3	0.9	0	91.0	95.0
4	1.5	0	99.6	100.0
5	0	0.5	89.5	92.5
6	0	0	88.1	90.6
Average : Male and female	0.43		90.4	

*Dental depigmentation.*—Repeated observations were made on the colour of the upper incisor teeth. The results were recorded according to the system of Moore & Mitchell,<sup>8</sup> in which 2 marks were given for each normal brown tooth, 1 mark for a pale brown or mottled tooth, and no marks for a white tooth. Average markings were calculated for the groups, with a range from 4 when all the rats had normal teeth down to 0 when all the rats had white teeth.

The average markings per rat for the combined groups of male and female rats at different stages of the experiment are shown graphically in Fig. 1. On day 124 the average markings were 4.0 in the group receiving untreated flour and 0 in the group receiving flour treated with chlorine dioxide. At this time the difference in the colour of the teeth between the two groups was significant according to statistical standards, with  $P \ll 0.01$ .

FIG. 1.—Comparison of average markings for dental pigmentation in rats given diets containing untreated flour or flour treated with chlorine dioxide

Maximum marking for normal brown upper incisors = 4  
 Marking for completely white incisors = 0  
 ○—○—○ Untreated flour  
 ●—●—●  $\text{ClO}_2$ -treated flour



After more prolonged deficiency an unexpected return towards normal pigmentation was seen in the teeth of the female group receiving treated flour. The males by this time had been killed, but the females were being retained for investigation of their breeding powers. The teeth started to become pigmented after 159 days, and after 208 days had become a uniform brown throughout the group, although they were decidedly lighter in shade than in the group receiving

untreated flour. The application of the haemolysis test at 194 days indicated clearly that the animals, in spite of the brown colour of their teeth, were still deficient in vitamin E. The cause of the resumption of pigmentation is at present obscure.

*Size and condition of testes.*—After receiving their diets for 125 days both groups of males were killed for the examination of their testes. The testes were weighed fresh, and were studied histologically in paraffin sections, stained by conventional methods with haematoxylin and eosin. From Table III it will be seen that the testes of the rats which had received untreated flour weighed from 2.40 to 2.93 g., as compared with 1.26 to 1.79 g. in the group given treated flour. The differences in the weights of the testes between the groups were statistically significant, with  $P \ll 0.01$ .

Table III

*Weights of testes of rats fed upon diets containing untreated or ClO<sub>2</sub>-treated flours*

Rat No.	Untreated flour		ClO <sub>2</sub> -treated flour	
	Wt. of rat, g.	Wt. of testes, g.	Wt. of rat, g.	Wt. of testes, g.
1	310	2.93	242	1.26
2	287	2.84	258	1.52
3	354	2.91	289	1.44
4	324	2.40	296	1.79
5	274	2.51	255	1.39
6	279	2.29	269	1.48
Average	305	2.65	268	1.48

The results of the histological examinations were in all instances consistent with the conclusions to be drawn from the weights of the testes. Thus in the animals given untreated flour the production of spermatozoa was proceeding normally (Fig. 2). In contrast the testes in the group given treated flour were all abnormal (Fig. 3). The seminiferous tubules usually contained only one or two layers of spermatogonia, with no spermocytes or spermatids. The basement membrane was thickened, but there were no changes in the interstitial tissues.

*Reproduction in the female.*—On the 125th day of the experiment the groups of females given untreated and treated flour were mated with normal males. During the next few weeks they were inspected and weighed frequently, but no instances of pregnancy were observed in either group. The matings were first made late in October, and it seems possible that the infertility was due to an unfavourable season of the year. On day 176 the males were removed, and one female from each group was killed. No evidence of placental sites was seen in their uteri, which implied that there had been failure in coitus or fertilization, and not resorption of foetuses which is characteristic of avitaminosis E.

The five remaining females in each group were mated again with fresh bucks on day 207, in January. During the next five weeks 3 litters were born to the females receiving untreated



FIG. 2.—Typical section of the seminiferous tubules in a rat which had been fed upon a diet containing untreated flour



FIG. 3.—Typical section of the seminiferous tubules in a rat which had been fed upon a diet containing flour treated with chlorine dioxide

flour. One rat in the group receiving flour treated with chlorine dioxide increased considerably in weight, but the weight eventually declined again gradually without the production of the litter.

Most of the rats were killed on day 253. Examination of the uteri gave no indication of resorption gestations in the two rats receiving untreated flour which had failed to have litters. It must be concluded that they had failed to conceive. In the rats which had been given flour treated with chlorine dioxide evidence of a resorption gestation was found in the animal which had increased temporarily in weight, but not in the remaining animals. Statistical treatment is obviously precluded by the small numbers of animals and by the failures in conception, but the findings are at least consistent with the presence of vitamin E in the untreated flour, and with its absence after the treatment of the flour with chlorine dioxide.

*Colour of the uterus.*—Prolonged avitaminosis E causes brown discoloration of the uterus even in virgin rats.<sup>9</sup> This abnormality, moreover, does not prevent the successful production of litters, provided that the subsequent intake of vitamin E is adequate. As a guide to the adequacy of the past diet in vitamin E, therefore, the colour of the uterus provides a criterion which can be considered without reference to the question of reproduction.

In the present investigation the uteri of all the six females that had received untreated flour were found at autopsy to be normally coloured. The uteri of the rats which had received the flour treated with chlorine dioxide were decidedly brown. This difference was statistically significant, with  $P \ll 0.01$ .

*Kidney degeneration.*—Degeneration of the kidneys in rats deficient in vitamin E was described by Martin & Moore<sup>9</sup> and was recently shown by Emmel<sup>10</sup> to be made manifest by *post mortem* autolysis. Thus the kidneys of normal rats remain virtually intact if left in the body for 3 hours after death, whereas those in deficient animals show a degeneration, caused by autolysis in the convoluted tubules.

Kidneys taken from 5 female rats receiving the flour treated with chlorine dioxide, and from 4 which had received untreated flour, were fixed in formol saline exactly 3 hours after death. Paraffin sections were cut and were stained with haematoxylin and eosin. In all those rats which had received untreated flour the kidney cortex remained intact, but tubular degeneration was observed in all the animals which had received flour treated with chlorine dioxide. Typical sections are shown in Figs. 4 and 5.

*Control groups.*—Throughout the investigation additional groups were included in which the diets containing flour were supplemented with adequate doses of tocopherol (2 mg. of DL- $\alpha$ -tocopherol acetate per rat weekly). In other groups the flour was replaced by casein and sugar, in amounts which were calculated to provide about the same amounts of protein and carbohydrates as were present in the replaced flour. The main intention in setting up these groups was to provide against any unexpected findings in the two main groups given flour without added tocopherol. Since there were no surprise findings in these groups, apart from the eventual return of pigmentation to the teeth in the group given treated flour, it seems unnecessary to present the results for the additional groups in detail.

Two points, however, do seem worthy of mention. Firstly the administration of tocopherol to the rats given the flour which had been treated with chlorine dioxide completely prevented signs of avitaminosis E. Thus the dosed animals were normal in regard to the haemolysis tests, the condition of their testes or uterus, and of their teeth during the first 5 months of the experiment. Secondly the testes and uteri of rats which received the diet containing extra sugar and casein, but without supplements of tocopherol, were closely similar in size and colour, respectively, to those of the animals which had been deprived of vitamin E and given the diet containing flour treated with chlorine dioxide. The abnormalities caused by the treated flour were, therefore, due to deficiency of vitamin E, as might have been expected, and not to any unsuspected toxic effect caused by the chlorine dioxide in some other way.

#### *Subsidiary experiments on bread made from flour treated with chlorine dioxide*

The destruction of vitamin E in flour treated by chlorine dioxide would lose its main practical interest if it could be shown that the vitamin is lost, in any case, during baking. The following preliminary experiments, therefore, were made.



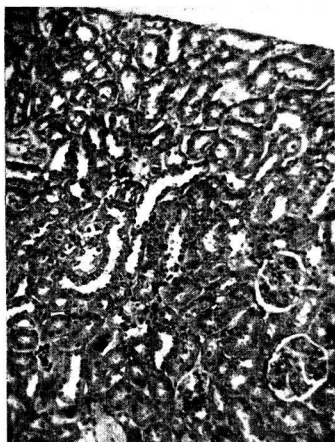


FIG. 4.—Kidney cortex from a rat which had received a diet containing untreated flour. The tissues were fixed 3 hours after death. The convoluted tubules have remained virtually intact



FIG. 5.—Kidney cortex from a rat which had received a diet containing flour which had been treated with chlorine dioxide. Tissues fixed 3 hours after death. Many of the convoluted tubules have lost their linings. The distended lumen within the denuded supporting membrane is filled with cellular debris

*Chemical assay.*—Untreated flour and a freshly baked loaf made from the same flour were examined for tocopherol content by the method described above, except that in the case of the bread extraction with ethanol for 3 h. in a Soxhlet was substituted for the percolation with light petroleum. In order to avoid complications which could arise from the different moisture contents of flour and bread the results in Table IV have been calculated on a basis of dry weights.

Table IV

The tocopherol contents of untreated flour and bread baked from it (mg./100 g. dry weight)

Tocopherol	Flour		Bread		% Loss	
	Tocopherols	Total vitamin-E activity	Tocopherols	Total vitamin-E activity	Tocopherols	Vitamin-E activity
$\alpha$	0.41	0.41	0.20	0.20	51	
$\beta$	0.29	0.09	0.18	0.05	38	
$\zeta$	0.09	0.08	0.05	0.04	44	
$\epsilon$	1.03	0.10	0.73	0.07	29	
Total	1.82	0.68	1.16	0.36	36	47

It will be seen that the baking process caused a loss of about 47% of the total biological activity of the original flour. This finding is in substantial agreement with the destruction of 40% of the vitamin during baking which has been reported by Moran *et al.*<sup>3, 4</sup> For the fresh bread, which contained 39.3% of moisture, the total vitamin-E equivalent may be calculated as 0.23 mg. per 100 g.

*Biological test.*—From the previous findings on the vitamin-E requirements of the rat it would be expected that a weekly intake of 100 g. of fresh bread would supply the minimum requirements. To test this conclusion bread was baked three times weekly at a small commercial bakery in Cambridge, from surplus supplies of flour that were left over from the main experiments.

Before baking, chalk was mixed into the flour at the rate of 0.31%, which is the proportion added to the national flour. The usual amount of yeast was included in making the dough. For the feeding trials the bread was given to male albino rats, weighing 79–121 g., and was supplemented only by water and one drop of halibut-liver oil per animal weekly.

In spite of their sparse diet the animals remained well at least during the first 70 days of the experiment. Growth over this period was relatively rapid, at the rate of 2–3 g. daily. Tests of the vitamin-E status were made by the haemolysis test on 6 animals in each group after the bread had been given for 59 or 64 days, with the results shown in Table V.

Table V

*Percentages of haemolysis in the dialuric acid test of erythrocytes taken from rats fed upon bread baked from untreated flour, or from flour treated with ClO<sub>2</sub>*

Rat No. in group	Untreated flour, % haemolysis	ClO <sub>2</sub> -treated flour, % haemolysis
1	3.5	86.5
2	9.1	84.9
3	4.8	92.5
4	9.7	71.4
5	0.7	87.1
6	0.4	93.1
Average	4.7	85.9

The difference between the groups given treated and untreated flours was statistically significant, with  $P \ll 0.01$ . According to the haemolysis test, therefore, enough vitamin E had survived in the bread to make it an adequate source of vitamin E, at least when it was the only major component of the diet. As expected the bread made from flour which had been treated with chlorine dioxide was inadequate as a source of vitamin E.

*Subsidiary experiments on bread made with the use of different methods of improvement*

In the course of a general investigation on the effect of flour improvers on the nutritive value of bread, in which one of us (T. M.) has collaborated with Dr. L. J. Harris, the opportunity occurred to examine the tocopherol contents of specimens of bread which had been made with the use of different methods of improvement. The agents or procedures used included chlorine dioxide (30 p.p.m.), potassium bromate (20 p.p.m.), ascorbic acid (20 p.p.m.) and the aeration or 'batter' process (for 7 minutes). The results, obtained by the usual chemical method, are given in Table VI.

Table VI

*Influence of the method of improvement of flour on the tocopherol contents of bread*  
(Values are the average for two extractions with two chromatograms on each, and are expressed as mg./100 g. of undried loaf)

Tocopherol	Untreated	Chlorine dioxide	Aeration	Potassium bromate	Ascorbic acid
$\alpha$	0.18	0.02	0.06	0.22	0.21
$\beta + \zeta$	0.21	0.02	0.08	0.19	0.18
$\epsilon$	0.60	0.07	0.35	0.49	0.58
Total	1.09	0.11	0.49	0.90	0.97
Total vitamin-E activity	0.33	0.04	0.12	0.35	0.34

Vitamin-E activity:  $\alpha = 100$ ;  $\beta + \zeta = 40$ ;  $\epsilon = 10$

It will be seen that bread from flour containing bromate and ascorbic acid, which are believed to be active as improvers when dissolved at dough making though they may be added during milling, contained as much vitamin E as bread made from untreated flour. A considerable part of the vitamin is shown to be lost in the aeration procedure, and again almost complete destruction followed the use of chlorine dioxide.

### Discussion

Flour is subjected to treatment with improvers for the purpose of making it more suitable for human food. The practical importance of the demonstration of the destruction of vitamin E by chlorine dioxide must, therefore, depend in the first place on whether vitamin E is essential for the health of the human subject. It seems most improbable that the human can dispense with a vitamin which has been proved necessary for the well-being of many other species. Secondly it may be important whether the vitamin E which could be contributed by flour would make up a large or a small fraction of our total intake of the vitamin from all foods. If an intake of 250 g. of flour per day is assumed, the total tocopherol intake from this source, in untreated form, would be about 4 mg., equivalent to about 1.5 mg. of vitamin-E activity without losses due to baking. Knowledge of the vitamin intakes in the human is scanty, but Hickman<sup>11</sup> gave the average intake of  $\alpha$ -tocopherol intake from all sources by American adults as 15 mg. daily. On this slender evidence, flour which has not been subjected to destructive improvement could make a significant, but not a predominant, contribution of vitamin E to our diet.

### Conclusions

The results of this investigation confirm the finding of previous workers that chlorine dioxide, at the concentration usually employed in commercial practice, is destructive of the vitamin E in flour. According to chemical tests, in which the various tocopherols have been separated by paper chromatography, the loss of the vitamin is virtually complete.

The results of biological tests, carried out by five different methods, are all concordant in indicating that untreated flour can supply adequate amounts of vitamin E to rats. By the same criteria, flour which has been treated with chlorine dioxide is quite inactive as a source of the vitamin.

The process of baking involves a loss of some 47% of the vitamin E of untreated flour, but enough vitamin survives in the bread to make it an adequate source of the vitamin for rats.

Preliminary chemical studies on bread made by different procedures for improvement have shown decided differences in the destruction of vitamin E. Bread made from dough treated with potassium bromate and ascorbic acid contained as much vitamin E as bread made from untreated flour. The 'aeration' process caused a considerable disappearance of the vitamin E, but this process was much less destructive than chlorine dioxide treatment.

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# JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE ABSTRACTS

FEBRUARY, 1957

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

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# JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

## ABSTRACTS

FEBRUARY, 1957

### I.—AGRICULTURE AND HORTICULTURE

#### General: Soils and Fertilizers

**Report on definitions (of soil terms) approved by the Committee on Terminology, Soil Science Society of America.** D. G. Aldrich (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 430—440).—The list of definitions is presented. A. H. CORNFIELD.

**Future applications of atomic energy to horticulture.** H. J. M. Bowen (*J. roy. hort. Soc.*, 1956, 81, 303—308).—The uses are reviewed of radioactive tracer techniques for such purposes as studying the behaviour of fertilizers in plants and soils, and of nuclear radiation for inducing mutation in plant breeding and for insecticidal and bactericidal purposes. J. S. C.

**Developments in the classification of the Sassafras soil series.** W. H. Lyford and G. A. Quakenbush (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 397—399).—Reasons for the separation of 30 new series from the original Sassafras series are presented. Sassafras soils are now classified as Grey-Brown Podzolic soils, but perhaps should be considered as intergrades towards Red-Yellow Podzolic soils. A. H. CORNFIELD.

**Nature of "chalk heath" soils.** R. M. S. Ferrin (*Nature, Lond.*, 1956, 178, 31—32).—A detailed examination of the sand and clay fractions of "chalk heath" soils suggests that they originate from deposition of wind-blown material on flinty pavements produced by local weathering of chalk. J. S. C.

**Sectioning of soil.** S. Hepple and A. Burges (*Nature, Lond.*, 1956, 177, 1186).—A technique for preparing thin soil sections for examination, using a polymeric resin impregnant, is described. It was primarily developed for the study of fungal hyphae in soil but can also be used to examine other organisms. J. S. C.

**Pore space in high- and low-moor peats.** H. Segeberg (*Z. PflErnähr. Düng.*, 1956, 74, 10—17).—Low-moor peats were divided into those with mineral contents above and below 30%. In the former the relationship between mineral content and pore space can be expressed by an exponential function, whilst the latter form a normal Gauss distribution, average 93.1. High-moor peats were divided into those containing above and below 5% of ash. A division of the latter group falls naturally into young and old moss peats, pore space averaging 95.1 and 93.3, respectively. In high-moor peats containing above 5% ash pore space and mineral content were unrelated. M. LONG.

**Plummet balance for measuring the size distribution of soil particles.** T. G. Marshall (*Aust. J. appl. Sci.*, 1956, 7, 142—147).—The  $d$  of soil suspensions (2—5%, particles  $\leq 20\mu$ , diameter) can be measured rapidly with the light-wt. plummet balance described. The only adjustment is for depth of immersion of the plummet. The % of soil in suspension at a given depth and time is recorded on a scale. Calibration procedure, including temp. correction, is described, and factors influencing the measurements are discussed. Accuracy is within  $\sim 0.5\%$ . W. J. BAKER.

**Predicting moisture in the surface foot of soil.** C. A. Carlson, K. G. Reinhart and J. S. Horton (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 412—415).—The method is based on a knowledge of known or estimated starting soil moisture contents, pptn. by calendar days, field max. moisture content, accretion and depletion relations, transition dates, and min. storm size. A. H. CORNFIELD.

**Tillage for soil and water conservation. I. Comparison of crop yields for contour versus up-and-down-slope tillage.** G. R. Free (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 427—429).—Yields of crops in all, with the exception of one, of the 13 years studied were higher on contour than on up-and-down-slope ploughed plots. The differences due to type of tillage tended to increase with years. A. H. CORNFIELD.

**Seed- and plant-soil relations as affected by seedbed firmness on a sandy loam range-land soil.** D. N. Hyder and F. A. Sneva (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 416—419).—The moisture content of the upper 1 in. of soil 16—28 days after sowing was highest where heavy rolling followed by harrowing, or where strip rolling was practised to cover the seed after broadcasting, and was relatively

low where harrowing or light rolling only was practised. Emergence of crested wheat grass and vigour of seedlings were higher with the heavy rolling-harrowing treatment than with any other treatment. A. H. CORNFIELD.

**Component and composite flux of matter through natural systems.** T. C. Broyer (*Plant & Soil*, 1956, 7, 377—388).—The movement of liquids through the soils and from soil to plant is considered mathematically. A. H. CORNFIELD.

**Sodium hyposulphite-soluble iron oxide and water retention by soils.** M. A. El Ashkar, G. B. Bodman and D. B. Peters (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 352—356).—Water retention at both 0.33 atm. and 15 atm. tension for a no. of soils increased with the  $\text{Na}_2\text{S}_2\text{O}_4$ -extractable  $\text{Fe}_2\text{O}_3$  (I) of the soils. The amount retained at 15 atm. tension increased more rapidly with I content than did that retained at 0.33 atm. The water retained between 0.33 and 15 atm. tensions increased as I was progressively removed. A. H. CORNFIELD.

**Moisture flow induced by thermal gradients within unsaturated soils.** W. L. Hutcheon (*Dissert. Abstr.*, 1956, 16, 612).—Qual. and quant. data are presented on the magnitude of thermally-induced moisture flow in unsaturated soils, with particular reference to moisture for plant growth. A multiple unit thermal apparatus is described for maintaining linear gradients of temp. across closed columns of soil, 30 cm. long, over a wide range of hot-cold conditions. Results are given and discussed. O. M. WHITTON.

**Sprinkling irrigation.** M. Medici (*Ric. sci.*, 1956, 26, 1678—1695).—Recent advances are described and the advantages of the system indicated. C. A. FINCH.

**Effect of soil conditioner-fertilizer interactions on biochemical and physical properties of some Ohio soils.** Milton B. Jones (*Dissert. Abstr.*, 1956, 16, 618—619).—A pouring method of adding conditioners to soils was developed. The aggregate stabilizing power of hydrolysed polyacrylonitrile on soil was reduced by  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{Mg}^{++}$  and  $\text{Ca}^{++}$  and increased by  $\text{OH}^-$ ,  $\text{PO}_4^{--}$ ,  $\text{K}^+$  or  $\text{Na}^+$  ions.  $\text{SO}_4^{--}$ ,  $\text{H}^+$  and  $\text{NH}_4^+$  ions were intermediate in effect. The influence of these fertilizers on the effectiveness of modified vinyl acetate-maleic acid and isobutyl-maleic acid was similar but not so marked. Conditioner treatments reduced K fixation, increased N fractions extracted, did not affect the amount of available P, increased plant yields and N contents, and reduced soil crusting. Conditioner treatment combined with K treatment produced higher K content than either treatment alone. O. M. WHITTON.

**Effect of a synthetic soil conditioner on intake, run-off and erosion.** F. L. Duley (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 420—422).—Run-off and erosion from a silty clay loam with 8.5% slope decreased with the amount of hydrolysed polyacrylonitrile applied (1000—4000 lb. per acre hoed to 3 in. depth). Differences due to treatments were still fairly high even during the second year after treatment. Water-stable aggregation in the 0—3-in. layer was improved. A straw mulch (2.5 tons per acre) was much more effective in reducing run-off and erosion in all three years than were any of the conditioner treatments. A. H. CORNFIELD.

**Distribution of important soil-forming minerals in Pennsylvania soils.** C. D. Jeffries, E. Grissinger and L. Johnson (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 400—403).—The mineral composition of 650 surface soils was correlated with the agricultural value of the soils. The most valuable agricultural soils are those in which the feldspar of the very fine sand fraction is  $>17\%$  and the predominant clay mineral is a poor fixer of K. A. H. CORNFIELD.

**Influence of sun heat on clays.** E. M. Winkler (*Soil Sci.*, 1956, 82, 193—200).—The physical properties of six clays were examined after heating to temp. up to  $200^\circ$ . In all cases the compressive strength increased with temp. but at different rates. The rate was highest between  $25^\circ$  and  $50^\circ$ , all clays responding similarly. Above  $100^\circ$  attapulgite and illite decreased in strength. Water resistance increased with temp. to a max. at  $100^\circ$ . T. G. MORRIS.

**Adsorption of sugars by montmorillonite. I. X-Ray studies. II. Chemical studies.** D. J. Greenland (*J. Soil Sci.*, 1956, 7, 319—328, 329—334).—I. X-Ray diffraction studies showed that montmorillonite formed complexes with a wide variety of sugars, each sugar forming complexes with one or two layers of mol. in each inter-lamellar region. For the single-layer complexes there was close

agreement between the interlamellar separation and the min. mol. thickness of the sugar mol. The extent of adsorption for a given sugar varied with the exchangeable cation adsorbed on the clay and increased in the order K, Ca, Mg, H, Na. In general, methylated glucoses were much more strongly adsorbed than were unsubstituted sugars, disaccharides more than monosaccharides and unsubstituted sugars more than COOH- or NH<sub>2</sub>-substituted sugars.

II. Glucosamine (I) was adsorbed more strongly than were gluconic acid (II) or gluconic acid (III). With increasing pH from 2.5 to 10 adsorption of I and II increased up to pH 4–5, decreased to pH 5.5–6.5 and then increased again. Adsorption of III increased to pH 6 and then decreased. A polysaccharide separated from a soil was adsorbed much more strongly than were glucose or methylated glucose. Soil polysaccharides are probably adsorbed preferentially to other constituents of soil org. matter. A. H. CORNFIELD.

**Influence of clay minerals on the breakdown of certain organic substrates.** D. L. Lynch and L. J. Cotoir, jun. (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 367–370).—The effect of clay minerals on the decomposition (as measured by CO<sub>2</sub> release) of a number of org. materials is reported. Addition of illitic or kaolinitic clay to simple (starch, gelatin) or complex (lucerne meal, oat straw) materials usually had no effect on the course of decomposition whilst montmorillonitic clay generally reduced the rate of decomposition of both simple and complex materials. The activity of the enzymes cellulase and hemicellulase was reduced in the presence of montmorillonite. The action of montmorillonite may be due to reduction of enzyme activity and/or to absorption of the org. material by the clay with subsequent protection from decomposition. A. H. CORNFIELD.

**Effect of dehydration-rehydration on cation-exchange capacity of Hawaiian soils.** Y. Kanehiro and G. D. Sherman (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 341–344).—Dehydration (sun-drying for 100 days or oven-drying at 100° for seven days) resulted in a considerable reduction in the exchange capacity (NH<sub>4</sub>OAc method) of Hawaiian soils. This reduction was greater with soils from wet than with those from drier regions and was greater with sub- than with topsoils. A Hydrol Humic Latosol subsoil showed a reduction in exchange capacity of about 200%. Drying of surface soil through removal of natural vegetation or intensive cultivation also reduced exchange capacity somewhat. At a given site exchange capacity after the rainy season was somewhat higher than after the dry season. Dehydrated soils from dry and moderately wet areas increased significantly in exchange capacity upon rehydration. Soils from wet areas did not show this effect. A. H. CORNFIELD.

**Reclamation of salt- and sodium-affected soils in the Mesilla Valley.** C. W. Chang and H. E. Dregne (*Bull. N. Mex. agric. Exp. Sta.*, 1955, No. 401, 26 pp.).—In greenhouse tests application of CaSO<sub>4</sub>, S, CaCl<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub> or HCl to a saline-Na loam increased barley yields in the year of treatment but not in the year after. Similar materials applied to a saline-Na clay soil gave erratic results. In field trials on the loam both CaSO<sub>4</sub> and S effectively reduced sol. salts and exchangeable Na, although the former material acted more quickly. Reduction in exchangeable Na proceeded as fast as, or faster than, did the reduction in sol. salt content. Yields of cotton increased with decreasing exchangeable Na or salt content of the soils. A. H. CORNFIELD.

**Reclamation of a saline-alkali soil in the upper Colorado River basin.** M. Amemiya, C. W. Robinson and E. W. Cowley (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 423–426).—The salt content (conductivity of saturation extract) and % Na saturation of a saline-alkali soil to 40 in. decreased to a greater extent where six than where 2 cu. ft. per acre of water was applied. Application of 4 tons of gypsum per acre in combination with the water treatments did not further reduce the salt content or the % Na saturation. Yields of lucerne hay in the year following treatment were higher where the heavier doses of water had been applied and were not affected where gypsum had been applied. A. H. CORNFIELD.

**Nitrogen studies on black soils from Darling Downs, Queensland.**  
I. Seasonal variations in moisture and mineral nitrogen fractions.  
II. Nitrifying activity of subsurface horizons. A. E. Martin and J. E. Cox (*Aust. J. agric. Res.*, 1956, 7, 169–183, 184–193).—I. Equilibrium levels of moisture, NO<sub>3</sub>-N and NH<sub>4</sub>-N are reported for two black soils during 1952–54. (13 references.)

II. The nitrifying capacity of the soil decreased markedly with increase in depth. Deeper samples accumulated large quantities of nitrite. The percolation of surface samples with aq. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with the pH increased by alkali additions resulted in marked nitrite accumulation comparable with that found in deeper layers in presence of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> only. (15 references.) R. H. HURST.

**Importance of subsoil phosphorus to agronomic crops.** J. T. Murdock (*Dissert. Abstr.*, 1956, 16, 621).—Subsoil P is important to some agronomic crops and the amount of available P in the lower

soil horizons, as well as that in the plough layer, should be considered when fertilizer recommendations are made. The recovery of P applied to different horizons of several soils was measured by radioactive tracer techniques. O. M. WHITTON.

**Factors affecting yields and uptake of phosphorus by different crops.**  
II. Rock phosphate and superphosphate, separate and in combination, under extended cropping. E. O. McLean (*Soil Sci.*, 1956, 82, 181–192).—Lucerne, oats and buckwheat were grown on soil supplied with rock phosphate, superphosphate and a combination of the two, together with K and N. After harvest the crops were incorporated in the soil and then oats were grown repeatedly and removed. In the initial cropping period, rock phosphate was less effective than superphosphate as a P source. In general the K, P, Mg and Ca contents of the crops were little affected by the treatments. The yield of the first test crop of oats was greatest in the soil treated previously with superphosphate but the effect of the incorporation of the initial crops was small compared with that of the phosphate treatment. In later test crops the superiority of superphosphate was lost and the persistence of rock phosphate was evident. T. G. MORRIS.

**Availability of calcium in calcareous soils.** W. J. Flocker and W. H. Fuller (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 387–391).—Available Ca, as determined by Fried and Dean's A-value method using <sup>45</sup>Ca, of soils containing 0–13% Ca was not related to CaCO<sub>3</sub> content. In some cases plants absorbed more Ca from acid than from calcareous soils. Available Ca was significantly correlated with exchangeable soil Ca, but not with soil Ca extractable with water or 1:1 HCl, or with Drouineau's active Ca (that which reacts with sol. oxalate). Addition of CaCO<sub>3</sub> to non-calcareous soils reduced slightly the uptake of Sr by some, but not by other, crops. A. H. CORNFIELD.

**Effects of slash burning on soils of the Douglas-fir region.** R. F. Tarrant (*Proc. Soil Sci. Soc. Amer.*, 1956, 20, 408–411).—Severe burning of logging slash, resulting in destruction of org. litter, reduced macroscopic pore vol. and percolation rate and increased microscopic pore vol. and bulk density of the soil. Soil pH increased in proportion to the severity of burning. One year after burning the pH was neutral or slightly acid; three years after burning the soils had returned to within the pH range normally found in the Douglas-fir region. Germination of fir seed and subsequent growth of seedlings were similar on burned and unburned soils. A. H. CORNFIELD.

**Ammonium ligninsulphonate as a chelating agent for supplying soluble iron to plants.** A. Wallace and R. T. Ashcroft (*Soil Sci.*, 1956, 82, 233–236).—Beans were grown in an acid (pH 5.8) clay and also in a calcareous loam. Ammonium ligninsulphonate, labelled with <sup>59</sup>Fe, and providing 0–230 lb. of Fe per acre, was added. On neither soil was growth promoted but in both soils the uptake of Fe from the chelate increased with the rate of application. No Fe migrated into leaves which had developed before the applications of the chelate on the acid soil. On the calcareous loam, lignin, EDTA and a polyamine-polyacetate as sources of Fe corrected chlorosis, but considerably larger amounts of lignin were required to give the same effect as the polyamine agents. T. G. MORRIS.

**Determination of very small amounts of zinc in soil, plant and animal matter and fertilizers.** H. Scharrer and H. Munk (*Z. PflErnähr. Düng.*, 1956, 74, 24–42).—The determination of Zn, using indoxin, was examined in pure solution, the error being 1.0% at concn. of Zn, 0.5 µg.—20 µg. in 5 ml. The blue coloration of the Zn-indoxin complex is determined photometrically. Dithion was used to separate Zn from interfering ions. The technique was extended to biological matter after wet ashing with HNO<sub>3</sub> and HClO<sub>4</sub>, the error in this case being 3.0%. In soils the max. error was 3.0%, common soil extractants being employed. Errors of 5.0% were obtained by a polarographic method. M. LONG.

**Application of a method of gas microanalysis to the study of soil air.** H. R. B. Hack (*Soil Sci.*, 1956, 82, 217–231).—Techniques are described for the micro- and macro-sampling of soil gases. The Scholander Evans micro-technique for analysis was used. Glasshouse soil was sampled at random by both techniques. In artificially compacted soil macro-samples gave higher O<sub>2</sub> and lower CO<sub>2</sub> values than did the micro-samples, at both 20 and 60 cm. depth. Better agreement was obtained between the methods in soils not artificially compacted. T. G. MORRIS.

**Influence of liming on the decomposition of organic matter in soils.** H. Kick (*Z. PflErnähr. Düng.*, 1956, 74, 1–10).—The liming of a slightly acid (pH 6) loam with quicklime, chalk and foundry chalk did not affect significantly the C content of the soil in the field. The decomposition of the added org. matter, including peat, straw, farmyard manure and sludge was in general little affected by liming. The C/N ratio increased from 7.2 in the original soil to 9.0–10.7. Three years after applying the org. matter, CO<sub>2</sub> evolution was



highest in the soil treated with straw, liming having little positive effect. M. LONG.

**Factor contributing to the pH of the John Innes composts.** A. C. Bunt (*J. hort. Sci.*, 1956, **31**, 258—271).—Mineralization of org. N, which can reach a max. in six weeks in glasshouse pots, results in wide fluctuations in pH; biological activity, which determines the rates of  $\text{NH}_3$  and  $\text{NO}_3^-$  production, is influenced by temp., moisture, soil reaction and re-inoculation following steam-sterilization. Subsequent pH changes are primarily dependent on the quality of the irrigation water and the quantity and type of supplementary fertilizer. E. G. BRICKELL.

**The carbohydrate component in leaf extracts and in leachates obtained under forest canopy.** K. D. MacLean and W. A. deLong (*Canad. J. agric. Sci.*, 1956, **36**, 267—275).—Rainfall passing through the canopy accumulated a carbohydrate component and the amounts of carbohydrate removed in leachates obtained from leaves appeared to increase when environmental conditions were favourable to microbiological activity. The major part of the carbohydrate component of leaf extracts is of relatively high mol. wt. E. G. BRICKELL.

**Distribution of alpha-radioactivity in native vegetation.** J. T. Curtis and R. Dix (*Bot. Gaz.*, 1956, **117**, 231—238).—The  $\text{A}_\alpha$  horizon of soils in the Appalachian Mountains contained appreciable amounts of material emitting  $\alpha$ -rays, the activity being greatest in coniferous forests on loess-free igneous soils in cool climates. The mor humus of coniferous forests also contained notable concn. of Cu, Ga, Pb and Zr. A. G. POLLARD.

**Formation of humic-acid-like polymers.** J. Procházka (*Chem. Tech., Berl.*, 1956, **8**, 259—260).—The formation of polymers with humic-acid-like properties by interaction of 1:2-di- or 1:2:3-tri-hydroxybenzene in alkaline media in presence of air is considered to proceed via an intermediate product 3-hydroxy-2-ketocyclopentane-3:5-diene-1-carboxylic acid. The polymerization is explained in terms of C-C coupling with intramol. dehydrogenation. Reaction mechanisms are discussed. H. L. WHITEHEAD.

**Effects of crop residue and fumigant applications on the decomposition of an Ohio muck soil.** G. Stotzky, W. P. Martin and J. L. Mortensen (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 392—396).—Decomposition, as measured by loss of  $\text{CO}_2$ , of young rye (C:N22) added to a muck soil was increased by CIPC [isopropyl *N*-(3-chlorophenyl)carbamate] treatment and slightly reduced by other fumigant treatments of the soil. The mineral N content of the soil increased during incubation when rye was present and decreased when straw (C:N73) was present. The mineral N content was significantly increased by chlorobromopropene, ethylene dibromide, and CRAG fungicide 974, and reduced by CIPC. The activity index of lignin was increased by CIPC and the residues treatments. A. H. CORNFIELD.

**Influence of temperature on nitrification in soils.** B. R. Sabej, W. V. Bartholomew, R. Shaw and J. Pesek (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 357—360).—In incubation tests the nitrification rate in three soils was about 50%, 25% and 6%, respectively, as rapid at 20°, 15° and 8° as at 25°. The rate was usually no greater at 30° than at 25°. The rate of nitrification of added  $\text{NH}_4^+$  varied between soils, but the temp.-nitrification rate curves for the three soils were similar and differed only by a constant factor. In field plots treated with  $\text{NH}_4^+$  in autumn, nitrification occurred from Oct. to Dec., although the rate decreased with decreasing soil temp. and was insignificant below 10°. A. H. CORNFIELD.

**Denitrification in soils.** R. D. Hauck and S. W. Melsted (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 361—364).—Incubation studies using  $\text{NO}_3^-$  labelled with  $^{15}\text{N}$  to determine denitrification in soils containing water in excess of field capacity are reported. During 25 days incubation at 30° the amount of  $\text{N}_2$  produced increased with time and was greater in limed (pH 7.2) than in unlimed (pH 6.0) soil. Most of the  $\text{N}_2\text{O}$  produced was evolved early in the incubation in the limed and late in the incubation in the unlimed soil. Most of the denitrified  $\text{NO}_3^-$  appeared as  $\text{N}_2$  from the limed and as  $\text{N}_2\text{O}$  from the unlimed soil. In limed soil 40% and in unlimed soil 60% of the added  $\text{NO}_3^-$  was denitrified; 97—100% of the N could be accounted for. A. H. CORNFIELD.

**Effect of deep horticultural cultivation on soil organisms.** A. Feldmann (*Z. Pflernähr. Düng.*, 1956, **74**, 17—24).—The effect of 60 cm. deep trenching on soil organisms was followed, using a fluorescent microscopical bacterial count. Before trenching, the bacterial counts varied irregularly with depth, as also did pore space and humus content. A few days after trenching, the no. of organisms increased in the surface layers and the depth distribution curve became more smoothly curvilinear with a sharper decrease in no. at the lower depths. M. LONG.

**Incidence in soil of bacteria requiring vitamin  $\text{B}_{12}$  and the terregens factor.** A. G. Lochhead and M. O. Burton (*Soil Sci.*, 1956, **82**, 237—245).—Bacteria for which vitamin  $\text{B}_{12}$  and the terregens factor (TF) were stimulatory or essential were isolated from soil and from rhizospheres under three crops. In all cases the proportion of these bacteria requiring vitamin  $\text{B}_{12}$  was lower in the rhizosphere than elsewhere. Bacteria requiring or stimulated by the TF were less abundant than those responding to vitamin  $\text{B}_{12}$ , but there was a higher relative incidence of these bacteria in the free soils and higher absolute no. in the rhizosphere. Estimations of vitamin  $\text{B}_{12}$  and the TF in the control and in rhizosphere soils showed no significant differences in levels. T. G. MORRIS.

**Production of antibiotics in soil. III. Production of gliotoxin in wheatstraw buried in soil.** J. M. Wright (*Ann. appl. Biol.*, 1956, **44**, 461—466).—Production of gliotoxin when wheatstraw was buried in soil inoculated with *Trichoderma viride* (which produces gliotoxin in culture media) occurred to a fair extent only where acid soils (pH < 5.2) were used or where the straw had been acidified prior to burial. Gliotoxin was not produced in uninoculated soils. A. H. CORNFIELD.

**Persistence and biological effects of surface-active agents in soil.** K. C. Ivarson and D. Pramer (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 371—374).—Addition of surface-active agents, e.g., Tween 80 (non-ionic), Ceepryn (cationic) or Nacconol NRSF (anionic) to a soil (10—100 p.p.m.) had no significant effect on soil respiration. Where larger amounts (up to 10,000 p.p.m.) were added Tween 80 and Ceepryn, after an initial lag, decomposed relatively rapidly, whilst Nacconol was resistant to decomposition. Tween 80 and Ceepryn in amounts exceeding 1000 p.p.m. and Nacconol in amount greater than 100 p.p.m. reduced nitrification. In dosages of 1000—10,000 p.p.m. Tween 80 increased, whilst Nacconol reduced, the no. of fungi, bacteria and actinomycetes in soil. Ceepryn reduced fungal no. but increased those of bacteria and actinomycetes. A. H. CORNFIELD.

**Orchard soil management.** D. W. P. Greenham (*Ann. appl. Biol.*, 1956, **44**, 521—525).—A general review, with particular reference to work at East Malling Research Station. A. H. CORNFIELD.

**Soil fertility investigations at Columbus Experimental Field, 1924—54.** F. W. Smith, F. E. Davidson and V. H. Peterson (*Kansas agric. Exp. Sta.*, 1955, Bull. 372, 23 pp.).—Maize benefited especially from applications of lime in the rotation including lucerne, and also responded better to K than did any other crop in the rotation. Soya-beans gave the lowest response. Flax benefited especially from legumes or farmyard manure in the rotation. Lucerne responded well to P and manure, but the soil was probably deficient in lime for lucerne. A. H. CORNFIELD.

**Assessing the nutrient status of soils and crops.** W. J. Hanna and E. R. Purvis (*New Jersey agric. Exp. Sta.*, 1955, Bull. 780, 15 pp.).—Extractable cations and P are separated from soils by electrodialysis in 0.05N- $\text{H}_2\text{BO}_3$ . Plant material is extracted with 2%  $\text{AcOH}$ . Rapid test techniques for assessing P, K, Na, Mg,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , B, org. matter, salt concn. and pH are described. Interpretation of soil-test data are presented. A. H. CORNFIELD.

**Fertility levels of New Mexico soils.** H. E. Dregne and H. J. Maker (*Bull. N. Mex. agric. Exp. Sta.*, 1955, No. 396, 11 pp.).—The pH and sol. salt, org. matter, and available P, K and Ca contents of several thousand soil samples throughout the State are summarized and discussed in relation to locality, parent material, climate and other soil characteristics. A. H. CORNFIELD.

**Significance of the coequal effect or balanced-baule-pounding ratio of major fertilizer nutrients to relative percentage growth response in crop plants.** H. P. Cooper and E. E. Hall (*Soil Sci.*, 1956, **82**, 201—216).—A discussion. T. G. MORRIS.

**Selection of nitrogenous side-dressing and top-dressing fertilizers.** E. R. Collins (*N. Carolina agric. Exp. Sta.*, 1955, Ext. Circ. 386, 12 pp.).—The use of  $\text{NH}_4\text{NO}_3$ , anhyd.  $\text{NH}_3$ , Calnitro,  $\text{CaCN}_2$ ,  $\text{NaNO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ , urea and conc. solutions containing  $\text{NH}_4^+$  and  $\text{NO}_3^-$  for side-dressing and top-dressing crops is described. Unit values and physiological acidity or basicity of the materials are given. A. H. CORNFIELD.

**Rapid determination of nitrogen and phosphorus pentoxide in ammonium phosphate fertilizer solutions.** D. N. Bernhart and R. S. Bryant (*J. agric. Food Chem.*, 1956, **4**, 688—689).—The method described is based on the fact that the 1:3N:P<sub>2</sub>O<sub>5</sub> ratio ammonium phosphate solution is a mixture of  $(\text{NH}_4)_2\text{HPO}_4$  and  $(\text{NH}_4)\text{H}_2\text{PO}_4$ . The  $(\text{NH}_4)_2\text{HPO}_4$  is titrated to  $(\text{NH}_4)\text{H}_2\text{PO}_4$  with standard acid. This is then converted into  $\text{NaH}_2\text{PO}_4$  by boiling with excess standard alkali and back-titrating with acid. Equations used in determining the N and P<sub>2</sub>O<sub>5</sub> content are given. Values obtained are accurate within 10 parts per 1000. N. M. WALLER.



**Crop response to metaphosphate fertilizers.** G. L. Terman and L. F. Seatz (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 375—378).—In 1092 tests over 18 years  $\text{Ca}(\text{PO}_3)_2$  compared favourably with concentrated superphosphate (applied at equiv. P rate) for maize, small grain, forage, cotton and tobacco crops but was slightly inferior for potato and vegetable crops. Liming acid soils resulted in similar increases in response to both types of P.  $\text{KPO}_3$  was as satisfactory as was concentrated superphosphate for maize and seed cotton and slightly more satisfactory for small grain and hay; it was less effective than ordinary superphosphate for most crops. The availability of P from 10-mesh  $\text{Ca}(\text{PO}_3)_2$  was generally as high as that of finer-ground material.  $\text{Ca}(\text{PO}_3)_2$  was a satisfactory source of P for lucerne and other hays on calcareous soils, but was usually inferior to superphosphate for potatoes, sugar beet and spring-sown small grains. A. H. CORNFIELD.

**Characteristics and fertilizer value of phosphate rock from different fields.** J. H. Caro and W. L. Hill (*J. agric. Food Chem.*, 1956, **4**, 684—687).—The solubility in fertilizer solvents, surface properties, physical properties of particles and chemical compositions are recorded of several phosphate rocks from Tunis, Curaçao, S. Carolina, Florida, Idaho, Tennessee and Virginia. Statistical comparison of these measurements with crop yields shows that the phosphate-bound carbonate content and citric acid solubility are the criteria most useful as measures of P availability. (20 references.) N. M. WALLER.

## Plant Physiology, Nutrition and Biochemistry

**Action of glycerol on leaf transpiration and on cuticular permeability.** J. Sivadjan (*C. R. Acad. Sci., Paris*, 1956, **242**, 2478—2479).—Treatment of the primary leaves of haricot-bean plants with 1% aq. glycerol increases considerably, within 24 hr., the cuticular permeability and, in consequence, the leaf transpiration. This effect also explains the increased absorption (10—30 times) of added antibiotics when the glycerol solution is spread over the leaves. W. J. BAKER.

**Responses of *Regnellidium diphylum* to nutrient supply and photoperiod.** W. W. Bloom and P. D. Voth (*Bot. Gaz.*, 1950, **117**, 173—193).—Optimum growth of *R. diphylum* was associated with high-Ca and -N and low-Ca, -K and -P nutrition. A chelated NaFe complex of ethylenediaminetetra-acetic acid satisfactorily prevented chlorosis. Plants grown with a long photoperiod (13 hr. 54 min.—14 hr. 40 min.) grew more vigorously and produced larger leaves and greater dry matter yields than did those grown under short-day (8 hr.) conditions. In long-day plants nutrient deficiency symptoms usually developed rapidly (7—10 days). A. G. POLLARD.

**Mechanisms of rootstock effect.** A. B. Beakbane (*Ann. appl. Biol.*, 1956, **44**, 517—521).—Certain rootstock effects and some relationships between anatomy and behaviour in rootstocks are considered. Several hypotheses for possible mechanisms of rootstock effect are formulated. A. H. CORNFIELD.

**Spectrophotometric study of modifications of chlorophylls A and B under the influence of various physical and chemical agents.** C. Lamort (*Rev. Ferment.*, 1956, **11**, 84—105).—A review of the literature relating to spectrophotometry of chlorophyll solutions under the influence of H<sup>+</sup> and OH<sup>-</sup> ions, metallic ions, temp., etc. and formation of chlorophyllins, pheophytins, metallic chelates, reactions with amino-acids and org. bases, etc. (86 references.) J. S. C.

**Penetration of seed tuber of potato by phosphate ion from external environment.** Y. Coïc and G. Vandewalle (*C. R. Acad. Sci., Paris*, 1956, **242**, 810—812).—<sup>32</sup>P-labelled  $\text{H}_2\text{PO}_4^-$  was used to follow the absorption of  $\text{PO}_4^{3-}$  by the seed-tuber of potato. The results are tabulated and show the movements and relative concn. of  $\text{PO}_4^{3-}$  in different parts of the plant at different stages of growth. J. S. C.

**Amino-acids of potato tubers, etiolated sprouts and foliage.** D. Le Tourneau (*Bot. Gaz.*, 1956, **117**, 238—242).—In potato tubers, etiolated sprouts and foliage the principal free amino-acids were, aspartic acid, glutamic acid, glutamine, alanine,  $\gamma$ -aminobutyric acid and valine. Asparagine occurred in notable proportions in tubers and sprouts but in only small amounts in foliage. Valine, the leucines, alanine, aspartic acid, glutamic acid, arginine and lysine were present in acid-hydrolysed fractions of tubers, sprouts and foliage; hydroxyproline occurred only in sprout and foliage hydrolysates. A. G. POLLARD.

**Factors influencing expression of the flowering stimulus in *Xanthium*.** I. Translocation and inhibition of the flowering stimulus. R. G. Lincoln, K. A. Raven and K. C. Hamner (*Bot. Gaz.*, 1956, **117**, 193—206).—The flowering response in a branch of *Xanthium* was inversely related to the amount of mature long-day leaf tissue on the branch. The inhibitory effect of long-day leaves disappeared

under carbohydrate-deficiency conditions (shading) and the branch flowered. Interrelationships between photoperiodic response, movement of carbohydrate and flowering response are examined. A. G. POLLARD.

**Comparative physiological study of certain members of the genus *Cellulomonas*.** R. G. Garrison and J. O. Harris (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 364—366).—Six species of *Cellulomonas* differed in ability to grow at 37° and also in lysozyme susceptibility. Proteolytic abilities also varied considerably; *C. rossica* utilized only gelatin, whilst *C. flavigena* digested eight protein substrates. Differences in the utilization of the salts of org. acids were also found. A. H. CORNFIELD.

**Cation-exchange capacities of plant roots as related to their nitrogen content.** E. O. McLean, D. Adams and R. E. Franklin, jun. (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 345—347).—There was a highly significant curvilinear regression ( $R = 0.866$ ) between the cation-exchange capacity of the roots of 20 agronomic crops (grown in gravel culture) and % of N in the roots. Where the N supply was varied both cation-exchange capacity and % of N in the roots increased with the N level. This effect was more pronounced with cereal than with other crops. A. H. CORNFIELD.

**Nitrogen-magnesium relationships in crop plants.** E. G. Mulder (*Plant & Soil*, 1956, **7**, 341—376).—In pot and field tests with wheat and oats in acid soils the plants became increasingly deficient in Mg with increasing rate of application of  $(\text{NH}_4)_2\text{SO}_4$  (I).  $\text{NH}_4\text{NO}_3$  was not quite as detrimental in this respect, whilst  $\text{NaNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$  applications did not induce Mg deficiency. Pot tests using the divided roots technique showed that the beneficial effects of  $\text{NO}_3^-$  on Mg uptake occurred only when both ions were taken up by the same roots.  $\text{NO}_3^-$  increased the uptake of Mg by potato plants. On acid soils of low Mg status application of I reduced uptake of Mg in the year of and in the year after application. The detrimental effect of I was due to leaching out of Mg by rainfall through reduction of soil pH. The direct detrimental effect of I on Mg uptake may be due to a competitive effect of  $\text{NH}_4^+$  and  $\text{H}^+$ ; these ions were present in great excess in root tissue soon after absorption of  $\text{NH}_4^+$ . A. H. CORNFIELD.

**Interaction between nickel and calcium in plants.** A. H. Knight and W. M. Crooke (*Nature, Lond.*, 1956, **178**, 220).—A split-root technique has been applied using the oat and tomato in solution culture and <sup>45</sup>Ca to identify the origin of Ca afterwards found in the plant. Two opposing phenomena may occur simultaneously when Ni is absorbed by plants: (1) the roots are damaged and the absorption and translocation of all major nutrients are reduced; (2) Ni in the leaves increases Ca absorption by the roots. The effects are described and discussed. O. M. WHITTON.

**Effect of the zinc chelate of ethylenediaminetetra-acetic acid on plant uptake of zinc and other heavy metals.** P. C. Butler and R. H. Bray (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 348—351).—Application of Zn ethylenediaminetetra-acetate (EDTA) (5—30 p.p.m. Zn on soil basis) or  $\text{Na}_2\text{EDTA}$  ( $\approx 5—50$  p.p.m. Zn) to a fine sand or silt loam soil had no effects on yields of ryegrass in pot tests. On the fine sand ZnEDTA increased the uptake of Zn and Cu but had no effect on uptake of Mn or Fe.  $\text{Na}_2\text{EDTA}$  increased the uptake of Fe only. On the silt loam neither treatment had any consistent effect on the uptake of any of the micro-nutrients. There was a close correlation between exchangeable Zn and % Zn in the plant in the case of the fine sand. In this soil some of the applied Zn remained exchangeable even after six months, whilst in the silt loam the added Zn was converted fairly rapidly into non-exchangeable form. A. H. CORNFIELD.

**Chlorine as a plant nutrient.** C. M. Johnson (*J. N.Z. Inst. Chem.*, 1956, **20**, 38—42).—Cf. J.S.F.A. Abstr., 1955, i, 340. J. S. C.

**Molybdenum as a plant nutrient. VII. Effects of different molybdenum and nitrogen supplies on yields and composition of tomato plants grown in sand culture.** E. J. Hewitt and C. C. McCready (*J. hort. Sci.*, 1956, **31**, 284—290).—Market King plants were grown in sand culture at deficiency and normal levels of Mo with  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4\text{NO}_2$ ,  $(\text{NH}_4)_2\text{SO}_4$ , urea or glutamic acid as N source. Mo is required for normal growth irrespective of the form in which N is supplied but the requirement is greater in the presence of  $\text{NO}_3^-$  or  $\text{NH}_4\text{NO}_3$ . E. G. BRICKELL.

**New methods of treating trace element deficiencies in plants.** L. W. Holdsworth (*J. roy. hort. Soc.*, 1956, **81**, 318—321).—The uses are reviewed of ion-exchange processes for hydroponic cultivation, of sequestered or chelated compounds for supplying available Fe to plants and of fritted trace elements (made by incorporating the required elements in fritted glass which is ground and incorporated in soil) for fertilizer placement. J. S. C.

**Plastic pails in trace element investigations.** H. Kick (*Z. Pflernähr. Düng.*, 1956, **74**, 60).—Polyethylene pails of 8/10 l.

capacity are proposed as being superior to enamel or glazed earthenware culture pots for use in trace element investigations. To aid aeration and water distribution, lengths of perforated polyethylene tubing were placed in the pails during filling. A cheaper method is the lining of ordinary pots with seamless polyethylene bags.

M. LONG.

**Small polystyrene and galalith beakers in trace element investigations.** W. Bussler (*Z. PflErnähr. Düng.*, 1956, **74**, 61).—Polystyrene beakers pierced at the bottom with a hot needle make excellent containers for investigations requiring a rapid appearance of deficiency symptoms, both in soil and solution culture. Galalith beakers, of similar appearance, are unsatisfactory due to the absorbed formaldehyde being released and killing the plants.

M. LONG.

**Role of auxin in plant flowering. IV. New unidentified naturally occurring indole hormone in normal and gamma-irradiated Maryland Mammoth tobacco.** A. J. Viitros, W. Meudt and R. Beimler (*Contr. Boyce Thompson Inst.*, 1956, **18**, 283—293).—Short-day Maryland Mammoth tobacco flowered under long daylengths when exposed to  $\gamma$ -irradiation for 60 days. An indole component was the predominant auxin in the tobacco. It is stable to treatment with alkali, and labile to acid. It was found in leaf and apical tissues but not in root or stem tissues of both normal and  $\gamma$ -irradiated plants. It is apparently not indol-3-ylacetic acid, its Et ester, or indol-3-ylacetonitrile. It is probably a single compound. R. H. HURST.

**Effect of growth regulators on ripening, split peel, reducing sugars and diastatic activity of bananas.** S. R. Freiberg (*Bot. Gaz.*, 1955, **117**, 113—119).—The ripening of stems of bananas in an improvised and well-ventilated room was hastened by immersion in a solution containing 2:4-dichloro-, 2:4:5-trichloro- or *p*-chloro-phenoxyacetic acid (1000 p.p.m.). The effect was not observed in a standard enclosed ripening room at a controlled temp. Treatment in either type of ripening room reduced the amount of longitudinal splitting of peel. Treated fruit showed greater water retention and higher reducing sugar content and diastatic activity than did controls.

A. G. POLLARD.

**Effects of maleic hydrazide on embryologic development. I. Avena sativa.** L. W. Mericle, A. M. Eunus and R. P. Mericle (*Bot. Gaz.*, 1955, **117**, 142—152).—Histological effects of spraying flowering oat panicles with maleic hydrazide (I) (0.03—0.75%) are described. Low concn. restrict cell division and high concn. may disrupt normal organogenesis. It is unlikely that I can be employed to prevent the sprouting of stored seed without undue loss of germinability.

A. G. POLLARD.

**Floral initiation and fruit-set in lychee, with special reference to the effect of sodium naphthylacetate.** S. Nakata (*Bot. Gaz.*, 1955, **117**, 126—134).—Autumn application of Na naphthylacetate (I) promoted the flowering of lychee only when the rainfall in Oct.—Nov. was heavy and moist conditions prevailed until Feb. No action of I was apparent in dry autumn seasons or on trees which had dropped heavily in the previous year. I restricts vegetative growth and in turn favours flower initiation.

A. G. POLLARD.

**Plant growth-regulating effect of 1:1-difluoroethylene (Genetron 150) applied to plants.** P. W. Zimmerman and A. E. Hitchcock (*Contr. Boyce Thompson Inst.*, 1956, **18**, 295—297).—The effect is similar to that of ethylene gas.

R. H. HURST.

**Preliminary screening tests of amino-acid derivatives of 2-(2':4'-dichlorophenoxy)propionic acid.** C. F. Krewson, T. F. Drake, J. W. Mitchell and W. H. Preston, jun. (*J. agric. Food Chem.*, 1956, **4**, 690—693).—Alanine, aspartic acid, leucine, methionine, phenylalanine and threonine derivatives of 2-(2':4'-dichlorophenoxy)propionic acid, in their D-, L- and DL-forms, are synthesized and screened for effectiveness as plant-growth regulators. The derivatives of DL- and L- amino-acids are generally active plant-growth regulators with high selectivity but those of the D-amino-acids are almost completely lacking in these properties. In general there is lower activity for these compounds than for other series of halogenated phenoxy-acids, but their behaviour varies considerably. The mode of action of growth regulators is discussed. (16 references.)

N. M. WALLER.

## Crops and Cropping

**Effect of high nitrogen applications on the quality of winter wheat varieties.** Z. Primost (*Z. PflErnähr. Düng.*, 1956, **74**, 42—59).—The gluten content of wheat varieties was considerably increased by three applications of N up to 200 kg./ha. (CaNH<sub>4</sub> nitrate), starting in early spring and ending when the ears appeared. Gluten strength was not affected, but the gluten quality index (as a measure of wheat quality) was raised. Three applications of N proved more effective than did one early application. Grain yields reached max., whilst

gluten content appeared to increase almost linearly with increasing N applications.

M. LONG.

**Storage of maize in N. Carolina.** S. H. Usry (*N. Carolina agric. Exp. Sta.*, 1955, Tech. Bull. 114, 44 pp.).—The problems associated with the storage of maize ears and types of storage containers are described.

A. H. CORNFIELD.

**Potato growing in the South.** T. P. Dykstra and W. J. Reid, jun. (*U.S. Dep. Agric.*, 1956, Fmrs Bull. 2098, 52 pp.).—Soil cultivation, fertilizers, crop varieties, seed disinfection, planting, harvesting, washing and storage are described, together with diseases and insects and their control.

E. G. BRICKELL.

**Variation of the retentive or absorptive powers for the phosphate ion of the potato during growth; agricultural consequences.** Y. Coïc and G. Vandewalle (*C. R. Acad. Sci., Paris*, 1956, **242**, 1763—1765).—The effect of P treatment on the growth of the plants and their PO<sub>4</sub>''' contents, in the roots, stems, leaves and new tubers, were measured at intervals of growth. Agricultural implications are discussed.

J. S. C.

**Sugar beet yields and mineral nutrient content as influenced by stands and fertilization.** S. McC. King (*Dissert. Abstr.*, 1956, **16**, 619).—The investigation described showed that (1) sugar beet crops assimilate much N, K and Mg but relatively little P; (2) seed should be coated with fungicide; (3) planting should be early and not more than  $\frac{1}{2}$  in. deep; (4) a yield increase is associated with a population increase, especially when recommended amounts of fertilizer, based on the soil test, are applied; (5) higher plant populations give increased yields when borate at the rate of 8% of the fertilizer or 16 lb./acre is applied.

O. M. WHITTON.

**Protein of pasture plants. Cytoplasmic protein of white clover and Italian ryegrass.** J. W. Lyttleton (*Biochem. J.*, 1956, **64**, 70—80).—The sol. cytoplasmic proteins extracted from leaf cells of white clover and Italian ryegrass are fractionated by high-speed centrifuging, salt pptn., acid pptn. and ethanol pptn. A monodisperse protein fraction isolated from clover leaf having mol. wt. approx. 600,000 resembles proteins extracted from other plants, but it differs from those from spinach and tobacco in not being associated with ribonucleic acid. Leaves of monocotyledonous and dicotyledonous plants contain the same types of sol. cytoplasmic proteins.

J. N. ASHLEY.

**Effect of molybdenum on reclaimed Welsh upland pastures.** J. H. Williams (*Plant & Soil*, 1956, **7**, 327—340).—The effects of lime, P and Mo on the yield and composition of herbage from reclaimed upland pasture on a soil of pH 4.8 and of low P status was studied. Application of Na<sub>2</sub>MoO<sub>4</sub> (1 lb. per acre) increased yields by 9—10% in the first year only. There was no response to Mo when lime was added. When P, but not when lime, was added, Mo increased yields by 15% in the first and 10% in the second year. P increased yields considerably in the first and second years, the response being greater when Mo was also applied. Where P was applied, Mo increased the N content of white clover. Liming increased uptake of both native and added Mo.

A. H. CORNFIELD.

**Permanent pasture compared with a five-year crop-and-cattle rotation for dairy cattle feed.** J. B. Shepherd, R. E. Ely, C. H. Gordon, C. G. Melin, R. E. Wagner and M. A. Hein (*U.S. Dep. Agric.*, 1956, Tech. Bull. 1144, 44 pp.).—Sown orchardgrass-ladino clover pasture, in a five-year rotation with maize for silage and wheat as nurse crop, provided a good means of utilizing the accumulating fertility reserves in old pasture sods. Grazing the wheat crop off or cutting it early for silage or hay reduces costs and labour requirements without decreasing the amount of feed nutrients produced.

E. G. BRICKELL.

**Fertilizer requirements of lucerne hay in Utah.** R. F. Nielsen, J. P. Thorne and G. T. Baird (*Utah agric. Exp. Sta.*, 1955, Bull. 374, 14 pp.).—Yields were usually markedly increased by application of P fertilizers. Method or time of application was usually relatively unimportant; 80 lb. of P<sub>2</sub>O<sub>5</sub> per acre was usually the most profitable rate. The need of lucerne for P or K could be predicted fairly well from plant or soil analysis. No economic yield increases have occurred due to applications of N or K to established stands.

A. H. CORNFIELD.

**Effect of fertilizers and manure on lucerne.** H. F. Murphy and J. Q. Lynd (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 385—387).—Yields of lucerne forage over three years on a very fine sandy loam (pH 6.0) increased with applications of P (32—96 lb. of P<sub>2</sub>O<sub>5</sub> per acre) and increased even further when 72 lb. of K<sub>2</sub>O were applied in addition. Manure (5 tons) gave yields similar to those obtained with the lowest P application. Highest yields were obtained where P, K and B (borax 40 lb.) were applied; Mg (MgSO<sub>4</sub> 480 lb.) in addition to these treatments reduced yields slightly. The B treatment increased water-sol. B content of the soils and these values were correlated with the total B content of lucerne.

A. H. CORNFIELD.

**Soil-moisture relationships in fruit plantations.** J. E. Goode (*Ann. appl. Biol.*, 1956, **44**, 525—530).—The vigour of growth of apple trees increased with the amount of water supplied (water was applied when soil-moisture tension reached 10, 20 or 50 cm. of Hg). The main response was in increased no. of shoots; shoot length and thickness were little affected. The treatments also increased trunk cross-sectional area. Yields of apples from trees receiving water when soil-moisture tension reached 20 or 50 cm. of Hg were 20% higher, whilst those from trees receiving water when soil-moisture tension reached 10 cm. were 10% higher, than were yields from non-irrigated trees. The increases were due more to increases in size of rather than no. of apples.

A. H. CORNFIELD.

**Effects of soil phosphorus on growth and minor element nutrition of citrus.** F. T. Bingham and J. P. Martin (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 382—385).—The effects of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  (0—900 lb. P per acre) on citrus soils (pH 5.3—6.0) of low P status on shoot growth and mineral composition of the leaves of pot-cultured citrus seedlings are reported. Shoot wt. increased with small and decreased with large application of P. Increasing P applications resulted in increasing leaf-P and -Mn and decreasing Cu and Zn (%); leaf-Fe was usually unaffected. Visual Cu deficiencies occurred in the leaves of plants where heavy P dressings were given. Addition of Cu (20 p.p.m.) to the soil increased shoot wt. and leaf-Cu, but leaf-Cu still decreased considerably with increasing P fertilization.

A. H. CORNFIELD.

**Nitrogenous reserves of apple trees.** K. Oland and E. W. Yemm (*Nature, Lond.*, 1956, **178**, 219).—Chromatographic separations of the amino-acids from the tissue extracts showed that arginine and asparagine were the chief reserves and represented 70—80% of total sol. N which could be extracted, the arginine accounting for more than 60%. None of the other amino-acids present exceeded 1% of total sol. N. Extractions were made with 70% ethanol followed by hot water or aq. NaCl buffered at pH 7.

O. M. WHITTON.

**Control of fruit-tree behaviour by the use of rootstocks.** A. P. Preston (*Ann. appl. Biol.*, 1956, **44**, 511—517).—A general account dealing with rootstock/scion behaviour and compatibility and with breeding and selection of new clones.

A. H. CORNFIELD.

**Spring frost damage to vines.** D. E. Angus (*Aust. J. agric. Res.*, 1956, **7**, 163—168).—The critical vine-shoot temp. for frost damage was about 28.5°F. The air temp. at the height of the vines was about 1°F. higher.

R. H. HURST.

**Influence of exchangeable sodium on the yield and chemical composition of plants.** I. Green beans, garden beets, clover and lucerne. L. Bernstein and G. A. Pearson (*Soil Sci.*, 1956, **82**, 247—258).—The crops were grown on two soils, a loam and a clay of different exchange capacities. The soils were treated with vinyl acetate-maleic acid copolymer (VAMA) and then with  $\text{NaHCO}_3$  to give five levels of exchangeable Na. Germination of beans and beet was unaffected by exchangeable Na in the presence of VAMA but that of clover and lucerne was decreased. Fresh wt. yields of beans, clover and lucerne decreased with increasing exchangeable Na either with or without VAMA. Beets showed increased yields at intermediate Na levels with VAMA but at higher Na levels the yields again decreased. With increasing exchangeable Na levels the Na content of all plants increased and the Ca and Mg contents decreased. The effect on the K content of the plant varied according to the species.

T. G. MORRIS.

**Influence of magnesium, potassium and lime on yield and chemical composition of beans.** W. S. Fraser (*Dissert. Abstr.*, 1956, **16**, 611—612).—Bean plants were grown on 16 different types of soil and treated with additions of  $\text{MgSO}_4$ , K and  $\text{CaCO}_3$ .  $\text{MgSO}_4$  significantly increased the Mg content of the plants, but had little effect on dry wt. yield. K had little effect on growth, increased the K uptake and decreased the Ca and Mg contents of the plants. Limestone increased growth and Ca content and markedly reduced the Mn content. Dolomite increased the Mg content of the plants while Ca lime had no effect. High rates of K application led to pale green or chlorotic plants; this condition was improved but not corrected by calcitic lime or  $\text{MgSO}_4$ . Plants treated with dolomite were normal in appearance at all levels of applied K.

O. M. WHITTON.

**Ascorbic acid, carotene, riboflavin and thiamine contents of turnip greens in relation to nitrogen fertilization.** E. V. Miller, T. J. Army and H. F. Krackenberg (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 379—382).—Although the yields of turnip greens on three soils increased with the level of applied N (0—120 lb. of N as  $\text{NaNO}_3$  or 120 lb. of N as  $\text{NH}_4\text{NO}_3$  per acre) the ascorbic acid content of the leaf blade was unaffected. The carotene, riboflavin and thiamine contents of the leaf increased with level of N, carotene being somewhat higher where  $\text{NH}_4\text{NO}_3$  than where an equiv. amount of  $\text{NaNO}_3$  was applied. The treatments involving side-dressings of N each week for four weeks resulted in a fairly constant level of vitamins in the leaf.

A. H. CORNFIELD.

**Effects of cultivation on growth of tobacco.** R. W. Wilson (*N. Carolina agric. Exp. Sta.*, 1955, Tech. Bull. 116, 28 pp.).—Mechanical soil disturbance did not improve tobacco growth. Deep cultivation did not improve yields in comparison with shallow cultivation. High layby was beneficial mainly as added insurance against lodging. Uncontrolled weeds reduced yields more than did any other factor.

A. H. CORNFIELD.

**Starter solutions in flue-cured tobacco production.** T. B. Hutcheson, jun. and W. G. Woltz (*N. Carolina agric. Exp. Sta.*, 1955, Tech. Bull. 112, 20 pp.).—In field and greenhouse tests addition of N, P or K to the transplanting water did not improve subsequent growth of tobacco plants. In some cases growth was reduced by the treatments. Laboratory tests indicated that, of the elements tested, K was the most beneficial to root development.

A. H. CORNFIELD.

**Burley tobacco plant bed studies.** L. Shaw and C. D. Welch (*N. Carolina agric. Exp. Sta.*, 1955, Bull. 395, 26 pp.).—On a clay loam stands of tobacco were usually better following a bare fallow than when following a turned-in crop of soya-beans. Stands were not affected by application of P, K or inorg. N but were reduced by cottonseed meal. Growth of seedlings was better where  $\text{NH}_4\text{NO}_3$  or urea than were  $\text{NaNO}_3$  or cottonseed meal were applied. Growth was improved by application of P. Growth was usually better following the turned-in soya-beans than following a bare fallow.

A. H. CORNFIELD.

**Field irrigation of tobacco.** R. R. Bennett, S. N. Hawks, jun., H. H. Nau, C. J. Nusbaum, M. S. Williams, C. W. Williams and H. M. Ellis (*N. Carolina agric. Exp. Sta.*, 1955, Ext. Circ. 388, 35 pp.).—Some tobacco irrigation principles, black shank disease as related to irrigation, irrigation rental agreement, and water supply and equipment are described.

A. H. CORNFIELD.

**Effect of fertilization on seedling weight and utilization of nitrogen, phosphorus and potassium by loblolly pine in the nursery.** G. L. Switzer and L. E. Nelson (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 404—408).—Growth of and % of N in loblolly pine seedlings on a sandy loam increased with application of N (150—300 lb. per acre). The % of P and K were not affected by N applications. Seedling growth was not affected by application of  $\text{K}_2\text{O}$  or  $\text{P}_2\text{O}_5$  (300 lb. per acre). The amounts of N, P and K removed by the plants over the season were similar to those removed by a crop of maize.

A. H. CORNFIELD.

**Effect of modified atmosphere storage at low temperature and treatments after low temperature storage which affect the keeping quality of cut flowers.** J. H. Tinga (*Dissert. Abstr.*, 1956, **16**, 623).—The effects of increasing the  $\text{CO}_2$  and decreasing the  $\text{O}_2$  contents of the atm. in the store and of pre-harvest and post-storage conditions on the keeping quality of cut flowers, e.g., roses, chrysanthemums, carnations and orchids, were investigated. Ordinary air in a high-humidity container and at 31°F. was most satisfactory for all flowers except orchids which were stored best at 50°F. The gain or loss in fresh wt., the change of colour and degree of opening were found to be reliable criteria for measuring the useful life of cut flowers. Wilting flowers recovered to a turgid condition by using a weak detergent solution for 1 hr. Several solutions, all of which contained sugar and a bactericide, increased the life of flowers.

O. M. WHITTON.

## Pest Control

**Parasite control in agriculture.** J. Jenny (*Mitt. Wein- u. Obstbau, Wien*, 1956, **6A**, 177—182).—A review covering the mechanics of various methods for spraying, with special reference to droplet-size, spraying range and spraying losses.

P. S. ARUP.

**Effects of feeding dieldrin and heptachlor-treated lucerne hay to dairy cows.** J. R. Harris, G. E. Stoddard, G. O. Bateman, J. L. Shupe, D. A. Greenwood, L. E. Harris, T. L. Bahler and F. V. Lieberman (*J. agric. Food Chem.*, 1956, **4**, 694—696).—Feeding trials on eight dairy cows during 112 days are reported. Dieldrin and heptachlor epoxide are present in milk and butter from cows fed with hay treated with 1 and 4 oz. of dieldrin per acre or 4 oz. of heptachlor per acre respectively. There is no detectable effect on the health of the cattle so fed, nor does histological examination reveal any abnormality. (12 references.)

N. M. WALLER.

**Effect of organophosphorus insecticides on the growth and phosphorus content of Brussels sprouts.** G. N. Thorne (*Ann. appl. Biol.*, 1956, **44**, 499—505).—Dry wt. yields of Brussels sprouts plants grown on soils of low- or high-P status were unaffected by spraying 6 times with schradan, demeton or  $\text{Na}_2\text{HPO}_4$  containing an equiv. amount of P. On the low-P soil, spraying with schradan or inorg. P increased the P content of the plant in only one test. On the high-P soil, none of the spraying treatments affected the P content of the plant.

A. H. CORNFIELD.

**Diseases of wheat in Kansas.** S. M. Pady, C. O. Johnson, W. C. Haskett, W. H. Sill, jun., E. D. Hansing, H. Fellows, C. T. Rogerson and J. C. Frazier (*Kansas agric. Exp. Sta.*, 1955, Bull. 368, 24 pp.).—The diseases and their methods of control are described.

A. H. CORNFIELD.

**Pests of farm-stored wheat and their control.** D. A. Wilbur and G. Halazon (*Kansas agric. Exp. Sta.*, 1955, Bull. 371, 28 pp.).—The characteristics of insects that infest stored wheat, the nature of damage, and sources of infection are described. The control of insects, particularly by fumigation, and of rodents is described.

A. H. CORNFIELD.

**Control of loose smut in wheat and barley.** C. C. V. Batts (*Ann. appl. Biol.*, 1956, 44, 437–452).—Following presoaking (4 hr. in cold water—3 hr. draining or 4 hr. at 32–2°), 10-min. soaking in water at 52.7–53.8° for wheat or 51.1–52.2° for barley controlled loose smut. Good control was also given by a 4-hr. dip in water at 45°. Germination was reduced with increasing temp. of treatment. Treated seeds emerged later than did control seeds, but there were no differences in growth after 1–2 weeks. Soaking in water alone or in chloranil (Spergon) suspensions, as recommended by Tyner (*Phytopathology*, 1951, 43, 313) gave poor control of loose smut in wheat and some control in barley, but with serious reduction in germination.

A. H. CORNFIELD.

**Insect pests of maize in Kansas.** Anon. (*Kansas agric. Exp. Sta.*, 1955, Bull. 373, 41–46).—Characteristics of the maize rootworm, autumn armyworm, and southwestern maize borer and methods of control are described.

A. H. CORNFIELD.

**Influence of nitrogen and potassium fertilization on the incidence of stalk rot of corn (maize) in New York.** H. J. Otto (*Dissert. Abstr.*, 1956, 16, 621–622).—The effects of two levels of K- and three levels of N-fertilization on the incidence of stalk rot of maize in New York was investigated. There was a trend toward increased susceptibility with increased N supply, but with K there was a definite decrease.

O. M. WHITTON.

**Influence of organic manuring on the development of the potato root eelworm, *Heterodera rostochiensis*.** P. A. van der Laan (*Nematologica*, 1956, 1, 112–125).—Plants treated with org. materials such as farmyard manure or compost, develop a slight resistance to nematodes. Physiological changes in the plant itself may be the reason.

E. G. BRICKELL.

**Overwintering and epidemiology of *Phytophthora infestans*, and some new possibilities of control.** D. E. van der Zaag (*Tijdsch. Plantenziekt.*, 1956, 62, 89–156).—Overwintering of *P. infestans* as a saprophyte in the soil or as oospores is not of practical significance, nor is there much evidence of its survival as mycelium in diseased tubers. The appearance of an epidemic is determined by the no. of infection sources (on an average one primary focus per sq. km.) and the rapidity with which the infection is spread; sporangia can be carried by wind over a distance of at least 11 km. without losing their viability. Some control is possible by phytosanitary measures such as collection of diseased tubers and field inspection, and by treating the seed potatoes with hot water (1 hr. at 43° or 30 min. at 45°).

E. G. BRICKELL.

**Brown root rot of tomatoes. I. Associated fungal flora.** M. H. Ebben and P. H. Williams (*Ann. appl. Biol.*, 1956, 44, 425–436).—Four species of fungi were frequently isolated from tomato roots, suffering from brown root rot. Five other species were isolated less frequently. *In vitro* inoculation tests with seedlings are described.

A. H. CORNFIELD.

**Control of the fruit tree red spider mite, *Metatetranychus ulmi*, Koch. II. Control by delayed-dormancy petroleum.** A. M. Masse and M. D. Austin (*J. hort. Sci.*, 1956, 31, 239–243).—Both stock emulsion and miscible petroleum washes containing 3 or 5% of oil were effective when applied in mid-March.

E. G. BRICKELL.

**Insecticides and varietal resistance in the control of the squash vine borer, *Melittia cucurbitae*, (Harr.), in S.W. Ontario.** L. A. Miller (*Canad. J. agric. Sci.*, 1956, 36, 309–313).—Dusts of aldrin (2½), dieldrin (1–2½), methoxychlor (3) and aerosol-grade DDT (3%) gave good control; rotenone (1%) was moderately effective. Immune and susceptible cucurbit varieties are listed.

E. G. BRICKELL.

**The pea weevil and methods for its control.** T. A. Brindley, J. C. Chamberlin and R. Schopp (*U.S. Dep. Agric.*, 1956, Frms Bull. 1971, 24 pp.).—*Bruchus pisorum* (L.) is described and control, by insecticide dusts, border trap strips, sanitation and related practices, is reviewed.

E. G. BRICKELL.

**Effect of schradan on growth and photosynthetic pigments of the cotton plant.** J. Hacsakaylo and D. R. Engle (*Bot. Gaz.*, 1955, 117, 120–126).—Addition of schradan (10 and 100 p.p.m.) to the nutrient solution for water-cultured cotton plants stimulated vegetative growth and reduced yields of seed cotton without affect-

ing the properties of seed or fibre. The concn. of chlorophyll and carotenoids in cotton leaves were directly related to the concn. of schradan (0–1000 p.p.m.) in the nutrient.

A. G. POLLARD.

**Control of tobacco blue mould.** F. A. Todd (*N. Carolina agric. Exp. Sta.*, 1944, Tech. Bull. 111, 17 pp.).—Zineb (2 lb. per 100 gal. spray or 6.5% dust), ferbam, 3–100 spray or 11.4% dust, Manzate (0.5–100 spray), Vancide (2–100 spray or 6% dust), and Cadminate (0.625–100 spray) effectively controlled blue mould, due to *Peronospora tabacina*, Adam, on tobacco without injuring the plants. Spergon (2–100 and 4–100 sprays or 6% dust), Orthocide 50-W (1–100 and 2–100 sprays), 2–20 p.p.m. actidione, and 240 p.p.m. Thiolutin were ineffective.

A. H. CORNFIELD.

**Tobacco insects of N. Carolina and their natural enemies.** R. L. Rabb, F. E. Guthrie, H. E. Scott and C. F. Smith (*N. Carolina agric. Exp. Sta.*, 1955, Bull. 394, 32 pp.).—Pests attacking tobacco plant beds, newly-planted tobacco, field tobacco and stored tobacco and their insect and disease enemies are described.

A. H. CORNFIELD.

**Control of coffee berry disease in Kenya.** K. R. Boch and R. W. Rayner (*Nature, Lond.*, 1956, 178, 217–218).—The effect of various fungicides on the control of coffee berry disease, attributed to a form of *Colletotrichum coffeanum*, Noack, was tested. Only Perenox applied at fortnightly intervals, and Verdasan applied at monthly intervals from March to May and from Nov. to Dec. were effective. The latter is the more practical. Repeated calcium sulphamate treatments at 1, 3 and 6% were highly phytotoxic. Preliminary laboratory tests using griseofulvin at a concentration of 880 p.p.m. have given results similar to those with "Verdasan."

O. M. WHITTON.

**Control of violet root rot in Ontario.** N. J. Whitney (*Canad. J. agric. Sci.*, 1956, 36, 276–283).—Control in the greenhouse was given by 4% formaldehyde solution and by pentachloronitrobenzene (20%); in the field the disease was suppressed in decreasing order of effectiveness by methyl bromide, formaldehyde, bleaching powder and thiram (50%).

E. G. BRICKELL.

**Insecticidal activity of some organophosphorus compounds against the migratory locust, *Locusta migratoria migratorioides*, Reiche & Fairm.** A. Stringer (*Ann. appl. Biol.*, 1956, 44, 506–510).—The toxicity of Paraoxon to adult male locusts was similar whether injected or applied topically and was greater than that of the other nitrophenyl P compounds tested. Parathion and its isomers and EPN had approx. equal toxicities, whilst malathion, with relatively low toxicity, was still effective. Methyl, ethyl and isopropyl esters were effective in that order of toxicity. Substitution of other alkoxy- or aryloxy-groups resulted in loss of activity.

A. H. CORNFIELD.

**Qualitative tests for rapid identification of chlorinated hydrocarbons in insecticide formulations.** D. P. Johnson (*J. Ass. off. agric. Chem. Wash.*, 1956, 39, 490–497).—The tests, which are designed primarily for dusts, may also be applied directly to liquid prep. in small vol., but if the % of insecticide in the latter is small, preliminary isolation by partition chromatography may be necessary. Rapid qual. tests for detecting and identifying the following substances are described: chlordane, methoxychlor, toxaphene, dichlorodiphenyldichloroethane (DDD), DDD in presence of methoxychlor, DDT, heptachlor, aldrin, dieldrin, endrin.

A. A. ELDRIDGE.

**Separation and identification of chlorinated organic pesticides by paper chromatography. VII. Aramite, captan, dieldrin, lindane, Spergon and Tritisan.** L. C. Mitchell (*J. Ass. off. agric. Chem. Wash.*, 1956, 39, 484–489).—Aramite, captan, dieldrin, lindane, Spergon and Tritisan can be separated and identified by a paper chromatographic technique. Procedure is detailed. The  $R_F$  values are shown in figures.

A. A. ELDRIDGE.

**Toxicity and breakdown of "hormone" herbicides.** W. W. Fletcher and J. C. Raymond (*Nature, Lond.*, 1956, 178, 151–152).—The effects on the growth and nodulation of white clover (*Trifolium repens*) of 2:4-D, MCPA, 2:4:5-T, 2:4-DB [ $\gamma$ -(2:4-dichlorophenoxy)butyric acid], and MCPB [ $\gamma$ -(2-methyl-4-chlorophenoxy)butyric acid] were examined in (a) aseptic agar culture in test-tubes, and (b) soil culture in pots. The (decreasing) order of toxicity in each case was: (a) 2:4-D, MCPA, 2:4:5-T, MCPB, and 2:4-DB, and (b) 2:4:5-T, MCPA, MCPB, 2:4-D, and 2:4-DB. The difference between these orders is attributed to the relative persistence of the various herbicides in soil, a factor which affects (b) but not the (a). Another factor briefly discussed is the microbial breakdown of the chemicals.

J. S. C.

**Control of weeds in cotton.** J. K. Leaseure (*Tenn. agric. Exp. Sta.*, 1955, Bull. 240, 11 pp.).—Application of CIPC (isopropyl N-(3-chlorophenyl)carbamate; 6–100 lb. per acre) usually gave satisfactory control of weeds in cotton for three or more weeks after planting. Resistant weeds are listed.

A. H. CORNFIELD.



**Controlling bindweed in cotton.** J. W. Whitworth (*Bull. N. Mex. agric. Exp. Sta.*, 1955, 397, 11 pp.).—A one-year stand of Sudangrass combined with cultivation before and after the crop together with application of 2:4-D (0.75 lb. acid-equiv. per acre) gave 95% reduction in stand of bindweed. In cotton planted the following year, four flammings gave adequate control of bindweed. Neither flaming, hoeing, nor application of DNOC alone had any effect on bindweed stands. Application of 2:4:5-trichlorophenoxypropionic acid amine (1 lb. acid-equiv. per acre) gave good control of bindweed, but resulted in moderate-to-severe injury to cotton.

A. H. CORNFIELD.

**Control of mule ear, *Wyethia amplexicaulis*.** D. C. Tingey and C. W. Cook (*Utah agric. Exp. Sta.*, 1955, Bull. 375, 16 pp.).—Satisfactory control of mule ear was obtained by applying sprays (10–160 gal. per acre) of ethyl, butyl or butoxyethanol esters of 2:4-D (<2 lb. of acid-equiv. per acre) in the pre-blossom stage. Yields of desirable forage were greatly increased following eradication of the weed.

A. H. CORNFIELD.

## Animal Husbandry

**Kinetics of microbial activity in the bovine rumen.** R. E. Hungate (*J. agric. Food Chem.*, 1956, 4, 701–703).—Manometric and chemical methods for measuring fermentation rates in *in vitro* incubation tests on rumen contents are described. Microbial activity in the living animal can be estimated from total methane if the ratio of methane to other products is determined in parallel *in vitro* experiments. The rates of fermentation before and after incubation can be used to estimate rumen synthesis. The efficiency of added feed constituents in promoting synthesis are compared. (18 references.)

N. M. WALLER.

**Digestion of cellulose from different sources by rumen organisms.** L. D. Kamstra (*Dissert. Abstr.*, 1956, 16, 645).—The studies on cellulose from different plant sources were made by the *in vitro* rumen technique. The cellulose, holo-cellulose and A.O.A.C. fibre extracted from roughage materials showed higher digestion than the whole plant, and cellulose fractions from mature plants were less digestible than those isolated at earlier stages of development. There appeared to be an inverse relation between lignin content of the whole plant and its digestion. Comparative tests with cattle and the *in vitro* technique suggest that the latter might be used to predict the feeding value of roughages.

O. M. WHITTON.

**Digestion and absorption of various carbohydrates posterior to the rumino-reticular area of the young bovine.** H. J. Larsen, G. E. Stoddard, N. L. Jacobson and R. S. Allen (*J. Anim. Sci.*, 1956, 15, 473–484).—Calves (8–9 months) provided with ruminal and caecal fistulas were used to compare the digestibility of glucose, maltose, starch and maize carbohydrates fed directly into the omaso-abomasal cavity. Reducing sugar levels in the blood demonstrated the ready absorption of glucose and of maltose (after hydrolysis). Very little digestion of starch or maize carbohydrates was apparent.

A. G. POLLARD.

**Effects of curing methods and stage of maturity upon feeding value of roughages.** G. W. Trimmerger, W. K. Kennedy, K. L. Turk, J. K. Loosli, J. T. Reid and S. T. Slack (*Cornell agric. Exp. Sta.*, 1955, Bull. 910, 43 pp.).—The feeding value of a clover-grass forage (a) cut early and ensiled, (b) cut at intermediate stage and barn-dried, (c) cut late and field-cured, (d) cut late and ensiled was compared. When fed with grain to cows dry matter intake decreased in the order, a, b, c, d. Digestibility decreased in the order a, b, d, c. The silages contained much more vitamin A than did the hays, but the vitamin-A content of the milk was only slightly higher where silages than where hays were supplied. Milk production decreased in the order a, b, d, c.

A. H. CORNFIELD.

**Digestibility of range grasses and grass-legume mixtures.** W. E. Watkins (*Bull. N. Mex. agric. Exp. Sta.*, 1955, No. 400, 21 pp.).—Feeding values of four native range grasses, three introduced grasses, and four legume-grass mixtures were determined in 22 metabolism trials with wethers. Total digestible nutrients of grass-legume mixtures ranged from 56% to 61% and of immature grasses (both native and introduced) from 51% to 71%. Weathered forage had much lower total digestible nutrient contents. Grasses containing less than 7% of protein usually did not provide enough protein to maintain wethers. The lignin content of grasses was closely correlated with crude fibre content.

A. H. CORNFIELD.

**Determination of total digestible nutrients in lucerne from its lignin and crude fibre contents.** J. H. Meyer and G. F. Lotgreen (*J. Anim. Sci.*, 1956, 15, 543–549).—Substantially straight-line relationships are established between the total digestible nutrient content of lucerne hays and the lignin, crude fibre and (less closely) N contents.

A. G. POLLARD.

**Effect of valeric and isovaleric acids on straw utilization by steers.** R. E. Hungate and I. A. Dyer (*J. Anim. Sci.*, 1956, 15, 485–488).—Addition of the acids to a steer ration containing a high proportion of wheat straw stimulated the appetite of the animals but did not affect the rate of increase in live wt. or the activity of rumen organisms.

A. G. POLLARD.

**Relationships between level of protein, molasses, trace minerals and quality of hay in rations for fattening cattle.** E. W. Klosterman, O. G. Bentley, A. L. Moxon and L. E. Kunkle (*J. Anim. Sci.*, 1956, 15, 456–463).—A fattening ration for steer calves (ground ear maize, soya-bean oil-meal, poor quality timothy hay) was improved by addition of trace elements or cane molasses. Molasses did not improve a ration already supplemented with trace elements or one including good quality mixed hay. The latter probably contains a beneficial factor other than protein or trace elements. The apparent protein-sparing action of molasses in a ration sub-optimal (but not markedly deficient) in protein results from its action in increasing food consumption and not in improving feed utilization.

A. G. POLLARD.

**Effects of preservatives upon red clover-grass forage ensiled without wilting. II. Feeding values.** L. S. Wittger, G. W. Trimmerger, W. K. Kennedy, K. R. Allred, J. T. Reid, J. K. Loosli and K. L. Turk (*Cornell agric. Exp. Sta.*, 1955, Bull. 913, 30 pp.).—The forage (red clover 75, grasses 25%) was ensiled with molasses, brewers' dried grains, NaHSO<sub>3</sub> or no preservative. There were no significant differences in milk production, roughage consumed and body-wt. changes or in vitamin-A and carotene content of blood plasma or milk fat between cows fed the different types of silages.

A. H. CORNFIELD.

**Feeding value and digestibility of cane molasses nutrients for dairy heifers.** R. F. Davis, G. W. Trimmerger, K. L. Turk and J. K. Loosli (*Cornell agric. Exp. Sta.*, 1955, Bull. 914, 27 pp.).—Total digestible nutrients from molasses were as effective as nutrients from maize (6 lb. molasses replaced 4 lb. maize daily per animal) in promoting growth of yearling heifers when fed in a ration containing ample protein and good quality hay and maize silage. High levels of molasses in the rations of steers depressed the digestibility of protein. Molasses did not provide as efficient a source of energy for the utilization of urea-N as did maize meal.

A. H. CORNFIELD.

**Role of carbohydrates in urea utilization, cellulose digestion and fatty acid formation.** I. J. Belasco (*J. Anim. Sci.*, 1956, 15, 496–508).—In artificial rumen experiments the nature and amount of carbohydrate present influences the extent of utilization of urea by the organisms and also the quantity and distribution of volatile fatty acids produced. Total fatty acid production in presence of individual carbohydrates decreased in the order starch, glucose, cellulose. Compared with cellulose, starch produced relatively more acetic, butyric and valeric acids but less propionic acid. High levels of glucose inhibited the digestion of cellulose and increased the relative proportions of butyric and valeric and lowered that of acetic acid. High starch levels favoured cellulose digestion.

A. G. POLLARD.

**Evaluation of urea and dicyanodiamide for milking cows.** C. L. Davis, C. A. Lassiter, D. M. Seath and J. W. Rust (*J. Anim. Sci.*, 1956, 15, 515–522).—About  $\frac{1}{3}$  of the N of a basal ration containing soya-bean meal as N source was replaced by urea or by dicyanodiamide (I). Differences in milk production were not statistically significant (although I produced an appreciable decrease) and no changes in body wt., feed consumption or digestibility of the ration were apparent. The levels of urea-N in blood and milk of cows receiving a low-protein (9%) ration or one containing I were less than those in animals given the full soya-bean ration (12.7% protein) or that partially substituted with urea.

A. G. POLLARD.

**Dicyandiamide and urea as supplements for dairy cattle.** C. A. Lassiter, D. M. Seath, F. B. Hamblin, B. M. Adams and G. M. Bastin (*Kentucky agric. Exp. Sta.*, 1955, Bull. 638, 7 pp.).—Dicyandiamide and urea, when replacing 33% of the protein in the concentrate mixture of dairy cattle, were equal in value to soya-bean meal for milk production, although the feeds containing the two chemicals were slightly less palatable. There were little differences in the digestibility of the feeds containing the various materials.

A. H. CORNFIELD.

**Effect of plane of nutrition on the mineral composition of blood serum and liver and on the growth of bone.** E. J. Thacker, M. L. Alderman and R. W. Bratton (*J. Anim. Sci.*, 1956, 15, 447–455).—In bull calves a parabolic relationship was established between bone characteristics and body wt. The stage of development of bones was the same in calves of the same body wt. regardless of age or plane of nutrition. The Cu, Mn and Mo concn. in blood and the Fe, Mo and Mn concn. in liver were unaffected by age or plane of nutrition.

A. G. POLLARD.

**Laboratory technique for measuring the phosphorus availability of feed supplements fed to ruminants.** R. Anderson, E. Cheng and

W. Burroughs (*J. Anim. Sci.*, 1956, **15**, 489—495).—An adaptation of the artificial rumen technique is described. A washed suspension of rumen organisms is incubated for 24 hr. in a cellulose-P-free (but otherwise adequate) medium. The P-depleted organisms were again incubated with cellulose and with the P source under examination, the extent of cellulose decomposition being determined by the method of Cheng *et al.* (*J. Dairy Sci.*, 1955, **38**, 1225). A curve relating cellulose digested and P supply using standard  $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$  serves to calibrate the method. Data for various commercial P products are recorded. A. G. POLLARD.

**Trichloroethylene-extracted feeds. IX. Trichloroethylene-extracted soya-bean meal for swine.** L. E. Hanson, W. R. Pritchard, C. E. Rehfeld, V. Perman, J. H. Sautter and M. O. Schuitze (*J. Anim. Sci.*, 1956, **15**, 368—375).—The meal, which caused aplastic anaemia in cattle, was included (10—20%) in a pig ration used throughout the growth and reproductive periods of one generation and for the growth period of the litters up to market wt. No toxic effects were apparent. A. G. POLLARD.

**Effect of unheated soya-bean meal on blood coagulation of chicks.** P. Griminger, W. D. Morrison and H. M. Scott (*Poultry Sci.*, 1956, **35**, 911—915).—Addition of 20% of unheated hexane-extracted soya-bean meal, replacing cerelose in a purified diet, had no effect on the blood-clotting or prothrombin times of chicks or on their response to supplemental vitamin K. A. H. CORNFIELD.

**Effect of water-soluble B-vitamins and energy level of the diet on the performance of laying pullets.** L. R. Berg and G. E. Bearse (*Poultry Sci.*, 1956, **35**, 945—951).—The effects of adding a mixture of the eight water-sol. B-vitamins to diets containing either 1148 or 1331 kg.-cal. of metabolizable energy per lb. on egg production of a meat-type breed and an egg-producing breed were studied. Energy level of the diet did not affect rate of lay, mortality, hatch of fertile eggs, egg wt. or quality of eggs produced by either breed. Feed efficiency with respect to egg production was higher with the high than with the low-energy ration. The additional B-vitamins depressed slightly the hatchability of fertile eggs, increased body-wt. gains and reduced egg wt., but had no effect on egg production, rate of growth of progeny, colour of egg yolks or quality of albumin. A. H. CORNFIELD.

**Synthesis of vitamin B<sub>12</sub> after oral and parenteral administration of inorganic cobalt to cobalt-deficient sheep.** C. J. Kercher and S. E. Smith (*J. Anim. Sci.*, 1956, **15**, 550—558).—The vitamin-B<sub>12</sub> content in the blood of sheep on a Co-deficient diet decreased rapidly but the animals continued to thrive for some time after the vitamin-B<sub>12</sub> level became very low. Oral administration of Co increased the vitamin B<sub>12</sub> in the blood and various organs but parenteral administration was ineffective in this respect. A. G. POLLARD.

**Effect of oral and subcutaneous oestrogen and androgen administration on growth and carcass quality of lambs.** F. N. Andrews, M. Stob, T. W. Perry and W. M. Beeson (*J. Anim. Sci.*, 1956, **15**, 575—588).—Implantation of diethylstilboestrol (12 mg.) or of progesterone (250 mg.) + oestradiol (10 mg.) or the oral administration of dienestrol (4 mg. daily) increased the gain in wt. of lambs but lowered carcass grades. The oestrogen activity of meat from treated animals was not significantly different from the controls, in the case of all treatments except progesterone + oestradiol (slight increase) and dienestrol, which significantly increased the activity of the liver. A. G. POLLARD.

**Urea and stilboestrol for fattening lambs.** M. R. Light, W. E. Dinusson, R. M. Richard and D. W. Bolin (*J. Anim. Sci.*, 1956, **15**, 570—574).—As a protein supplement in a fattening ration soya-bean meal was superior to urea when the latter provided 41% of the N of the ration. Stilboestrol (0.5—2.0 mg. daily) increased live-wt. gains with both N sources without affecting carcass grades or yields or causing any ill effects. A. G. POLLARD.

**Influence of an aureomycin feed supplement on growth and thrift of dairy calves and on ration digestibility.** V. S. Logan, V. J. Miles and G. J. Brisson (*Canad. J. agric. Sci.*, 1956, **36**, 302—308).—Aurofac A was added in part to the milk and in part to the calf starter in a diet consisting of whole milk, calf starter and hay feed to Holstein and Ayrshire calves. The digestibility of the ration was unaffected and there was no influence on the growth, thrift or feed consumption of the calves. E. G. BRICKELL.

**Effect of chlortetracycline feeding on *in vitro* cellulose digestion by rumen micro-organisms.** M. R. Lambert and N. L. Jacobson (*J. Anim. Sci.*, 1956, **15**, 509—514).—Administration of chlortetracycline (240 mg. daily) to cows over long periods (<8 months) caused a somewhat lowered capacity for cellulose digestion in artificial rumen tests. Three weeks after the cessation of feeding the antibiotic, digestive activity was regained. A. G. POLLARD.

**Antibiotic concentration in eggs from hens on chlortetracycline-supplemented diets.** N. Raica, B. W. Heywang and A. R. Kemmerer (*Poultry Sci.*, 1956, **35**, 884—888).—Approx. 1-year-old laying hens were fed chlortetracycline (I) (50—200 g. per ton of feed). No antibiotic was found in the eggs. With the higher levels of I (up to 2000 g.), 0.018—0.141  $\mu\text{g}$ . per g. of egg was found. All eggs were free of chlortetracycline during the first week after removal of the antibiotic from the dams' diet. A. H. CORNFIELD.

**Effect of aureomycin (chlortetracycline) on egg production.** H. D. Branion, D. C. Hill and H. G. Jukes (*Poultry Sci.*, 1956, **35**, 783—789).—Addition of chlortetracycline hydrochloride (10 p.p.m.) to the diet of heavy breed laying chickens had no effect on, whilst addition of 100 p.p.m. significantly increased, egg production over six months. The greatest effect occurred in the first two months. The higher level of antibiotic increased feed efficiency and reduced mortality. A. H. CORNFIELD.

**Effects of chlortetracycline (aureomycin) feeding on swine carcass quality, and method of determining the composition of swine carcasses.** F. C. Wingert (*Dissert. Abstr.*, 1956, **16**, 616—617).—Feeding with chlortetracycline (a) resulted in a highly significant decrease in the length of time required to reach market wt., all of which occurred prior to 125 lb. live wt.; (b) did not significantly influence backfat thickness, carcass length, ratio of fat to lean in a tissue curve or dressing percentage, and had little effect on chemical composition; (c) did not significantly influence levels of thiamine or riboflavin in loin or ham muscle or in meat of boned and skinned half carcasses. The correlations of the various results are discussed. O. M. WHITTON.

**Influence of environment, diet and mode of administration on the response of chicks to antibiotics.** R. E. Moreng, D. W. Bolin, R. L. Bryant and D. G. Gosslee (*N. Dak. agric. Exp. Sta.*, 1955, Bull. 398, 11 pp.).—Oral administration of achromycin, bacitracin or aureomycin increased the wt. gains and feed efficiency of chicks to five weeks of age. When chicks were removed from batteries to pens there was no significant change in response to antibiotics. When bacitracin was implanted subcutaneously at the base of the skull a depression in growth resulted. Oral administration of another antibiotic tended to eliminate this growth depression. Male and female chicks responded similarly to antibiotics. A. H. CORNFIELD.

**Effects of 3-nitro-4-hydroxyphenylarsonic acid and antibiotics in broiler rations.** J. W. West (*Poultry Sci.*, 1956, **35**, 835—842).—Addition of 3-nitro-4-hydroxyphenylarsonic acid (I) (45 g. per ton of feed) to the diet of broilers to nine weeks of age resulted in 8% increase in growth rate and feed efficiency when no antibiotic was supplied, a 6% increase when low levels of antibiotic (e.g. Terramycin 10 p.p.m.) was supplied in the feed, and a 2% increase when high levels (e.g. 100 p.p.m. Terramycin) was supplied. Mortality, uniformity of body wt., and extent of yellow pigment deposition were unaffected by addition of I to the diet. A. H. CORNFIELD.

**Antibiotics for the prevention of bloat in cattle grazing ladino clover.** B. F. Barrentine, C. B. Shawver and L. W. Williams (*J. Anim. Sci.*, 1956, **15**, 440—446).—In steers grazing ladino clover oral administration of penicillin (300 mg. in a single dose) prevented bloat. Proportionate doses for younger animals were determined. In equivalent amounts K penicillin and procaine penicillin were equally effective. A. G. POLLARD.

**Effect of antibacterial agents on the growth of suckling pigs.** L. E. Hanson and E. F. Ferrin (*J. Anim. Sci.*, 1956, **15**, 376—391).—The growth of pigs (3—8 weeks) was increased by addition of oxytetracycline (5 mg.), procaine penicillin (5 mg.), chlortetracycline (5 mg.) or arsenic acid (30 mg. per lb. of feed) to the ration. The amount of food consumed per unit gain in wt. was lowered. Bacitracin, given orally or by implantation, produced no significant effect. A. G. POLLARD.

**Effect of texture on the nutritive value of feeding-stuff for dairy cattle.** N. F. Colovos, H. A. Keener, H. A. Davies, K. S. Morrow and K. S. Gibson (*New Hampshire agric. Exp. Sta.*, 1955, Bull. 419, 11 pp.).—Fine-textured concentrates were superior in digestible protein and total digestible nutrients to comparable coarse-textured or pelleted materials. Ground maize had a higher nutritive value than had flaked maize, whilst crimped oats were superior to ground oats. There were no significant differences in grain or roughage consumption or in milk or fat production between cows receiving fine or coarse concentrate mixtures. A. H. CORNFIELD.

**Buffalo milk and milk products.** N. N. Dastur (*Dairy Sci. Abstr.*, Rev. Art. No. 55, 1956, **18**, 970—1008).—The production composition and physicochemical characteristics of buffalo milk and its products are reviewed. (About 200 references.) A. G. POLLARD.

**Sulphur requirement of growing-fattening lambs in terms of methionine, sodium sulphate and elemental sulphur.** W. W. Albert,

U. S. Garrigus, R. M. Forbes and H. W. Norton (*J. Anim. Sci.*, 1956, **15**, 559—569).—A purified diet containing urea (4%) and supplemented with S as the element, Na<sub>2</sub>SO<sub>4</sub> or methionine was fed to fattening lambs. The % of S in the ration calculated to give the highest daily increase in wt. were 0.47, 1.27 and 0.64 for the respective S sources.  
A. G. POLLARD.

**Artificial insemination of Merino sheep following synchronization of oestrus and ovulation by progesterone injected alone and with pregnant mare serum gonadotrophin.** T. J. Robinson (*Aust. J. agric. Res.*, 1956, **7**, 194—210).—Progesterone, particularly when used in conjunction with pregnant mare serum, is of value in synchronizing oestrus in the breeding season for precisely-planned artificial insemination. (20 references.)  
R. H. HURST.

**Lucerne cube mixtures for fattening lambs.** P. E. Neale (*Bull. N. Mex. agric. Exp. Sta.*, 1955, 398, 14 pp.).—The effects of cubed mixtures containing molasses 10, coarse lucerne hay 50—70, and sorghum grain 20—40% on fattening of lambs were studied. The 70—20—10 cubes gave better wt. gains and feed efficiency than did the 60—30—10 or 50—40—10 cubes.  
A. H. CORNFIELD.

**Protein levels for pigs as studied by nitrogen balance.** J. W. Lassiter, S. W. Terrill, D. E. Becker and H. W. Norton (*J. Anim. Sci.*, 1956, **15**, 392—399).—Retention of N by 50-lb. pigs increased with the protein level of the diet up to approx. 18%. Protein levels producing max. growth may be lower than those causing max. retention of N. In N-balance trials, after a preliminary period of 10 days no advantage was apparent in collecting excreta for >3 days, especially for large (150 lb.) animals.  
A. G. POLLARD.

**Effect of added soluble carbohydrate upon the digestibility of protein and fibre in rations for swine.** C. N. Skipitaris (*Dissert. Abstr.*, 1956, **16**, 616).—High sugar feeding, by altering intestinal bacterial growth, contributed indirectly to a depression of protein digestibility by depressing the crude fibre digestibility, and also directly by increasing the elimination of bacterial protein.  
O. M. WHITTON.

**Relationship between blood plasma-vitamin A levels of sows and of their suckling pigs.** I. R. Sibbald, J. P. Bowland and R. T. Berg (*J. Anim. Sci.*, 1956, **15**, 400—406).—The relationship established between the plasma-vitamin A of suckling pigs and that of their dams permits the prediction of the former value from the latter. No sex differences between plasma-vitamin A values for piglings were apparent.  
A. G. POLLARD.

**Mineral interrelationships in parakeratosis of swine.** R. W. Luecke, J. A. Hoefler, W. S. Brammel and F. Thorp, jun. (*J. Anim. Sci.*, 1956, **15**, 347—351).—High mineral contents (notably Ca and P) in pig rations predisposed the animals to this form of dermatosis. A ration containing Ca 1.5, P 0.8% and Zn 31 p.p.m. induced 100% incidence of parakeratosis; addition of more Zn 20 p.p.m. prevented the disease.  
A. G. POLLARD.

**Effects of a manganese deficiency on growth, development and reproduction in swine.** M. P. Plumlee, D. M. Thrasher, W. M. Beeson, F. N. Andrews and H. E. Parker (*J. Anim. Sci.*, 1956, **15**, 352—367).—A ration having Mn content 1.0—1.5 p.p.m. produced normal growth in pigs starting at 30—43 lb. live wt. In younger piglings (9.5 lb. live wt.) the ration caused reduced skeletal growth and increased deposition of fat. Prolonged feeding of low-Mn rations (0.5—2.5 p.p.m.) resulted in gradual depletion of Mn from bones and tissues (notably liver).  
A. G. POLLARD.

**Nutritional factors required for growth of the chick.** J. E. Savage (*Dissert. Abstr.*, 1956, **16**, 615).—Nutritional factors required by the chick for rapid growth were determined. In synthetic diets, casein is inferior to soya-bean protein as a source of protein, whilst lactalbumin has low biological value. Crude supplements to synthetic diets which accelerated rate of growth were identified and fractionated to concentrate the active nutrient from soya-bean meal. Supplementation with amino-acids (especially arginine and glycine) of a casein-gelatin diet gave growth responses equal to that produced by supplements of liver residue.  
O. M. WHITTON.

**Influence of feed intake during the growing period on the subsequent reproductive performance of laying hens.** T. T. Milby and D. H. Sherwood (*Poultry Sci.*, 1956, **35**, 863—869).—Restricting the feed of pullets from six weeks of age until the beginning of production to 85% of that consumed by full-fed birds in confinement and 75% of that of full-fed birds on range, significantly reduced growth rate and increased the age to sexual maturity by 10—15 days. The restriction in feeding did not result in any saving in feed cost to time of first egg. The restriction of feeding during the growth period had no effect on subsequent egg production, egg wt., body wt., fertility, hatchability, or laying house mortality.  
A. H. CORNFIELD.

**Farm-raised poultry-feeding supplements.** H. W. Scharpenseel and F. C. Anastasio (*Avaneta. J. Agric.*, 1955, **2**, No. 4, 16—41).—Sun-dried cow or carabao dung when mixed with a poultry ration acted as a growth-promoter probably because of its vitamin-B<sub>12</sub> content rather than its direct action on the digestibility of nutrients. Co exerted a stimulating effect only if added to a ration deficient in animal protein. Administration of Co in drinking water is preferred to that in feeds. Papain (15 mg. per chick, daily) had a better growth-promoting action than did a chicken manure extract. In a comparative growth-promoting trial, leaves of *Tropaeolum maius* (antibiotic), Aurolfac, chicken manure extract + *T. maius* leaves and papain, in decreasing order of efficiency, gave the best results.  
A. G. POLLARD.

**Arginine requirement of chicks fed purified and maize-soya-bean diets.** J. M. Snyder, W. D. Morrison and H. M. Scott (*Poultry Sci.*, 1956, **35**, 852—855).—A purified diet containing casein 18 and gelatin 10% was deficient in arginine. When the gelatin was omitted and the casein increased to 22%, 1.1% supplemental L-arginine HCl (1.73% total arginine) was required for max. growth. A practical maize-soya-bean diet, containing 1.11% of arginine, was not improved by supplementation with gelatin. Neither arginine nor glycine alone or in combination improved the growth-promoting ability of a practical diet.  
A. H. CORNFIELD.

**Effect of vitamin E, dehydrated lucerne meal and condensed fish solubles upon hatchability of eggs from Broad Breasted Bronze hens maintained on litter.** T. M. Ferguson, H. P. Vaught, B. L. Reid and J. R. Couch (*Poultry Sci.*, 1956, **35**, 872—875).—Addition of vitamin E (DL- $\alpha$ -tocopheryl acetate, 0.02 g. per lb. of feed), 5% of dehydrated lucerne meal, and 5% of condensed fish solubles in combination to an all-vegetable diet increased the hatchability of turkey eggs by 21%. Addition of any supplement alone or any two in combination increased hatchability by 4—12%. The tocopherol content of egg yolks was increased when vitamin E was added to the dam's diet, but was unaffected by the two other supplements.  
A. H. CORNFIELD.

**Feather meal and poultry meat scrap in chick starter rations.** E. C. Naber and C. L. Morgan (*Poultry Sci.*, 1956, **35**, 888—895).—Feather meal and poultry meat scraps replaced 25% of the protein in a maize-soya-bean meal ration without any adverse effects on chick growth to nine weeks of age. Feather meal to provide 5% of protein in the diet could replace 2% of fish meal and 2% of dried whey products. Feather meal and poultry meat scraps supplied to give 5% of protein in the diet also supplied sufficient vitamin B<sub>12</sub> for optimum growth.  
A. H. CORNFIELD.

**Response of chicks to fish meal in relation to the composition of the basal diet.** H. M. Scott, B. C. Johnson and M. W. Moeller (*Poultry Sci.*, 1956, **35**, 924—925).—The growth of male chicks on a purified diet was very similar whether sucrose or glucose was used as the carbohydrate source. The growth response due to the addition of 10% of fish meal to the diet was similar with both carbohydrates. Addition of 0.03% of iodinated casein failed to accentuate the chick's requirement for the unidentified factor in fish meal.  
A. H. CORNFIELD.

**Effect of zinc on the growth of chicks.** A. L. Mehring, jun., J. H. Brumbaugh and H. W. Titus (*Poultry Sci.*, 1956, **35**, 956—958).—Addition of ZnO (Zn 15—778 p.p.m.) to an all-mash diet (maize-soya-bean meal, containing Zn 36 p.p.m.) for New Hampshire chicks up to nine weeks of age had no effect on growth or feed efficiency.  
A. H. CORNFIELD.

**Nutrition of breeding turkeys. I. Need to supplement practical turkey rations with vitamin E.** L. S. Jensen, M. L. Scott, G. F. Heuser, L. C. Norris and T. S. Nelson (*Poultry Sci.*, 1956, **35**, 810—816).—For maintenance of high hatchability practical turkey breeding rations often required supplementation with vitamin E. The vitamin-E requirements of breeding turkey hens is <13.6 i.u. per lb. of diet. An unknown factor present in fresh forage juice is necessary for hatchability.  
A. H. CORNFIELD.

**Resistance of the chicken embryo to low-temperature exposure.** R. E. Moreng and R. L. Bryant (*Poultry Sci.*, 1956, **35**, 753—757).—Eggs at daily stages of incubation from 1 to 15 days were exposed to still-air temp. of 10—12.8° for 19 to 120 hr. Resistance to cold, as measured by embryo survival and hatchability, decreased with advancing age at which exposure was carried out and with increasing exposure time; 11-day-old embryos withstood 19 hr. exposure and a limited number hatched. Only 1-day-old embryos withstood 120 hr. exposure and showed embryo survival of 40% and hatchability of 20%.  
A. H. CORNFIELD.

**Effect of furazolidone and other drugs on the growth of chicks raised on old litter containing coccidia.** L. R. Berg, C. M. Hamilton and G. E. Pearce (*Poultry Sci.*, 1956, **35**, 876—884).—Addition of furazolidone (55—110 p.p.m.) to the diet enhanced the growth rate of chicks reared on coccidia-infected litter and was more effective

than was the addition of penicillin (3 p.p.m.) to the diet. Nitrophenide (250 p.p.m.) was not as effective as was furazolidone, whilst sulphathiazole (175 p.p.m.) and nitrofurazone (55 p.p.m.) were ineffective. A. H. CORNFIELD.

**Determination of NN-diphenyl-p-phenylenediamine (DPPD) in [a] chicken fat and eggs, [b] feeds.** [A] W. Pudelkiewicz, L. M. Potter, L. D. Matterson and E. P. Singen. [B] R. H. Bunnell (*Poultry Sci.*, 1956, **35**, 959—960, 960—961).—[A] The method of isolating DPPD from chicken fat and eggs and the determination, based on the red colour formed with  $\text{HNO}_3$  in acetone, are described. DPPD was detected in egg yolk and depot fat from hens receiving 0.0125% of the material in the diet.

[B] The method of extracting DPPD from feeds and its estimation, based on the red colour formed with  $\text{HNO}_3$ , are described. A. H. CORNFIELD.

**Colorimetric determination of nitrofurazone and furazolidone in feeds and premixes.** J. A. Buzard, V. R. Eils and M. F. Paul (*J. Ass. agric. Chem. Wash.*, 1956, **39**, 512—518).—Nitrofurazone (5-nitro-2-furfuraldehyde semicarbazone) and furazolidone [N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone] are converted into 5-nitrofurural phenylhydrazine by extraction with alcohol and treatment with phenylhydrazine hydrochloride and HCl. The absorbance of the red colour is measured photoelectrically. The method gives reproducible results; recoveries of 91.0—104.5% of added furazolidone and 92.9—101.9% of added nitrofurazone are reported. Many other substances likely to be found in poultry feeds do not interfere. A. A. ELDRIDGE.

**Olfactory behaviour of *Lucilia* species (Diptera) under natural conditions.** J. B. Cragg (*Ann. appl. Biol.*, 1956, **44**, 467—477).—A wool-factor, which could not be wholly replaced by  $\text{NH}_4^+$  or sulphhydryl-type attractants, played an important part in the attraction of female *Lucilia sericata* to sheep. *L. sericata* was strongly attracted by wool-( $\text{NH}_4$ )<sub>2</sub>CO<sub>3</sub>/indole and less strongly by wool-ethyl mercaptan/ $\text{H}_2\text{S}_a$  mixtures. *L. caesar* and *L. illustris* were more strongly attracted by the latter mixture. Sheep wool kept moist and incubated at 38° under field conditions for three weeks did not attract *L. sericata* and oviposition did not occur on such wool. A. H. CORNFIELD.

**Chemosensory reactions of blowflies.** J. B. Cragg and P. Cole (*Ann. appl. Biol.*, 1956, **44**, 478—491).—The attractive factor in sheep wool to blowflies did not disappear during storage or washing of the wool. Only female flies were attracted to wool, the highest degree of attraction being obtained from fertilized females which had daily access to meat. There were differences due to species and strains of *Lucilia* in the strength of attraction to wool. *Calliphora vomitoria* was not attracted. The olfactory organs involved in the response to wool are mainly on the antennae, but antennaeless *L. sericata* and *L. cuprina* with some tarsi removed still showed some response provided they were tested on a damp floor. A. H. CORNFIELD.

**Incidence and economic importance of poultry parasites under different ecological and geographical situations in Egypt.** W. M. Reid (*Poultry Sci.*, 1956, **35**, 926—933).—Data are presented and discussed. A. H. CORNFIELD.

**Coliforms as related to the hæmorrhagic syndrome.** G. W. Anderson, S. J. Slinger and J. R. Couch (*Poultry Sci.*, 1956, **35**, 933—936).—An amount of liquid culture of a penicillin-resistant strain of *Escherichia coli* sufficient to supply 0.1% of dry matter in the diet was somewhat less effective than was menadione (0.005 g. per lb. of feed) in reducing the blood-clotting time of chicks receiving a vitamin K-deficient diet. The culture was more effective than menadione in reducing the extent and severity of subcutaneous and intramuscular hæmorrhages. Coliform organisms may contain a factor(s), other than vitamin K, which prevent hæmorrhagic lesions. A. H. CORNFIELD.

**Nicarbazin as a prophylactic drug in caecal coccidiosis of chickens.** R. Rubin, D. K. McLoughlin, L. C. Costello and E. E. Wehr (*Poultry Sci.*, 1956, **35**, 856—860).—Addition of 0.00625—0.0125% of Nicarbazin to the diet of birds 4—5 weeks old over a two-week period prevented mortality from caecal coccidiosis and was more effective in this respect than were either sulphathiazole 0.0125 or nitrofurazone 0.0055%. With Nicarbazin in excess of 0.0125%, wt. gains and feed efficiency were reduced, whilst with 0.1%, wt. loss and death due to apparent starvation, arising from reluctance to consume the feed, occurred. A. H. CORNFIELD.

**Furoxone for treating infectious enterhepatitis (blackhead) of turkeys.** L. C. Costello and H. M. DeVolt (*Poultry Sci.*, 1956, **35**, 952—955).—Addition of 0.011—0.033% Furoxone for 8—10 weeks to the diet of turkeys, commencing at 3—4 weeks of age, had no effect on growth rate, suppressed clinical blackhead, and prevented mortality, but failed to prevent the incidence of slight lesions in some cases. A. H. CORNFIELD.

**Insecticide-impregnated litter for control of chicken body lice, *Eomenacanthus stramineus*, Nitz., on poultry.** E. H. Floyd and B. A. Tower (*Poultry Sci.*, 1956, **35**, 896—900).—Dried ground sugar-cane bagasse was treated with BHC (0.5—36.0 g. per 100 lb.), baled, and stored for 16 months. The material was then used as poultry-house litter. In comparison with untreated material, the treated material gave very effective control of body lice for up to 5.5 months; 90—100% control was obtained where 4.5—36.0 g. of BHC had been used per 100 lb. of bagasse and 50% control where 0.5 g. had been used. A. H. CORNFIELD.

**Effects of infectious bronchitis in baby chicks.** D. I. Broadfoot, B. S. Pomeroy and W. M. Smith, jun. (*Poultry Sci.*, 1956, **35**, 757—762).—Exposure of susceptible chicks during their first 18 days of life to a virulent field strain of infectious bronchitis virus caused permanent abnormalities of egg-producing organs. This effect increased with age at which the chicks were exposed to the infection. A. H. CORNFIELD.

***Escherichia coli* as a complicating factor in chronic respiratory disease of chickens and infectious sinusitis of turkeys.** W. B. Gross (*Poultry Sci.*, 1956, **35**, 765—771).—The lesions on a no. of organs which are seen in field cases of "air-sac disease" alone or as complications of chronic respiratory disease (CRD) were reproduced by injecting *Escherichia coli* alone or with CRD agent into the air-sacs of chickens and turkeys. Five serological types of *Esch. coli* were isolated from field cases of pericarditis. The CRD agent greatly increased the pathogenicity of *Esch. coli*. Chloromycetin, neomycin, Terramycin, streptomycin and aureomycin were effective against *Esch. coli* *in vitro*. A. H. CORNFIELD.

## 2.—FOODS

**Review of carbohydrates of wheat and other cereal grains.** R. Montgomery and Fred Smith (*J. agric. Food Chem.*, 1956, **4**, 716—720).—The carbohydrates known to be present in wheat are listed to illustrate the diversity of components in cereal grains. Experimental work elucidating the nature of wheat and other grains is reviewed. (63 references.) N. M. WALLER.

**Estimation of protein in wheat and flour by ion-binding.** D. C. Udy (*Cereal Chem.*, 1956, **33**, 190—197).—The method is dependent on the reaction of wheat proteins with the disulphonic acid dye, Orange G, at pH 2.2, to form an insol. complex. The estimate is based on the concn. of unbound dye measured colorimetrically at 470  $\mu\mu$ . Equations relating protein content to concn. of unbound dye are presented for samples of wheat and flour. N. M. WALLER.

**Review of proteins in wheat flour.** J. W. Pence, D. K. Mecham and H. S. Olcott (*J. agric. Food Chem.*, 1956, **4**, 712—716).—A review of the present knowledge of the nature of flour proteins, particularly with respect to the sol. proteins. The multicomponent albumin and globulin proteins vary significantly in amount among different flours and are of potential importance as enzymic or structural modifiers of the complete complex of proteins in doughs. (45 references.) N. M. WALLER.

**Glutamic acid decarboxylase in corn. I. Paper chromatographic detection of the formation of  $\gamma$ -aminobutyric acid.** M. Rohrich and R. Rasmus (*Z. Lebensmittelforsch.*, 1956, **104**, 313—316).—A method is described for the detection and quant. determination of  $\gamma$ -aminobutyric acid in aq. extracts of crushed wheat germ by means of a circular chromatogram with isopropanol/glacial acetic acid/water (25:6:5) as solvent mixture and development with ninhydrin. The  $\gamma$ -aminobutyric acid formed by the decarboxylation of glutamic acid was found in quantity of approx. 2.6 mg./g. of wheat germ. E. M. J.

**Effects of variations in temperature of the breaking process.** N. L. Kent, G. J. Baker and C. R. Jones (*Milling Production*, 1956, Aug. repr.; *Mühle*, 1956, **93**, No. 17 repr.).—In a systematic laboratory study of variation in temp. of mill feed, the best temp. at which to send Manitoba wheat to the first break rolls was 38°. The endosperm was permanently softened and the total % release of flour from the small middlings rose from 90.5 to 91.8%. The straight run flour extraction rose from 69.3 to 70.5%. The differences observed, mainly due to the modification of the endosperm properties, are discussed. Findings were tested on a commercial scale and data are presented on cold (25°) and warm (38—41°) tests and discussed. By controlling the temp. immediately before the first break the size of the break chop could be varied at will. No significant effect was produced on physical properties of dough. E. M. J.

**Paper chromatographic detection of ascorbic acid in flours and floury foods.** E. Becker and H. Hoppe (*Z. Lebensmittelforsch.*, 1956, **104**, 21—23).—The detection of 2 mg. of ascorbic acid per 100 g. of flour is readily achieved by the technique described which



is based on the chromatographic analysis of methanolic or ethanolic extracts by known methods with appropriate minor modifications.  
P. S. ARUP.

**Surplus agricultural products utilization, with particular reference to wet milling of maize.** N. A. C. Pryce (*S. Afr. industr. Chem.*, 1956, 10, 125—127).—Maize products arising from the wet milling industry are listed and their production described. They include industrial starch, cornflour, laundry starch, dextrans for use in adhesive manufacture, liquid and solid glucose, medical glucose, oil and the maize-steep liquor which is of value in stimulating certain fermentations. Other sources of starch and oil mentioned are potatoes, cassava, groundnuts and sunflower seeds.  
N. M. WALLER.

**High-frequency drying of starch.** E. Maes and F. P. Pietermaat (*Rev. Ferment.*, 1956, 11, 106—110).—Rapid desiccation of starch and related products by high-frequency induction, for moisture determinations, is described and some typical results on maize starch are reported and discussed.  
J. S. C.

**Action of gamma-radiation on dilute aqueous solutions of amylose.** E. J. Bourne, M. Stacey and G. Vaughan (*Chem. & Ind.*, 1956, 573—574).—The degradation of amylose, irradiated from a  $^{60}\text{Co}$  source, was followed by measurement of the light absorption of the solution when treated with I and of the Cu reducing power determined by the method of Shaffer and Hartmann (*J. biol. Chem.*, 1921, 45, 377).—Chromatographic examination indicated the presence of glucose, maltose, maltotriose, etc. (13 references.)  
J. S. C.

**Light scattering study of maize amylopectin and its beta-amylase limit dextrin.** C. J. Stacy and Joseph F. Foster (*J. Polym. Sci.*, 1956, 20, 57—65).—The degree of dispersion of a maize amylopectin and its  $\beta$ -amylase limit dextrin were studied in 1-N-alkali, anhyd. ethylenediamine, ethylenediamine hydrate, formamide and water, by measuring the concn. and angular dependence of scattered light. The degree of dispersion in the first three solvents was essentially the same. The mol. wt. of the amylopectin was 80 million and of the limit dextrin, 29 million. The dispersion was molecular and the mol. wt. distributions of the amylopectin and the limit dextrin are similar in form.  
J. S. C.

**Light scattering investigation of the retrogradation of amylose.** J. F. Foster and M. D. Sterman (*J. Polym. Sci.*, 1956, 21, 91—101).—Turbidity studies of the retrogradation (pptn.) rate of amylose from aq. solution indicate a marked dependence on prior physical state. It is suggested that rate of retrogradation is primarily determined by rate of disruption of the intramolecularly H-bonded form and is thus analogous to protein denaturation. (12 references.)  
J. S. C.

**Controlled degradation of waxy-maize starch by malt alpha-amylase.** R. L. Lohmar, F. B. Weakley and G. E. Lauterbach (*Cereal Chem.*, 1956, 33, 198—206).—The degradation at 60° was followed viscometrically. The inherent viscosity (0.21—0.44) of the dextrin precipitable by alcohol bears a definite ratio to the relative viscosity of the conversion liquors. An empirical relationship was found between enzyme concn., specific viscosity of the conversion liquor and time of conversion. Conversions on a pilot-plant scale are reported. Some of the dextrans are effective in sedimentation of red blood cells.  
N. M. WALLER.

**Determination of dialdehyde units in periodate-oxidized maize starches.** J. C. Rankin and C. L. Mehlretter (*Analyt. Chem.*, 1956, 28, 1012—1014).—Two methods are described; the first involves reduction of dialdehyde groups with sodium borohydride and the second the oxidation of oxystarch with sodium metaperiodate. Both agreed within 4.0%, the standard deviations being ~1.0% at the 95, 62 and 12% dialdehyde levels on oxystarch. Accuracy is also good.  
G. P. COOK.

**Iodine-binding capacity of some native starches of Indian food grains.** S. Singh, N. Nath and H. P. Nath (*Biochem. J.*, 1956, 63, 718—720).—The I-binding capacities of several native starches of Indian food grains (pulses, beans, millets, cereals, seeds and tubers) are determined. Most of the starches are normal and absorb 2.72—6.14 g. of I per 100 g. of carbohydrate. *Colocasia* starch contains appreciably more amylopectin than the other starches. Pulse and bean starches contain more amylose than do starches from cereals, millets and tubers.  
J. N. ASHLEY.

**Time of loading and strain retardation in starch jelly candy.** C. Sterling (*Food Res.*, 1956, 21, 546—554).—When time of loading of a starch gel is varied, crystallization, or the formation of new micellar bonds, displacement of isolated and lightly attached polysaccharide molecules ("brush heap") and breakage of weaker bonds are involved. With increased time of stress application, there is an increase in the time of retardation of the strain and in the permanent deformation of the gel. Resilience decreases with increased loading time. (13 references.)  
E. M. J.

**Nutritive value of the protein of white and wholemeal bread in relation to the growth of rats.** J. B. Hutchinson, T. Moran and J. Pace (*Proc. roy. Soc.*, 1956, 145B, 270—279).—Re-examination of the comparative value of the protein of wholemeal bread and white bread, in promoting the growth of young rats of both sexes, indicated that the better growth-rate is due to the higher lysine content of the wholemeal bread. The weanling rat is extremely sensitive to small changes in the lysine concn. in diets when the lysine content is in the range of 0.3 to 0.5%. (22 references.)  
E. M. J.

**Effect on the growth-rate of weanling rats of supplementing the protein of white bread with L-lysine.** J. B. Hutchinson, T. Moran and J. Pace (*Nature, Lond.*, 1956, 178, 46—47).—The growth-rate was substantially increased, optimum response being provided by an addition of 0.25% of L-lysine.  
J. S. C.

**Effects on flour, dough and bread quality of moulds grown in wheat and those added to flour in the form of specific cultures.** Y. Pomeranz, P. Halton and F. G. Peers (*Cereal Chem.*, 1956, 33, 157—169).—The effects produced by mould growth on damp wheat, stored at high temp. include increased riboflavin content and fat acidity and decreased thiamine, nicotinic acid and fat content. Flour produced from such wheat in early stages of storing shows some improvement in baking quality but at later stages the water absorption, dough characteristics and bread quality deteriorate. The effects of pure cultures of certain fungi are compared.  
N. M. WALLER.

**Effect of calcium stearyl-2-lactylate in bread made with non-fat milk solids of varying baking quality.** W. G. Bechtel, G. E. Hammer and J. G. Ponte, jun. (*Cereal Chem.*, 1956, 33, 206—212).—The addition of Ca stearyl-2-lactylate to doughs containing 3.0—8.5% non-fat milk solids of good baking quality improves the bread quality. When 8.5% non-fat milk solids of poor baking quality are used the addition of Ca stearyl-2 lactylate results in a good loaf provided optimum mixing time is allowed. At lower levels of milk solids mixing time is not so critical.  
N. M. WALLER.

**Effect of light on vitamin retention in enriched white bread.** K. Morgareidge (*Cereal Chem.*, 1956, 33, 213—220).—The vitamin content of bread was studied to determine the effect of transparent and semi-opaque wrapping papers. No loss of thiamine, riboflavin or niacin was observed in any case, when bread was subjected to normal intermittent illumination for periods up to five days. The crust is sufficient protection under normal conditions.  
N. M. WALLER.

**Effects of temperature and relative humidity on the rate of defrosting of commercial bread.** J. W. Pence, N. N. Standridge and M. J. Copley (*Food Technol.*, 1956, 10, 492—495).—Best overall defrosting results were obtained at 120°F. and 50—60% R.H. Warming of air for defrosting caused faster defrosting and a lower R.H. with less trouble from condensed moisture. Rapid defrosting of bread to an internal temp. of 120—140°F. by micro-wave heating produced incomplete refreshing of the bread and caused marked damage to the wrappers. (13 references.)  
E. M. J.

**Effects on baking powder biscuits of four flour components used in two proportions.** M. V. Zaehring, A. M. Briant and C. J. Personius (*Cereal Chem.*, 1956, 33, 170—180).—A commercial hard-wheat bread flour and a soft-wheat pastry flour were each separated into four fractions—gluten, water-solubles, starch and "amylopectin"—and these eight fractions were combined into 32 different flours. The properties of the flours and doughs and biscuits prepared from them were compared. The proportion of the components used is more significant than their source but certain properties are affected by source.  
N. M. WALLER.

**Influence of texturation, occluded gas content and emulsifier content on shortening performance in cake making.** S. W. Thompson and J. E. Gannon (*Cereal Chem.*, 1956, 33, 181—189).—Conventional non-emulsifier and emulsifier shortenings, solidified with and without aeration and also solidified by slow cooling without agitation, are compared by testing in several cake recipes and using various mixing methods. Emulsified gas in the shortening improves performance but its omission can be compensated for by additional mixing of the batters. The optimum level for the conventional type of mono-glyceride is not critical in the range 2—4% and depends on the cake recipe used.  
N. M. WALLER.

**Polyoxyethylene monostearate in the bakery.** E. S. Lower and S. C. Cressey (*Food Manuf.*, 1956, 31, 277—279).—The use of polyoxyethylene monostearate for improving the quality of bread and the objections which have been raised to its use are reviewed. (13 references.)  
J. S. C.

**Preservative and antiseptic agents for flours and bakery products.** F. Hernández-Gutiérrez (*An. Bromatologia*, 1956, 8, 39—72).—The sample is acidified and acids likely to be present (acetic, propionic) are identified in the distillate. Acetic acid is identified by the

indigo reaction of Feigl or by pptn. of sodium uranyl acetate, and propionic as its mercurous salt. A quant. estimation, based on the difference of solubility in butanol of the Cu salts of the acids, is presented. (119 references.) L. G. L. UNSTEAD-JOSS.

**Sucrose double compound formation.** F. H. C. Kelly (*Int. Sug. J.*, 1956, **58**, 128—129, 190—192).—The literature relating to preparation of double compounds of sucrose and inorg. salts is reviewed and a study is made of the liquid phase composition at the ternary point, i.e., when the solution is saturated with respect to the second solute as well as sucrose. The significance of the results is briefly discussed in relation to molasses formation and practical crystallization problems. (17 references.) J. S. C.

**Nylon for filtration in beet sugar manufacture.** N. M. Adams (*Int. Sug. J.*, 1956, **58**, 192—193).—Experience in British beet-sugar factories of the use of nylon cloth in filtration is briefly summarized. In second carbonation and thick juice-syrup presses, results have been satisfactory. In rotary vacuum filters, damage caused during cake release is such that the additional life obtained with nylon offers no advantage over the use of cotton cloth. J. S. C.

**Viscosity of technical sugar solutions.** H. Breitung (*Z. Zuckerind.*, 1956, **6**, 185—193, 254—260).—The Umstätter free-flow capillary viscometer is described and a large number of viscosity measurements on pure sucrose, factory thick juices from various European countries with a 50—70° Brix range and molasses are reported. The results are statistically examined in relation to other analytical data, including conductimetric ash, CaO, non-sugars, pH, etc. and correlations were found between viscosity and (a) conductimetric ash and CaO of thick juices, and (b) electrolyte non-sugars in diluted molasses. SUG. IND. ABSTR. (J. S. C.)

**Phosphoric esters and sugars in growth of sugar beet.** E. Bougy (*C. R. Acad. Sci., Paris*, 1956, **242**, 667—669).—Development of phosphoric esters in the leaves and stems was apparently directly linked to that of saccharose. In the roots and seeds, in conditions where there is no change in sugar-content, phosphoric esters disappear. J. S. C.

**Influence of neutral salts on hydrolysis of sugars by dilute acids. I. Action of alkali chlorides on the inversion of saccharose by hydrochloric acid.** A. de Grandchamp-Chaudun (*C. R. Acad. Sci., Paris*, 1956, **242**, 690—692).—The addition of 1 g.-mol./l. of KCl, NaCl and LiCl increases the rate of inversion of 5% aq. sucrose by 0.1N-HCl by an identical amount for each salt. J. S. C.

**Quantitative chromatographic estimation of amino-acids in molasses.** E. Mariani, A. Ciferri and G. Torracca (*Int. Sug. J.*, 1956, **58**, 156—158).—Values of concn. of  $\gamma$ -aminobutyric acid (I) and tyrosine (II) obtained by elution chromatography have been found to differ from those of paper chromatographic and ion-exchange adsorption techniques. It is now shown that I (in pH 5 solution) does not give a separate peak and cannot be separated from II in a 20-cm. column. The position and intensity of the II peak from the 110-cm. column is confirmed. I may be estimated either from the difference in peak area of the 20-cm. and 110-cm. columns or by elution at 30° with (i) 60 ml. of pH 4.25 buffer, then (ii) 40 ml. of pH 5 buffer. A revised table of amino-acid concn. in beet molasses is given (cf. J.S.F.A. Abstr., 1955, ii, 237).

SUG. IND. ABSTR. (J. S. C.)

**Recovery of acetic acid from molasses.** E. A. Regna and P. F. Burns (*Industr. Engng Chem.*, 1956, **48**, 1268—1277).—Two processes developed and described were MeOH precipitation of over 90% of the acetic acid (I) as Ca salt and solvent extraction with methyl ethyl ketone. Ternary solubility data were determined for several systems. Pseudo-counter-current extractions resulted in over 90% recovery of I. Preliminary cost estimates were prepared to produce 1 million and 4 million lb. of I annually by these processes and by an ion-exchange process described. The solvent extraction process appears most economical. O. M. WHITTON.

**Detection and paper chromatography of sugars and sugar phosphates in picric acid system.** H. S. Loring, L. W. Levy and L. K. Moss (*Analyt. Chem.*, 1956, **28**, 539—540).—The use of the *tert.*-butanol-picric acid-H<sub>2</sub>O (80:4:20) solvent system for the paper chromatography of sugar phosphates (cf. Hanes and Isherwood, *Nature*, 1949, **164**, 1107) has been extended to the separation of the sugars themselves. Sugars and sugar phosphates are readily detected on the chromatograms by spraying with ethanolic NaOH and heating; they appear as red-brown spots against a yellow background. Values of  $R_F$  are listed for 14 sugars and 6 sugar phosphates. A. R. ROGERS.

**Cacao polysaccharides.** R. L. Whistler, E. Masak, jun. and R. A. Plunkett (*J. Amer. chem. Soc.*, 1956, **78**, 2851—2853).—Two hot-water-sol. polysaccharides were extracted from mature fruit husk and seed of *Theobroma cacao*, respectively, I (2.0) and II (0.3%

yield). I is composed mainly of L-rhamnose, L-arabinose, D-galactose and D-mannose, plus small amounts of glucose, xylose and an unidentified pentose. II contains the same major components but in different proportions. M. DAVIS.

**Determination of degree of polymerization of reducing oligosaccharides.** S. Peat, W. J. Whelan and J. G. Roberts (*J. chem. Soc.*, 1956, 2258—2260).—A microchemical method is developed involving reduction with NaBH<sub>4</sub> and colorimetric determination of reducing power with anthrone-H<sub>2</sub>SO<sub>4</sub> reagent. The method is not suitable for pentose oligosaccharides. M. DAVIS.

**Paper chromatographic identification of thickening materials in foods.** E. Becker and M. Eder (*Z. LebensmittlUntersuch.*, 1956, **104**, 187—192).—Since most thickening substances consist of carbohydrate material, paper chromatography offers a means of identification of sugars and similar substances. The substance is hydrolysed for 3 hr. with H<sub>2</sub>SO<sub>4</sub>, the H<sub>2</sub>SO<sub>4</sub> is removed with Ba(OH)<sub>2</sub> and the clear filtrate is concentrated in vac. Using *n*-butanol/pyridine/water and phthalic acid/aniline, chromatograms for carob-beanseed meal, agar agar, pectin, Tylose, cellulose glycolate, alginate, etc., with suitable control solutions are illustrated. E. M. J.

**Carbon dioxide injury and presence of succinic acid in apples.** A. C. Hulme (*Nature, Lond.*, 1956, **178**, 218—219).—Succinate applied to respiring discs of apple peel (in a suitable buffer system at pH 4.1) at concn. > about 0.025M reduces respiration to zero and kills the tissue. At this external concn. the internal concn. is < 0.001M. Succinic acid is only occasionally found and in trace amounts in normal healthy apples. CO<sub>2</sub> injury in apples is accompanied by an increase in succinic acid in the tissue. O. M. WHITTON.

**Microscopical detection of apple pulp in preserved fruits and jams.** H. Ludwig (*Z. LebensmittlUntersuch.*, 1956, **104**, 327—335).—The starch and fibre contents of various kinds of commercial apple sauces and apple pulp used in jam manufacture were studied, using 50-g. samples. To identify starch-free apple pulp and also to distinguish between that and other added fruit pulps, a double staining method using methylene blue and Ruthenium red was satisfactory in most cases in differentiation and in permitting the detection of small amounts of apple pulp. The shape of the fruit cells must also be considered. The method is not quant. but the behaviour of many separate kinds of fruit pulp with the dye mixture is described. E. M. J.

**Changes in cell wall of pear during ripening.** M. A. Jermyn and F. A. Isherwood (*Biochem. J.*, 1956, **64**, 123—132).—The changes that occur in the amounts of the simple-sugar polysaccharides (xylan, araban, polygalacturonic acid, galactan, and cellulose) in the cell wall of the Conference pear (as reflected in the amounts of simple sugars obtained after hydrolysis) are followed during storage at 15 and 5°. The cell wall appears to be in dynamic equilibrium with the cytoplasm, and polysaccharides are both broken down and synthesized during the physiological changes that occur during ripening. J. N. ASHLEY.

**Activity of starch-hydrolysing enzymes in pears during development and cold storage.** G. W. F. Maris McArthur-Hespe (*Acta bot. neerl.*, 1956, **5**, 200—213).—The changes in the activity of  $\alpha$ - and  $\beta$ -amylase in three varieties of pears gathered at different dates were examined. During cold storage  $\alpha$ -amylase continues to increase, the increase being different in the three varieties; if activity is approx. 6—8 times the value at commercial picking-time, ripening after cold storage does not occur. Such a comparison of values at any stage during cold storage gives an indication as to whether pears can ripen or not. Each variety possibly has its own critical ratio value. (12 references.) E. M. J.

**Grower handling of red cherries.** J. H. Levin and H. P. Gaston (*U.S. Dep. Agric.*, 1956, Circ. 981, 20 pp.).—A method for handling cherries in water at the orchard in reviewed. Costs are reduced and management is simplified. E. G. BRICKELL.

**Breakdown of strawberry anthocyanin pigment.** A. Lukton, C. O. Chichester and G. Mackinney (*Food Technol.*, 1956, **10**, 427—432).—The effect of pH on the rate of pelargonidin-3-glucoside destruction in pure pigment solutions and strawberry juice is discussed. (17 references.) E. M. J.

**Effect of 2:4:5-trichlorophenoxyacetic acid spray on organic acids, pectin and quality of canned apricots.** J. W. Hoos, S. J. Leonard and B. S. Luh (*Food Res.*, 1956, **21**, 571—581).—Malic acid is the dominant org. acid in apricots, with a smaller amount of citric acid, both acids decreasing as the fruit matures on the tree. Both decrease more rapidly in samples which had been sprayed with 2:4:5-T at the beginning of the pit-hardening period. The influence of 2:4:5-T spray and maturity of the fresh fruit on flavour, colour, texture and syrup viscosity of the canned product is discussed. E. M. J.

**Consumer survey on the dessert quality of canned apricots.** R. M. Valdés and E. B. Roessler (*Food Technol.*, 1956, **10**, 481—486). (37 references.) E. M. J.

**Dehydrofrozen apricots: preparation.** M. J. Powers, D. H. Taylor, W. F. Talburt and L. H. Walker (*Food Technol.*, 1956, **10**, 489—492).—Apricots are partially dehydrated, then frozen, treated with SO<sub>2</sub> and dried on trays to a 50% wt. reduction. A second treatment with SO<sub>2</sub> is given; the resulting product is of a bright colour and rich flavour. (10 references.) E. M. J.

**Detection of cherry, plum, apricot, peach, black and red currants in preserved fruits and jams.** A. T. Czaja (*Z. Lebensmittl. Untersuch.*, 1956, **104**, 269—275).—A method of detecting constituent fruits in jams etc. by means of histological characteristics is described, e.g., the stone cells of the mesocarp of cherries and plums, and of the flesh of apricots and the distinctive sclerenchymatous fibres of the various species. E. M. J.

**Chemical aspects of pectin gel darkening.** M. A. Steinberg, G. E. Livingston and C. R. Fellers (*Food Technol.*, 1956, **10**, 470—476).—Fourteen crystalline aldehyde 2:4-dinitrophenylhydrazones were isolated from a gel heated for 5 hr. at 110° and their u.v. spectra examined. Three crystalline compounds were isolated from a citric acid-fructose solution heated for 5 hr. at 110°. Such substances isolated: e.g., *n*-butyraldehyde, furfural and 2-hydroxymethyl-5-furfuraldehyde, when added back to their parent systems and heated, produced more darkening than that in the controls. (18 references.) E. M. J.

**Yeasts occurring in brines during the fermentation and storage of green olives.** E. M. Mrak, R. H. Vaughan, M. W. Miller and H. J. Phaff (*Food Technol.*, 1956, **10**, 416—419).—The qual. changes of the yeast flora during fermentation and subsequent storage comprise fermentative yeast species in the first seven weeks, e.g., *Candida krusei*, *C. solani*, *Hansenula subpelliculosa* etc.; and, a more aerobic flora in the period 9—16 weeks, e.g., *Pichia membranifaciens*. (14 references.) E. M. J.

**Shear-press: an instrument for measuring the quality of foods. IV. Application to asparagus.** R. C. Wiley, N. Elehwany, A. Kramer and F. J. Hager (*Food Technol.*, 1956, **10**, 439—443).—Fibre changes in raw green asparagus as affected by duration of storage and pre- and storage treatments are described. (16 references.) E. M. J.

**Determination of tomato paste solids by vacuum drying and by refractive index.** S. H. Judd (*J. Ass. off. agric. Chem. Wash.*, 1956, **39**, 445—453).—The results of numerous determinations have been compared. The differences observed were particularly large for samples containing more than about 26% of solids. A. A. ELDRIDGE.

**Suppression of sprouting of potatoes by vapour of alcohols.** W. G. Burton (*Nature, Lond.*, 1956, **178**, 218).—Test results are presented for various alcohols (1—10C). Nonyl alcohol gave promising results. O. M. WHITTON.

**Objective measurements of the maturity of raw and canned field peas *Vigna sinensis*.** H. R. Malcom, J. J. Powers, A. Lopez and D. E. Pratt (*Food Technol.*, 1956, **10**, 463—469).—The moisture-, or the alcohol-insol.-solids content of raw field peas was a satisfactory index of the maturity; the starch-content, resistance of the pea to puncturing by a penetrometer plunger and the % juice expressible were also correlated with maturity. (26 references.) E. M. J.

**Essential amino-acid composition of pulses and rice.** K. P. Chatterjee, A. Ray and S. Banerjee (*Food Res.*, 1956, **21**, 569—570).—Analytical data on the essential amino-acids present in seven varieties of pulses and rice, determined by microbiological assay, and protein contents of pulses and rice are presented. E. M. J.

**Chemical and flavour study of off-flavour due to artificial pepper.** E. L. Wick and S. E. Cairncross (*Food Technol.*, 1956, **10**, 423—427).—Examination of an abnormal black pepper oleoresin resulted in the isolation of a sesquiterpene hydrocarbon fraction from the volatile portion; 0.0002% of this fraction added to a commercial product, prepared without pepper, reproduced the off-flavour previously found. The non-volatile portion of the oleoresin contained less than the normal quantity of piperine and a synthetic compound, 1-cinnamoylpiperidine. (16 references.) E. M. J.

**Ascorbic acid content of fruits and vegetables of Rajasthan.** G. Sitaramaiah and K. I. Mathai (*J. Indian chem. Soc. industr. Edn.*, 1956, **19**, 15—17).—The ascorbic acid content of 23 varieties of fruit and vegetables is estimated and found to vary from province to province even for the same material. I. JONES.

**Filth test in preserved vegetables.** G. Peeters (*Rev. Ferment.*, 1956, **11**, 70—74).—Samples of tomato purée and preserves of peas, spinach, celery and carrots were examined by the methods previously described for fruits (cf. J.S.F.A. Abstr., 1956, i, 274). The

only serious contamination was found in spinach and tomatoes and, in both cases, this is shown to be due to the presence of insects prior to processing. J. S. C.

**Vegetable soup.** M. P. Charro (*An. Bromatologia*, 1956, **8**, 73—107).—Formulae, photomicrographs of constituents, together with moisture, protein, fat, carbohydrate, cellulose and ash contents of typical dried vegetable soups are given, together with a range of kg.-cal. contents. (11 references.) L. G. L. UNSTEAD-JOSS.

**Preparation of sodium alginate from rockweed.** D. L. Vincent (*Canad. J. Technol.*, 1956, **34**, 220—226).—Various methods of extracting alginate from rockweed (*Fucus vesiculosus*) (*R*) and kelp (*Laminaria digitata*) have been compared. By the usual extraction procedures, the product from *R* developed a brown colour during treatment with Na<sub>2</sub>CO<sub>3</sub> solution. A colourless product was obtained with little or no loss in  $\eta$  by (i) passing the crude alginate solution through a column of activated charcoal and diatomaceous earth (Celite), (ii) carefully controlled bleaching with NaOCl, and (iii) exhaustive extraction of *R* with methanol prior to extracting the alginate; exposure to formaldehyde was not effective. Extraction of dried *R* at 10—20° followed by pptn. with ethanol yielded a product of higher  $\eta$  than that given by the usual extraction procedures. Generally yields were lower from *R* than from kelp. S. C. JOLLY.

**Additions to fruit juice drinks and lemonades.** E. Benk (*Reichstoffe u. Avomen*, 1956, **6**, 174—175).—A review dealing chiefly with legal aspects in Germany. H. L. WHITEHEAD.

**Spectrophotometric determination of silica in mineral waters.** C. Milani (*Chim. e Industr.*, 1956, **38**, 587—590).—The determination is usually made gravimetrically, making no distinction between ionic and colloidal forms by spectrophotometric determination of the known silicomolybdic complex (I). The ionic portion is measured, and colloidal SiO<sub>2</sub> is calculated by difference from the total SiO<sub>2</sub> content. The absorption spectrum of I and the calibration curve are reported, as well as typical experimental results. C. A. FINCH.

**Chemical test for screw stoppers.** K. W. Allen (*J. Inst. Brew.*, 1956, **62**, [New Series 53], 337—338).—Ebonite screw stoppers used for mineral water bottles can, in some cases, cause tainting due to the presence of compounds of S. When boiled with aq. citric acid, a stopper should yield in the distillate  $>15 \mu\text{g.}$  of sulphide-S, determined colorimetrically as PbS. Stoppers which cause tainting can be improved in this respect by washing in dil. H<sub>2</sub>SO<sub>4</sub>. P. S. ARUP.

**Emulsifying agents in beverages.** I. G. Janssens and Y. Lutz (*Ann. Falsif., Paris*, 1956, **49**, 206—223).—The chemical composition, synthesis and principal uses of emulsifying agents are reviewed, and the chief characteristics (including solubilities in various solvents) are tabulated for Swiss and American products proposed for the dispersal of essential oils in beverages. American investigations indicating the harmlessness of some of these substances are cited. Dispersing agents can be separated from beverages as supernatant viscous layers by centrifuging at 5000 r.p.m. during 30 min. (repeatedly if necessary). These phases show characteristic microcryst. structures which display characteristic transformations in various liquid media. Provided that sufficient of the viscous phase can be separated, indications of the class of dispersing agent present can be obtained by means of solubility tests. P. S. ARUP.

**Sulphur content of sulphite alcohol and alcohols from other sources.** R. J. Peltonen, P. Neuenchwander and H. Suomalainen (*Z. Lebensmittl. Untersuch.*, 1956, **104**, 335—339).—The total S contents of sulphite alcohol and alcohols from other sources determined by the "lamp" method (cf. J.S.F.A. Abstr., 1956, ii, 66) were: crude sulphite alcohol 95, crude methanol-free sulphite alcohol 47, alcohol from potatoes, barley and maize 125—193, yeast prep. 400, refined sulphite alcohol 22, and refined potato alcohol 118  $\mu\text{g./100 ml.}$  The contained S indicated varied distribution on fractional distillation of products from different factories; easily and difficultly distilled S compounds varied considerably while the S content of the middle fraction was generally small. E. M. J.

**Routine determination of sulphate in wines.** J. Schneyder (*Mitt. Wein- u. Obstbau, Wien*, 1956, **6A**, 155—157).—A rapid method is described which depends on the pptn. from the acidified sample of the SO<sub>4</sub><sup>2-</sup> as PbSO<sub>4</sub>, dissolution of the ppt. in a known excess of aq. EDTA (disodium salt), and back-titration of excess EDTA with 0.1M-ZnCl<sub>2</sub>, with Eriochrome black-T as indicator. The results show agreement with gravimetric results. P. S. ARUP.

**Methanol content of wines.** J. Amiel, M. Nortz and J. Puisais (*C. R. Acad. Sci., Paris*, 1956, **242**, 1646—1647).—The methanol contents of a series of red and white wines of different native and hybrid vines grown in 1953 were measured and are tabulated. They were 137—370 mg./l. for red and 36—133 mg./l. for white wines. In general, the methanol content is higher in wines of deeper colour but the relationship is not an exact one. J. S. C.

**Elimination of yeast by filtration in preparation of sparkling wines.** M. Mayer-Oberplan (*Mitt. Wein- u. Obstbau, Wien*, 1956, **6A**, 182—187).—Two filtration methods are briefly described. The advantages of filtration over the conventional methods for yeast elimination are pointed out. P. S. ARUP.

**Determination of acetal in wines, alcoholic drinks and analogous solutions.** I. Mareca Cortés and M. de Campos Salcedo (*An. real Soc. esp. Fis. Quim.*, 1955, **51B**, 85—90).—Acetal is hydrolysed with  $H_2PO_4$  in presence of  $NaHSO_3$  and distilled at pH 9 together with free  $CH_3CHO$ . It is collected and reacted with excess of  $NaHSO_3$ ; the excess is then determined iodometrically. Free  $CH_3CHO$  is determined by distillation without prior hydrolysis, and the acetal content of the wine calculated from the difference. D. LEIGHTON.

**Content of difficultly volatile esters in fruit brandies.** L. Torbágyi-Novák and J. Verhás (*Z. Lebensmittelforsch.*, 1956, **104**, 182—187).—The total ester content of various wines (German, Spanish etc.) were examined, and six fruit brandies were studied. The total ester contents of fruit brandies, not always in close agreement with the established quality, when judged organoleptically, were determined by fractional distillation according to Micko's method. The separation of the easily volatile (considered as ethyl acetate) and difficultly volatile esters (containing the aromas of the brandies) is critically examined. The difficultly volatile esters found by total esters—ethyl acetate amounted to 100—200 mg./100 ml. of alcohol. E. M. J.

**Rapid determination of egg yolk content of emulsion liquors.** J. Kottász (*Z. Lebensmittelforsch.*, 1956, **104**, 266—268).—The fat content (determined by the Röse-Gottlieb method using 10-ml. or 10-g. samples) and the determination of the fat refraction value are discussed. Data are given of the egg yolk no. for various egg-liquors found by determinations of total  $H_2PO_4$ , lecithin phosphoric acid, protein content and refraction; and correction of the refractometrically determined values of egg yolk contents. In the determination of egg yolk contents of emulsion liquors the calculation of the fat content is adapted to the refraction value found. E. M. J.

**Determination of added distinctive cations in whisky. III. Flame spectrophotometric determination of caesium and rubidium.** M. J. Pro, R. A. Nelson and A. P. Mathers (*J. Ass. off. agric. Chem. Wash.*, 1956, **39**, 506—512).—Flame spectrophotometry affords the most satisfactory method for determining traces of Co and Rb added to whisky as brand labels. Both metals can be determined with precision and accuracy in the presence of a large excess of K and Na (which enhance emission) by the use of standards which contain K and Na in the concn. found in whisky. Other cations examined had no effect; phosphate and perchlorate reduced emission. A. A. ELDRIDGE.

**High-boiling substances present in fusel oil.** W. E. Poles, N. J. O'Connor and F. T. Riley (*Sci. Proc. R. Dublin Soc.*, 1956, **27**, 69—73).—The high-boiling substances present in fusel oil from Irish alcohol factories were identified. The high-boiling fraction was separated by distillation and steam distillation and chemically separated into neutral, basic and acidic fractions. These were then fractionally distilled at reduced pressure. The following compounds were identified: caprylic, capric and lauric acids, ethyl caprate, ethyl laurate, isopentyl caprylate, isopentyl caprate, isopentyl laurate, isopentyl myristate and isopentyl palmitate. The presence of two unidentified hydrocarbons was established. N. M. WALLER.

**Detection of higher alcohols in alcoholic ferments.** A. Daghetta (*Chimie Industr.*, 1956, **38**, 576—579).—Procedures for the chromatographic separation of aliphatic alcohols are examined, with reference to their applicability in the analysis of alcoholic ferments. A simple procedure is suggested for the concentration of higher alcohols in mixtures, which improves the sensitivity of the methods examined. Full procedures are given. (14 references.) C. A. FINCH.

**Determination of alcohols in aqueous ethanol-isopropanol mixtures.** F. Strache and E. Martienssen (*Z. Lebensmittelforsch.*, 1956, **104**, 339—344).—A method for determining ethanol and isopropanol in an aq. mixture, depending on the measurement of the  $d$  of the mixture and oxidimetric titration is described. Mathematical and graphical determinations of the analytical results are presented. Test analyses indicating the limit of error of the method are given. E. M. J.

**Utilization of agricultural raw materials in fermentation. Products and their use in industry.** F. W. Hayes (*S. Afr. industr. Chem.*, 1956, **10**, 122—124).—Fermentation processes using molasses are reviewed. The products include ethyl alcohol, butanol and acetone, yeast, organic acids, antibiotics, enzymes and vitamins. N. M. WALLER.

**Simple device for counting barley corns.** S. Learner (*J. Inst. Brew.*, 1956, **62** [New Series **53**], 322).—The rapid and random sampling of 50 corns is achieved by a device consisting in a slightly

tilted tray into which have been cut 50 cavities of size and shape suitable for the reception of one grain each. The sampling operation consists in a simple scooping action followed by a slight agitation of the tray. P. S. ARUP.

**Studies in barley and malt. VI. Stimulation of germination of freshly harvested barley.** J. R. A. Pollock and B. H. Kirsop. **VII. Disappearance of dormancy in barley on storage.** R. E. Essery and J. R. A. Pollock. **VIII. Survey of dormancy of barleys harvested in Britain in 1955.** J. R. A. Pollock (*J. Inst. Brew.*, 1956, **62** [New Series **53**], 323—327, 327—330, 331—333; cf. J.S.F.A. Abstr., 1956, ii, 29).—VI. In a study of the effect of germination of freshly harvested barley of additions of various substances to the steeping water, sulphhydryl compounds (including  $H_2S$ ) are the most efficient. Possible explanations for the observed effects are discussed. (18 references.)

VII. The increase in germinative energy of undried barley samples is considerably greater after storage at 18° than at 0°. Storage at 25° during periods >40 days tends to cause degeneration. Kiln drying before storage does not improve germinative energy, but increases the rate of disappearance of water-sensitivity in the germination test. (24 references.)

VIII. The survey includes 15 varieties, each represented by a no. of samples, which are classified into five groups according to the results of tests for germinative energy and water-sensitivity. Variations in dormancy, which in some cases is absent, are discussed. Water sensitivity prevails in varying degrees, but is absent in two varieties which show low germinative energy. P. S. ARUP.

**Relationship between the inhibitory action of butyric acid and pH on alcoholic fermentation.** K. Holtegaard (*Int. Sug. J.*, 1956, **68**, 221—223).—In laboratory fermentations of molasses containing butyric acid, the inhibitory action is affected by the pH of the wort. By increasing pH, normal fermentations can be obtained with concn. of butyric acid in wort as high as 0.75%. The tolerable concn. of undissociated butyric acid in the pH range 4.8—5.5 is ~0.08—0.09%. Outside these limits, the concn. should not exceed 0.05%. J. S. C.

**Brewery yeast propagation with special reference to flocculence in its relationship to attenuation.** E. J. Jeffery (*J. Inst. Brew.*, 1956, **62** [New Series **53**], 309—320).—For the propagation of yeast without increase in flocculence and concomitant loss of attenuative capacity, the Burton system is shown, in laboratory experiments, to be superior to other systems in use. In the single vessel skimming system, these changes are due to fractional separation which leads to the predominance of flocculating cells, and not to any changes in the cells themselves. No cells of intermediate flocculating or attenuative capacity can be found. The close relationship between changes in the Burns value, flocculence, and attenuative capacity are confirmed. Continuous propagation, in which fractional separation cannot occur unduly, can improve attenuative capacity, but the Burton Union system is preferred on account of its simplicity. P. S. ARUP.

**Role of humic acid in flocculation of yeast.** A. A. Eddy (*J. Inst. Brew.*, 1956, **62** [New Series **53**], 320—321).—Experiments with five bottom yeasts indicate that humic acids have no influence on flocculation, which thus appears to depend on innate cell characteristics. P. S. ARUP.

**Resolution of mixtures of cohumulone, humulone and adhumulone by reversed phase partition chromatography.** L. O. Spetsig and M. Steninger (*J. Inst. Brew.*, 1956, **62** [New Series **53**], 333—336).—The chromatographic procedure of Howard and Martin, as extended by Silk and Hahn (cf. *Biochem. J.*, 1950, **46**, 532; 1954, **56**, 407) proves, with minor modifications, to be a simple and convenient means for the separate quant. determination of cohumulone, humulone and adhumulone. (10 references.) P. S. ARUP.

**Effect of oxygen in bottled beer on ITT measurement.** K. Silbereisen and G. Wittmann (*Mtschr. Brauerei, wissen. Beil.*, 1956, **9**, 87—90).—The authors' procedure (cf. J.S.F.A. Abstr., 1956, i, 227) is described, and the superior accuracy of objective as compared with subjective colorimetric measurement is shown. Rates of decolorization are increasingly, but not linearly reduced with increasing absorption of  $O_2$  (0—2.5 ml. per 165 ml. of beer) during storage (for 1—12 days). The ratio between the times for 80% and 50% decolorization is reduced by approx. 50% within the above limits of oxidation. P. S. ARUP.

**Surface protection of malt beverage containers with silicones.** J. P. Poole (*Glass Ind.*, 1956, **37**, 195—200, 220, 222).—Silicone treatment of the bottles gives a surface lubrication which minimizes reduction in strength caused by rubbing and bumping during filling and transit. The life of the silicone-treated bottles was found to be extended to up to 20 trips. Other advantages are improved drain-out, pressure-retention and chemical resistance. The treatment,



which has no adverse effect on the beer, should enable lighter and cheaper packing to be used.  
J. A. SUGDEN.

**Differentiation between fermentation and synthetic vinegars by amino-acid content.** H. Schanderl and T. Staudenmayer (*Z. Lebensmittelforsch.*, 1956, 104, 26—28).—The sample is steam-distilled to remove acetic acid (with precautions against the introduction of extraneous org. matter), and the residual liquid, after concn. by evaporation, is tested on filter-paper with ninhydrin. Fermentation vinegars (I) show, in contrast to synthetic vinegars, a distinct violet coloration. Several common amino-acids have been identified in the test liquors from I.  
P. S. ARUP.

**Colour of coffee.** A. C. Little and G. Mackinney (*Food Technol.*, 1956, 10, 503—506).—A relationship was established between roasting temp. and (a) the colour of the roasted ground coffee, (b) the relative concentrations of the u.v. absorbing components in the aq. extracts prepared under standardized conditions and (c) the colour of the aq. extracts.  
E. M. J.

**Chlorogenic acid in raw coffee.** C. Griebel (*Z. Lebensmittelforsch.*, 1956, 104, 173—182).—In *Coffea arabica*, L. about half of the total chlorogenic acid (6—7%) and less in *C. robusta*, Chev. crystallized as K caffeinate chlorogenate. A non-crystallizing form isochlorogenic acid with high rotatory power occurs also, both forms being in combination with K, or Mg or Ca. Caffeic acid (0.5—0.6%) in raw coffee, as reported by the method of Slotta and Neisser, was not found. Aq. solutions of raw and roasted coffee contain small quantities of a substance, precipitable by egg albumin solution or alcohol, which is adsorbed on the chlorogenic acid ppt.  
E. M. J.

**Rapid volumetric determination of caffeine in coffee extracts.** G. Prange and H. Walther (*Z. Lebensmittelforsch.*, 1956, 104, 261—268).—The ground coffee is extracted with boiling water and the aq. extract is shaken with NaOH and chloroform. The separated chloroform solution, covered with 5 ml. of water in a small evaporating dish, is heated gently to remove the chloroform. A caffeine periodide ppt. is formed on a Jena glass filter crucible by adding 2 ml. of 16% H<sub>2</sub>SO<sub>4</sub>, 2 ml. of 0.1N-I solution and the cooled aq. solution of caffeine. This ppt. is dissolved in methanol, water is added and the mixture is titrated with 0.01N-thiosulphate and starch indicator. Results, obtainable within 30 min., compare favourably in accuracy with other methods.  
E. M. J.

**Relative merits of various tests for determining bacteriological quality of milk for pasteurization.** Committee on Applied Laboratory Methods, International Association of Milk and Food Sanitarians, Inc. (*J. Milk Tech.*, 1956, 19, 7—11, 17).—Reports are given on (a) relationships between various bacteriological methods for examining raw milk, including comparisons of tests with 700 producers of the direct microscopical count, standard plate raw count and laboratory pasteurized count techniques, the second of these appearing significantly superior, (b) fat tests for dairy produce, (c) methods for detection of proteolytic organisms (17 references), and (d) a survey of legal coliform standards for pasteurized milk and other dairy products in force in U.S.A.  
J. S. C.

**Come-up time method of milk pasteurization. I. Laboratory instrument.** R. B. Read, jun., T. C. Boyd, W. Litsky and D. J. Hankinson. **II. Investigation of milk properties and some bacteriological studies.** R. B. Read, jun., N. L. Norcross, D. J. Hankinson and W. Litsky (*J. Milk Tech.*, 1956, 19, 39—44, 45—49).—I. A heating test apparatus, designed to study in detail the effects of various pasteurization procedures on milk inoculated with a given organism, is described.

II. An evaluation was carried out of rapid heat treatment of milk. Phosphatase is inactivated in 0.25 sec. at 182.3°F. and in 0.5 sec. at 178.5°F. No significant pH changes occur. Creaming is markedly impaired at temp. >185°F. Curd formation tests showed no marked protein denaturation. (11 references.)  
J. S. C.

**Test for keeping quality of pasteurized milk.** E. A. Day and F. J. Doan (*J. Milk Tech.*, 1956, 19, 63—66).—Neotetrazolium [i.e., *pp'*-diphenylene-bis-2-(3:5-diphenyltetrazolium)chloride] dye (0.2% aq. solution) is mixed with milk (0.5 ml. to 5 ml. milk) in a test tube which is evacuated, sealed, and incubated at 37° for 4 hr. A pink colour indicates that the milk will undergo flavour spoilage within four days. The test apparently measures psychrophilic activity. (12 references.)  
J. S. C.

**Temperature rise of milk stored overnight in cans under hot weather conditions.** J. Hutchinson and J. B. Hoyle (*J. Soc. Dairy Tech.*, 1956, 9, 60—63).—Detailed measurements of the temp. variations of canned milk during overnight storage show that thermal insulation procedures are necessary if temp. >50° (at which bacterial proliferation becomes rapid) are to be avoided.  
J. S. C.

**A critical look at pasteurization standards.** E. B. Collins and W. L. Dunkley (*J. Milk Tech.*, 1956, 19, 285—289).—A discussion of limits for establishing min. pasteurization standards.  
J. S. C.

**Ultra-high-temperature sterilization of milk.** L. F. L. Clegg (*J. Soc. Dairy Tech.*, 1956, 9, 95—105).—The question of whether ultra-high temp. sterilization is desirable in British dairies is discussed in the light of detailed tests and experiments, the results of which are tabulated. (27 references.)  
J. S. C.

**Effect of heat on the colour of milk.** H. Burton (*J. Soc. Dairy Tech.*, 1956, 9, 146—147).—Browning of milk during sterilization, caused by reaction between lactose and milk protein in water, and the use of reflectance curves obtained with a spectrophotometer to measure it objectively, are discussed.  
J. S. C.

**Hydrogen-ion concentration of milk. II. Effect of some factors on the pH of milk.** M. B. Rao and N. N. Dastur (*Indian J. Dairy Sci.*, 1956, 9, 114—123; cf. *J.S.F.A. Abstr.*, 1956, i, 281).—The effects of stage of lactation, dilution, skimming and processing at different temp. on the pH of milk were examined and data are presented. Colostrum had low pH and high acidity; from the third day after parturition the pH steadily increased until ~20th day, and afterwards there was no change in pH to the end of lactation. The average pH of goats', sheep's, asses' and human milk were 6.54, 6.54, 6.97 and 7.27 respectively. Skimming, dilution with water, pasteurization and boiling increased the pH. In sterilized milk, the pH was decreased. (19 references.)  
E. M. J.

**Hydroxamate method of characterizing milk fat.** R. Basoette and M. Keeney (*J. Ass. off. agric. Chem. Wash.*, 1956, 39, 469—475).—Characterization of milk fat is based on the reaction between org. esters with hydroxylamine under alkaline conditions whereby hydroxamic acid deriv. of the corresponding org. acids are formed; these substances form coloured complexes with ferric ions in acid media and may be determined spectrophotometrically. A detailed description of the recommended technique is given. Values for 34 examples define the normal range of hydroxamic acid index of mixed herd milk fat. The method gives reproducible results which have a correlation coefficient of 0.88 with determinations of butyric acid content. The fact that the max. colour development under ideal conditions is not attained is not critical.  
A. A. ELDRIDGE.

**Variation in fat content of herd bulk milks.** R. A. Edwards (*J. Soc. Dairy Tech.*, 1956, 9, 63—68).—The mixed milks of 28 herds were analysed daily for fat content over a period of 13 days. Daily figures, together with max. and min. and mean daily variations, are given and coeff. of variation are calculated for individual herds. Estimates are made of the accuracy of the fat % figure for a single sample of mixed herd milk, and of a mean value derived from the fat contents of four random samples of such milk, with respect to the true mean fat % for individual herds and for grouped herds.  
J. S. C.

**Size of fat globules and the creaming power of cow, buffalo, sheep and goat milk.** A. H. Fahmi, I. Sirry and A. Safwat (*Indian J. Dairy Sci.*, 1956, 9, 124—130).—The average size of fat globules was 3.30, 3.49, 4.55 and 5.92 $\mu$ . for sheep's, goats', cows' and buffalo milk respectively; the higher the % of small-sized globules in the milk, the lower the rate of fat separation. The relative efficiency of skimming is mainly determined by the effective size of the fat globules. (11 references.)  
E. M. J.

**Seasonal variations in the Reichert and iodine values of milk fat.** S. R. Sampath and C. P. Anantkrishnan (*Indian J. Dairy Sci.*, 1956, 9, 135—141).—From analysis of many samples of market milk (cows', buffalo and mixed) the Reichert value of the fat ranged from 20.0—31.9; 23.5—34.4; and 23.5—34.2 units respectively and corresponding I val. from 20.9—47.0; 21.8—46.3 and 22.2—48.1 units respectively. The differences in the monthly variations in Reichert and I val. were statistically significant. The differences between the Reichert, but not between the I val., of cows' and buffalo milkfat were statistically significant. (11 references.)  
E. M. J.

**Direct microdetermination of calcium in milk.** J. R. Marier and M. A. Boulet (*J. agric. Food Chem.*, 1956, 4, 720—722).—The turbidimetric method described directly measures 40—100  $\mu$ g. of Ca in a sample contained in a colorimetric tube. The turbidity is developed using potassium oleate solution. Citrate-phosphate reagent is added to stabilize the suspension and gelatin is added since it contributes to sensitivity, improves stability and helps to reduce interference from other ions. The turbidity is measured at 420  $\mu$ ., 20—60 min. after addition of the reagent. Results reported average 0.8% above those obtained by a KMnO<sub>4</sub> titration method. The coeff. of variability of single determinations on standard solutions was  $\pm 3.2\%$ .  
N. M. WALLER.

**Characterization by paper electrophoresis of milk proteins and their transformation products.** H. Schober and H. Hetzel (*Z. Lebensmittelforsch.*, 1956, 104, 323—327).—In sterile milk casein, no changes

in solubility, P content or electrical behaviour, as compared with native casein were found on examination by paper electrophoresis, whereas with high temp. treatment essential changes appear in the protein composition of milk serum. With various kinds of cheese the first whey (broken whey) was compared with that collected after five hours; the observed changes of the % constituents of separate protein components were discussed. In Limburg cheese ripening, no change in P content of the separate casein components was found. The sources of error in the dyeing of casein with Azocarmine B were discussed. E. M. J.

**Density of processed milk.** A. L. Short (*J. Soc. Dairy Tech.*, 1956, 9, 81—86).—Small permanent changes in  $d$  of milk are caused by homogenization and sterilization but are not big enough to explain the difference in  $d$  between raw and sterilized milk. This is attributed to the lag in the phase change of fat after a change of temp. This phase lag is exaggerated on dispersion of the fat by homogenization. J. S. C.

**Antibiotic detection in milk.** L. A. Nutting and F. W. Barber (*J. Milk Tech.*, 1956, 19, 162—164).—Most procedures for detection of antibiotics in milk are based on the inhibition of metabolism of dairy streptococci as evidenced by decrease of acid production rate or reductase activity. These methods are compared and discussed in a review of the relevant literature. (19 references.) J. S. C.

**Determination of antibiotics in milk.** C. K. Johns and I. Berzuis (*J. Milk Tech.*, 1956, 19, 14—17).—Various methods are compared. The sensitivity of the disc assay procedure (cf. J.S.F.A. Abstr., 1956, i, 227) can be increased by (1) reducing the vol. of seeded agar per plate to 4 ml., (2) refrigerating "planted" plates for 2—6 hr., (3) avoiding overnight incubation at 37°, and (4) using larger (0.5 cm.) paper discs. The methylene blue test was found to be sensitive to aureomycin, *Bacillus cereus* proving a more sensitive test organism than *B. mesentericus*, and broth cultures more satisfactory than spore suspensions. J. S. C.

**An effective "single solution" stain.** D. Levowitz and M. Weber (*J. Milk Tech.*, 1956, 19, 121, 127).—A modification of Newman's No. 2 Stain for examination of milk samples, which avoids some of the drawbacks of the original stain (e.g., loss of smears at rinsing, dye pptn., mottled or too deeply coloured backgrounds) is described. J. S. C.

**Statistical analysis of reduction times in relation to plate counts.** Eugene K. Harris, Robert C. Thomas and Luther A. Black (*J. Milk Tech.*, 1956, 19, 243—247).—Some 400 results of plate counts and dye reduction times in split milk samples were statistically analysed and a procedure proposed for estimating disagreements in grading when accepted plate count and various dye reduction time limits are applied. J. S. C.

**Efficacy of chlorine, a quaternary ammonium compound and a new disinfectant (Chlorhexidine) in test cup decontamination.** J. H. Stewart, G. R. Spencer, J. Lasmanis and D. M. Holliday (*J. Milk Tech.*, 1956, 19, 89—93).—The activities of the three disinfectants were compared against organisms capable of causing bovine mastitis. Chlorhexidine was found to be the most effective. (10 references.) J. S. C.

**Use of silicones in the dairy and food processing industries.** C. W. Todd (*J. Soc. Dairy Tech.*, 1956, 19, 94—99).—The main uses of silicones in the food industry are in foam control, prevention of adhesion in processing or packaging, and as heat-stable, moisture-resistant paints or greases. They are also being used as a scratch-resistant coating for glassware. (12 references.) J. S. C.

**Riboflavin content of buffalo milk curds.** T. J. Boman and U. C. Dalal (*Indian J. Dairy Sci.*, 1956, 9, 131—134).—In buffalo milk curds (a) from skimmed and (b) from whole milk, the riboflavin content was from 1.0 to 1.66 and 5.74 to 7.36  $\mu\text{g.}/\text{g.}$  of curds respectively, using *Lactobacillus casei* as the test organism in microbiological assay. (11 references.) E. M. J.

**Yoghurt and other cultured milks. I.** J. G. Davis (*J. Soc. Dairy Tech.*, 1956, 9, 69—74).—The manufacture of yoghurt, now almost invariably carried out with a 50:50 mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, is described. J. S. C.

**Yoghurt and other cultured milks. II, III.** J. G. Davis (*J. Soc. Dairy Tech.*, 1956, 9, 117—122, 160—165).—II. Laboratory control procedures in manufacture of yoghurt, including microbiological examination, testing of culture purities, flavour and odour assessments, acidity and consistency, are reviewed.

III. Defects encountered in commercial yoghurt are briefly summarized. The medical aspects of yoghurt consumption are reviewed both in regard to its effects on the alimentary tract, its nutritive value and digestibility. Other cultured milks, as produced in the U.S.A., U.S.S.R. and other countries are briefly described. (53 references.) J. S. C.

**Structure of butter as revealed by observations on microtome sections.** L. von Gavél (*Z. Lebensmittelforsch.*, 1956, 104, 1—21).

—The prep. and microscopical examination of the sections (of ripened cream butter) are described. The dispersal of the air in butter is promoted on working and moulding. The size of the water-droplets is increased on immersing the sections in water, but diminished on immersion in sugar solution or on air-drying. The aq. phase forms a continuous system with connecting channels between the droplets, formed by hygroscopic constituents. The mobility of the water is greatest in badly worked or brittle butter. The fat globules vary greatly in size, and form a loose network embedded in structureless fat. Cryst. formations occur at the surfaces of the globules, and extend inwards in varying extent. Butter samples from various creameries show characteristic variation in the fat-globule picture. Unusually large areas of structureless fat result from inadequate working. Defatted and stained sections show a continuous residual network (probably consisting of protein-phosphatide compounds), which increases in extent with the acidity of the cream from which the butter has been made. (77 references.) P. S. ARUP.

**Phage control during the production of commercial cheese starters.** J. E. Lewis (*J. Soc. Dairy Tech.*, 1956, 9, 123—128).—Failure of starters due to destruction of lactic organisms by bacteriophage is examined and a technique of laboratory control over the whole process from mother culture to bulk starter, designed to exclude air from containers at all stages of production, is described. J. S. C.

**Influence of environment and processing on spoilage organisms in cottage cheese.** L. G. Harmon and C. K. Smith (*J. Milk Tech.*, 1956, 19, 252—259).—Improperly pasteurized milk and cream, coagulator, starter, water, air and contaminated equipment were all sources of spoilage organisms. The results are discussed as a basis for recommending preventive procedures. (12 references.) J. S. C.

**Vitamin B<sub>2</sub> potency of Cheddar, Swiss and cottage cheese.** A. M. Hartman, L. P. Dryden and R. E. Hargrove (*Food Res.*, 1956, 21, 540—545).—The vitamin B<sub>2</sub> potency of samples of Cheddar, Swiss and cottage cheese was determined by a microbiological and by a rat assay method. Agreement of results by the two methods was better with cottage cheese than with other types. On a dry matter basis, natural Cheddar, Process Swiss and cottage cheese had average potencies of 33, 32 and 38  $\mu\text{g. per kg.}$ , respectively, as determined by rat assay; natural Swiss 58 and Process Cheddar 19  $\mu\text{g. per kg.}$  E. M. J.

**Use of sequestering agents to solubilize proteins in determination of extraneous materials in foods by direct filtration methods.** M. G. Yakowitz (*J. Ass. off. agric. Chem. Wash.*, 1956, 39, 532—533).—Cheese is soaked in water at 33—40° and the stirred mixture is maintained at pH 7.5 with Na<sub>2</sub>PO<sub>4</sub> for 2 hr. while pancreatin extract is added. Then, at pH 6—7, Chelox B 14 (a solution of amino-carboxylates) is added. After dilution a surface-active preparation (Igepon TK 32) is added and the mixture kept overnight before filtration. A. A. ELDRIDGE.

**Sucrose loss from ice cream on storage.** H. J. Evans, W. Kwantes, D. C. Jenkins and J. I. Phillips (*Analyst*, 1956, 81, 204—210).—In a bacteriological examination of stored samples showing loss of sucrose *Leuconostoc mesenteroides* was isolated. The ability of the organism to produce dextran in sucrose solution and to cause reduction of the sucrose content of ice cream was confirmed. *L. mesenteroides* survives pasteurization or so may be derived from the ingredients of the original mix or from outside contamination. Ice cream may be stored without change in "deep freeze." Since ice cream must contain  $\leq 7.5\%$  of sucrose, determination of sucrose should be made preferably while the sample is still frozen solid and certainly within a few hr. of its storage at room temp. A. O. JONES.

**Microchemical determination of iodine in milk and milk products.** H. Hänni (*Mikrochemie*, 1956 [1—3], 257—262).—The method involves pre-treatment of the sample with Na<sub>2</sub>CO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub> at relatively low temp., followed by incineration with acid KMnO<sub>4</sub> solution, I being subsequently liberated by reduction with H<sub>2</sub>PO<sub>3</sub> and distilled. No results, accuracy or precision stated. D. F. PHILLIPS.

**Causes and control of corrosion of stainless steel, especially in conjunction with milk and other food equipment.** G. M. Riegel (*J. Milk Tech.*, 1956, 19, 149—152).—The types of corrosion experienced with stainless steel are classified and methods of prevention described. Particular reference is made to detergent cleaning and examples of dairy equipment corrosion are described and discussed. The advantages of "in-place" cleaning in minimizing corrosion risks are emphasized. J. S. C.

**Effect of several organic and inorganic acids in inhibiting the heat precipitation of calcium hardness salts from water.** D. A. Evans and G. H. Watrons, jun. (*J. Milk Tech.*, 1956, 19, 213—216).—With

water of Ca hardness  $>20$  grains/gal., acidified to pH 5 with gluconic or levulinic acid, waterstone deposition is prevented. The acids studied were found to cause negligible corrosion in stainless steel.

**Penetration and growth of *Salmonella* in shell eggs.** J. L. Stokes, W. W. Osborne and H. G. Bayne (*Food Res.*, 1956, **21**, 510—518).—Shell eggs were contaminated with five strains of *Salmonella* and were incubated at 29° for 3 to 4 weeks. All the strains penetrated the shell membranes and multiplied to very high bacterial populations. No sign of infection appeared in identically treated eggs stored at 1° for six months. Min. temp. for growth of *S. oranienburg* and other *Salmonella* strains is  $\sim 10^\circ$ . (16 references.) E. M. J.

**Treatment and conservation of beef carcass for industrial refrigeration.** M. María Beltran (*An. Bromatologia*, 1956, **8**, 109—122).—An account is given of the preparation and slaughtering of cattle for refrigeration, and of handling and process temp. Physical and microbiological changes in frozen meat are mentioned, together with directions for successful thawing. (15 references.)

**Isolation and identification of nitrogenous components in meat.** J. C. Alexander and C. A. Elvehjem (*J. agric. Food Chem.*, 1956, **4**, 708—711).—The nitrogenous components of meat are identified using ion-exchange and paper chromatography procedures. Approx. 100% of the N content is accounted for by 21 amino-acids, ammonia and other constituents such as vitamins, purines, creatine, carnitine and methylguanidine. (15 references.) N. M. WALLER.

**Sodium ascorbate in stabilizing cured meat colour.** R. L. Henrickson, R. B. Sleeth and D. E. Brady (*Food Technol.*, 1956, **10**, 500—503).—The simultaneous injection method of incorporation of  $\text{NaNO}_2$  and Na ascorbate into hams is discussed. Hams cured in a brine containing ascorbic acid or Na ascorbate had a deeper red colour than the controls. Pork muscle cured in brine containing monosodium glutamate and Na ascorbate had a light grey pink colour, more characteristic of cured ham. E. M. J.

**Chemical and organoleptic changes in gamma-irradiated meats.** H. S. Groninger, A. L. Tappel and F. W. Knapp (*Food Res.*, 1956, **21**, 555—564).—The flavour of irradiated- $\gamma$  was significantly different from that of un-irradiated meat. Thiamine is destroyed by radiation. An inert atm. or vac. is recommended to prevent formation of lipid peroxides and carbonyls in beef and pork. Oxymyoglobin is increased in fresh meat, but there was loss of nitrohaemochrome pigment in cured ham. The carotenoid pigment of salmon was destroyed, but at the sterilizing dose the succinoxidase system was still active in beef, pork and tuna. (23 references.) E. M. J.

**Penetration of aureomycin into fish muscle and its destruction by heat.** G. Steiner and H. L. A. Tarr (*Canad. J. Technol.*, 1956, **34**, 215—219).—The grey cod (*Gadus macrocephalus*) rarely contains natural substances that interfere with the microbiological determination of aureomycin (I) in fish extracts. After storage for five days in ice containing  $1 \mu\text{g}$  of I per g., relatively small amounts of I were found in the flesh of eviscerated fish, and these were mainly in the visceral cavity walls and the skin; it is unlikely that the total amount of I absorbed would be  $>0.01$ — $0.2 \mu\text{g}$  after  $>10$  days. Absorption of I was rather greater in fish stored in refrigerated sea water containing  $2 \mu\text{g}$  of I per ml. Heating such as occurs in the rather mild cooking procedures recommended for fish destroys a very large proportion of the absorbed I. S. C. JOLLY.

**Biochemical processes in the decomposition of the hake.** G. Varela and R. Wojciech (*An. Bromatologia*, 1956, **8**, 5—18).—Decomposing hake produces trimethylamine and tyrosine in increasing amounts, but dimethylamine was found to be the most reliable indicator of extent of decomposition. The iodimetric method of Trutwin (cf. J.S.F.A. Abstr., 1954, i, 102; 1955, i, 324) is also a fairly good index of the extent of ageing, but is susceptible to the mode of preparation of the sample. (58 references.)

**Quality of sardines [*Clupea pilchardus* (Walb.) held unfrozen and frozen prior to canning.** W. A. MacCallum *et al.* (*Food Technol.*, 1956, **10**, 432—438).—Recommendations include icing the sardines in boxes within 2—3 hr. of catching and canning after not more than three days, storing at 30—31°F. (24 references.) E. M. J.

**Chemical changes in fish actomyosin during freezing and storage.** H. L. Seagran (*Food Res.*, 1956, **21**, 505—509).—After freezing and thawing fish, the fraction of actomyosin still sol. in salt solution had an increase in reactive SH groups of from 52.4 to 55.6% of total SH groups; the insol. fraction had an increase from 52.4 to 55.6%. Total SH groups remained constant on freezing (0.76 g. of cysteine per 100 g. of protein). On freezing actomyosin intrinsic viscosity immediately decreased from 1.70 to 1.17. Max. solubility was reached after storage for one month. E. M. J.

**South African pilchard oil. V. Isolation and structure of an eicosapentaenoic acid from South African pilchard oil.** J. M. Whitcutt and D. A. Sutton (*Biochem. J.*, 1956, **63**, 469—475).—All *cis-n*-eicosapenta-5:8:11:14:17-enoic acid is isolated from S. African pilchard oil by methods that involve Li salt-acetone separation, fractionation as the urea complex, mol. distillation, and reversed phase partition chromatography. J. N. ASHLEY.

**Technical innovations in olive oil processing.** P. G. Garoglio (*Olii min.*, 1956, **33**, 181—203).—Technical progress during the last three years is reviewed. Recent studies are described, dealing especially with the most suitable means for the preservation of olive oil from rancidity and from alterations, the identification of metal impurities, and the protection of its nutritional value from fraud. Some points of view on regulations governing Italian olive oil classification are described; the analytical method and the control are considered to be insufficient and obsolete. Various types of apparatus are illustrated. (26 references.) C. A. FINCH.

**Metal ion catalysis and polarity of environment in the aerobic oxidation of unsaturated fatty acids.** N. Uri (*Nature, Lond.*, 1956, **177**, 1177—1178).—The catalytic effects are described of various heavy-metal stearates, phthalocyanines and octamethyltetra-azaporphins on the aerobic oxidation of linoleic acid. Various polar and non-polar solvents were used. J. S. C.

**Iodimetric processes for determination of peroxide number in edible oils.** H. Hadorn, K. W. Bießer and H. Suter (*Z. Lebensmittelforsch.*, 1956, **104**, 316—323).—The method of Hadorn and Kungkuz, which yielded low results with oils having a very high peroxide no., was re-examined and compared with the methods of Sully and of Wheeler. With usual commercial oils having a peroxide no. of  $\sim 20$ , all methods were in good agreement. The size of the granules rather than the quantity of KI used, affected the results. With the finest powdered KI almost without exception, the right values were obtained, and also when the quantity of KI was reduced from 1.0 to 0.1 g. The modified method of Wheeler is recommended, using small quantities of reagents, at room temp. Reproducible results in good agreement with those of other methods are obtained. E. M. J.

**Identification of fat-soluble synthetic colouring and colouring prepared from annatto seeds in margarine.** F. Hoeke and H. Onrust (*Chem. Weekbl.*, 1956, **52**, 186—187).—The margarine is melted, mixed with tragacanth powder and filtered. The filtrate is dissolved in light petroleum and shaken with  $\text{Al}_2\text{O}_3$ , which is then extracted with EtOH. The extract if yellow is treated with  $\text{SnCl}_4$  and left 1 hr.; fading indicates presence of synthetic dyes. H. A. FISHER.

**Identification and determination of vitamin A and  $\beta$ -carotene in an animal-fat mixture.** J. Gillman, K. B. Norton, D. E. A. Rivett and D. A. Sutton (*Biochem. J.*, 1956, **63**, 458—460).—The vitamin A activity (as indicated by bioassay) of a mixture of rendered beef and mutton fats (4:1) is due entirely to vitamin A together with small amounts of  $\beta$ -carotene. An enormous concn. of vitamin A is effected in the unsaponified portions by reversed-phase partition chromatography. A chemical method is described for the determination of vitamin A in beef fat-mutton fat mixtures. It depends on hydrolysis of the fats by the method of Bolding *et al.* (*Brit. Abstr. C*, 1952, 113), separation of vitamin A by chromatography on  $\text{Al}_2\text{O}_3$  and determination of the vitamin by the Carr-Price method. J. N. ASHLEY.

**Compensating for vitamin A deficiency in the normal diet.** S. Borrell (*An. Bromatologia*, 1956, **8**, 19—35).—Carotene is extracted from the sample by several portions of 1:1 light petroleum-acetone mixture, the solvent washed 10 or 15 times with water to remove the acetone, dried with sodium sulphite, and poured through a dried alumina-sodium sulphate column. The adsorbed carotenoids are eluted with a 2 or 3% v/v solution of acetone in light petroleum, and estimated spectrophotometrically at 436 m $\mu$ . The method has been applied to a wide variety of fresh and preserved fruits and to greenstuffs and spices. L. G. L. UNSTEAD-JOSS.

**Colorimetric determination of L-ascorbic acid.** K. Kalinowski (*Roczn. Chem.*, 1956, **30**, 269—274).—Solutions of known iodine concn. are prepared by electrolysis of 10% aq. KI for 2 to 3 min., reading a microammeter at 30 sec. intervals. The solution is added to 5 ml. of 5% NaCl in 0.01N-HCl, containing 0.1—1 mg. of L-ascorbic acid, and the microammeter is again read, giving the excess of I not reduced by ascorbic acid. (12 references.) R. TRUSCOE.

**Use of homocysteine in determination of dehydroascorbic acid.** R. E. Hughes (*Biochem. J.*, 1956, **64**, 203—208).—At pH value above 6.8 homocysteine rapidly reduces dehydroascorbic acid to ascorbic acid which can be determined by the 2:6-dichlorophenolindophenol method, without appreciable interference from the excess of homocysteine present, provided that the reaction is carried out within 30 sec. at pH 2.5. Based on this, a routine method is described for

determination of dehydroascorbic acid which is more rapid and sp. than other methods. It involves titration with 2:6-dichlorophenol-indophenol before and after reaction with homocysteine.

J. N. ASHLEY.

**Rôle of dehydroascorbic and dehydroreductive acids in the browning reaction.** S. I. Dulkin and T. E. Friedemann (*Food Res.*, 1956, **21**, 519—527).—Reductic and ascorbic acids undergo a type of browning reaction analogous to that in heat-processed and stored foods, resulting in increased colour, fluorescence, reducing properties and acidity. Stages in the oxidation-reduction and oxidation reactions of both acids are discussed.

E. M. J.

**Vitamin E content of paprika fruits.** I. W. Feldheim (*Z. Lebensmitteluntersuch.*, 1956, **104**, 24—26).—Vitamin E contents (determined by the Emmerich and Engel method) of the various parts of the fruits increase to a max. with maturity, and show considerable varietal differences.

P. S. ARUP.

**Denaturation of pepsin in solution and at a surface. II. Denaturation at a surface.** H. A. Dieu (*Bull. Soc. chim. belg.*, 1956, **65**, 740—752).—Pepsin dissolved at pH 5.5 and spread on HCl at pH 2 has a film mol. wt. of 34,800 and a gaseous area of 1.91 sq. m. per mg. at low surface pressures (0.00005—0.02 dynes per cm.) and at higher pressures (0.02—0.6 dyne per cm.) a mol. wt. of 38,000 and an area of 0.71 sq. m. per mg. Changes of area and mol. wt. have been studied at a function of pH in the higher pressure range and are attributed to mol. swelling. Measurements are made of sedimentation and viscosity constants of collected films which exhibit no more proteolytic activity when spread at a pressure lower than 0.6 dyne per cm.

A. JOBLING.

**Amino-acid proportions in food proteins compared with proportions utilized in rat growth.** N. Raica, J. Heimann and A. R. Kemmerer (*J. agric. Food Chem.*, 1956, **4**, 704—707).—The essential amino-acids contents of thirty foodstuffs are determined and compared with the requirements of the growing rat. (17 references.)

N. M. WALLER.

**Electrophoresis cells modified for determination of labelled protein fractions.** R. E. Hein, R. E. Clegg, T. J. Clark and R. H. McFarland (*Analyt. Chem.*, 1956, **28**, 544—545).—The modification of the ascending arm of the centre cell of a Tiselius electrophoretic apparatus is described. The modification does not affect electrophoretic patterns, protein mobilities or concn. of separated protein fractions.

K. A. PROCTOR.

**Modified method for protein separation by zone electrophoresis on starch gel.** P. Bernfeld and J. S. Nisselbaum (*J. biol. Chem.*, 1956, **220**, 851—860).—A starch gel that consists of 3 maize starch, 1.5 additional amylose, 3% Hyflo Supercel and the appropriate buffer solution is an improved supporting medium in zone electrophoresis for fractionation of plasma proteins. This medium forms a stiff gel which is easily cut into small sections. Electro-osmotic flow is negligible because of the low content of solids. This gel-medium allows considerable increase in the capacity of the cell. The method of fractionation is given in detail and two simple devices are described: a small cell for the separation of 2 ml. and a large cell for fractionation of 50 ml. of a 5—10% protein solution. (19 references.)

J. N. ASHLEY.

**Quality control in the food industry.** C. A. Bassett (*Chem. & Ind.*, 1956, 786—788).—A review of general principles and requirements.

J. S. C.

**Effect of various methods on the nitrogen and fat contents of biological materials.** J. H. Watts, L. K. Booker, W. G. Wright and E. G. Williams (*Food Res.*, 1956, **21**, 528—533).—Statistically, there was no difference at the 1% level in % N and fat retained in egg, ground beef and whole milk on drying by the air oven, vac. oven, i.r. rays, or lyophilizer.

E. M. J.

**Micro-determination of iodine in dietary foods, vitamin-mineral preparations, and drugs.** D. Menschenfreund (*J. Ass. off. agric. Chem. Wash.*, 1956, **39**, 523—528).—I is converted into KI which is oxidized with  $KMnO_4$  to  $KIO_3$ ; this is then treated with KI and the liberated I is extracted twice with xylene and determined spectrophotometrically. Using 1 or 2 tablets containing 0.018—0.173 mg. I consistent recoveries of approx. 100% were obtained.

A. A. ELDRIDGE.

**Moisture determination of foods by hydrogen nuclei magnetic resonance.** R. H. Elsen and C. H. Kunsman (*J. Ass. off. agric. Chem. Wash.*, 1956, **39**, 434—444).—In the determination of water by the nuclear magnetic resonance method, only one absorption band, that of the hydrogen nuclei, is utilized. The method is satisfactory in the absence of an appreciable quantity of sol. solids (I). If the amount of I is small and constant a calibration curve can be constructed. For potatoes, more than 90% of measurements agreed with vac. oven determinations to within  $\pm 2\%$ . The standard deviation was 1.2%. That for apples, 7.7%, was reduced to 1.3% by applying a correction for I.

A. A. ELDRIDGE.

**Surface-active agents in the food industries. I.** F. Aylward (*Rev. Ferment.*, 1956, **11**, 75—83).—A general classification and review of the chemical structures of both polar and non-polar surface-active agents used in food processes. (11 references.)

J. S. C.

**Certified food colours.** A. T. Schramm (*Dyestuffs*, 1956, **41**, 146—156).—A historical review of the use of dyes for colouring foods.

J. S. C.

**The enumeration of *Staphylococcus aureus* in foods.** D. A. A. Mossell and C. M. A. Vendrig (*Leeuwenhoek Ned. Tijdschr.*, 1956, **22**, 205—208).—*S. aureus* is enumerated using a 7.5% NaCl-medium, at pH 7.0  $\pm$  0.1, containing 2 g. of lactose, 10 g. of D-mannitol, 10 g. of tryptone, 2.5 g. of yeast extract, 30 g. of gelatin and 15 g. of agar per l. of water. The drop plate technique of inoculation is used. Colonies so obtained may be used immediately for testing their coagulase reaction.

N. M. WALLER.

**Procedure for evaluating the efficiency of bactericidal agents.** C. W. Chambers (*J. Milk Tech.*, 1956, **19**, 183—187).—The method described is based on growing test organisms (*Escherichia coli* or *Micrococcus pyogenes* var. *aureus*) on agar, suspended in buffered water and filtered through sterile filter paper. It combines flexibility with a considerable degree of bacteriological control and provides a test volume large enough to enable a reasonable no. of aliquots to be withdrawn for chemical examination. (10 references.)

J. S. C.

**Thermal resistance of food poisoning organisms in poultry stuffing.** R. C. Webster and W. B. Esselen (*J. Milk Tech.*, 1956, **19**, 209—212).—The thermal death times of *Salmonella enteritidis*, *Micrococcus pyogenes* var. *aureus* and *Streptococcus faecalis*, in poultry stuffing, were investigated and results are tabulated and shown in graphical form. *Str. faecalis* was considerably more resistant to heat than the other two organisms. The data indicate that roasting procedures for stuffed poultry, based on attaining a centre stuffing temp. of 165°F., will destroy these organisms.

J. S. C.

**Resistance of bacterial spores to superheated steam.** C. P. Collier and C. T. Townsend (*Food Technol.*, 1956, **10**, 477—481).—Using standard test organisms: Putrefactive Anaerobe 3679, Flat Sour 1518 (*Bacillus stearothermophilus*) and *B. polymyxa*, sterilization of surfaces was achieved in the canning industry by heating with super-heated steam to a temp.  $>350^\circ\text{F}$ . for 1 min. or  $>320^\circ\text{F}$ . for 10 min.

E. M. J.

**Use of ionizing radiation in food industry.** P. Chovin (*Ann. Falsif., Paris*, 1956, **49**, 203—206).—A review covering bactericidal, pesticidal, and (for vegetables) anti-sprouting effects of the radiation, concomitant effects on the irradiated foods, and possible future developments of the technique.

P. S. ARUP.

**Organoleptic studies of irradiated foods.** G. E. Pratt and O. F. Ecklund (*Food Technol.*, 1956, **10**, 496—499).—Statistical analysis of taste test scores indicated significant off-flavour in all samples of irradiated meats and vegetables. Changes in appearance or flavour developed on storage. (15 references.)

E. M. J.

**Rôle of enzymes in food flavours.** E. J. Hewitt, D. A. M. Mackay, K. Konigsbacher and T. Hasselstrom (*Food Technol.*, 1956, **10**, 487—489).—Flavour development in foods and restoration of flavour in processed foods are discussed.

E. M. J.

**Bread-making.** Baker Perkins, Ltd. (Inventor: A. R. Palmer) (B.P. 744,366, 5.11.52).—A cutter and depositor for dough in bread-making are described.

K. RIDGWAY.

**Crystallization of sugar from molasses.** N.V. Centrale Suiker Maats. (B.P. 744,114, 24.11.53. Neth., 11.12.52 and 25.6.53).—Recovery of high quality sugar from molasses is effected by admixing with MeOH (0.6—2 pt.) and acid (to give pH  $<$  4, preferably  $<$  3), then removing pptd. salts (above room temp., at e.g., 35—80°), and allowing the sugar to crystallize from the filtered solution, preferably after diluting with a MeOH-miscible liquid which neither dissolves sugar nor flocculates molasses, e.g., ethyl acetate or benzene (45—200 g. per kg. of filtrate).

F. R. BASFORD.

**Dry coffee extract.** Aktieselskabet Niro Atomizer (Inventor: J. E. Nyrop) (B.P. 744,757, 29.9.52).—Ground roasted coffee is treated at 90° with a current of  $CHCl_3$  vapour, which is subsequently condensed and the condensate mixed with cocoa butter (e.g., 100 kg. of coffee, 10 kg. of  $CHCl_3$  and 0.5 kg. of cocoa butter). The coffee residue is extracted counter-currently with water at 110° (130 kg.) to yield an extract with 30% total solids. This is emulsified with the cocoa butter containing the volatile matter from the coffee and the mixture is spray-dried to give small flavour-containing particles enveloped in sol. coffee solids.

F. R. BASFORD.



**Vitaminized products.** Institut National de la Recherche Agronomique (Inventor : A. C. G. François) (B.P. 744,091, 9.7.53).—Vitamin-active material (I) is homogeneously distributed in an aq. dispersion of protein (II), optionally containing lipidic, glucidic, or mineral substances and antioxidant, then II is coagulated (by adding non-toxic, chemical coagulant) to enclose I, and give a stabilized product. The coagulated material is washed with solvent for I, then dried as quickly as possible. F. R. BASFORD.

**[Silo] structures for the storage or treatment of grain and other crops or granular material.** J. H. de W. Waller (B.P. 744,193, 22.7.53). K. RIDGWAY.

### 3.—SANITATION

**Methods and equipment used for sanitation analysis [of flour].** R. H. Cory (*Cereal Sci.*, 1956, 1, 68—71).—A survey has been made of the methods and equipment used in the U.S.A. for examination of wheat flour for contamination with insect fragments and rodent fith. S. C. JOLLY.

**Counting insect fragments and rodent hairs [in flour].** R. H. Cory (*Cereal Sci.*, 1956, 1, 71—74).—The results obtained in a collaborative experiment on residues from the digestion of contaminated flour have been examined by an improvised statistical method in order to allocate accuracy ratings to the collaborators. Correlation coefficients have been calculated between no. of fragments or hairs reported, time taken and visual acuity, and each factor correlated with the accuracy ratings. S. C. JOLLY.

**Serum alkaline phosphatase levels weight changes and mortality rates of rats fed endrin.** S. C. Nelson, T. L. Bahler, W. V. Hartwell, D. A. Greenwood and L. E. Harris (*J. agric. Food Chem.*, 1956, 4, 696—700).—Serum alkaline phosphatase values are higher among rats consuming endrin (1, 5, 25, 50 and 100 p.p.m. in basal diet), than in a control group. Rats receiving 100 p.p.m. of endrin died within two weeks. All rats consuming endrin showed hypersensitivity to various stimuli and wt. loss. Male rats are significantly more susceptible to the toxic effects than female rats. (20 references.) N. M. WALLER.

**Toxic effect of 2-ethylhexane-1:3-diol, used as disinfectant.** G. Bornmann and A. Loeser (*Z. Lebensmittelforsch.*, 1956, 104, 28—32).—From experiments in which rats were exposed to the atomized insecticide, in admixture with other glycols, it is concluded that no harmful effects on personnel or farm animals will ensue during normal application. P. S. ARUP.

**Bacteriological standards for bathing waters.** W. F. Garber (*Sewage industr. Wastes*, 1956, 23, 795—807).—The bacteriological standards relating to surface bathing waters in U.S. are reviewed and compared and an attempt is made to define minimum requirements. (20 references.) J. S. C.

**Survival of Coxsackie virus in water and sewage.** N. A. Clarke, R. E. Stevenson and P. W. Kabler (*J. Amer. Wat. Wks Ass.*, 1956, 48, 677—682).—A series of tests were made to determine the relative survival times of Coxsackie virus in raw sewage, distilled water, and raw and autoclaved river waters. The rate of inactivation of the virus increases with temp. and is also correlated with the degree of pollution of the water. (17 references.) J. S. C.

**Waste treatment by optimal aeration.** N. Porges (*J. Milk Tech.*, 1956, 19, 34—38).—The accelerated oxidation process of dairy waste treatment (cf. J.S.F.A. Abstr., 1956, i, 176, 296) is based on the principle of satisfying a high O<sub>2</sub> demand during a short assimilation period and utilizing endogenous respiration at a low rate of O<sub>2</sub> demand for subsequent sludge digestion. (17 references.) J. S. C.

**Biology, biologists and industrial waste treatment.** W. B. Hart (*Industr. Engng Chem.*, 1956, 48, No. 7, 81—83A).—The function of biology and biochemistry in industrial waste treatment is discussed. O. M. WHITTON.

**Process design of aeration systems for biological wastes.** W. W. Eckenfelder, jun. (*Chem. Engng Progr.*, 1956, 52, 286—292).—Operating figures for various forms of aerator are given, with final reduction to the form of power required per unit of oxygen absorbed. Normal figures vary from 0.2 to 0.5 kw.-hr./lb. of O<sub>2</sub>; a general equation for air flow, power and oxygen absorption is derived. (28 references.) R. RUMFORD.

**Agricultural wastes.** S. J. J. de Swardt (*S. Afr. industr. Chem.*, 1956, 10, 128).—Waste materials of potential value are listed. They include undergrade citrus fruits and potatoes, citrus and pineapple peel and groundnut and egg shells. N. M. WALLER.

**Industrial use of agricultural products.** W. S. Rapson (*S. Afr. industr. Chem.*, 1956, 10, 136—138).—Possible liaison between agricultural, industrial and scientific interests in S. Africa is proposed. N. M. WALLER.

**Plastic rodent-repellent composition.** B. F. Goodrich Co. (B.P. 744,993, 12.2.53. U.S., 10.3.52).—Complex obtained by interaction of a Zn dithiocarbamate, e.g., Zn dimethyldithiocarbamate, with a primary or sec.-amine of ionization constant  $>1 \times 10^{-7}$ , e.g., cyclohexylamine, is uniformly dispersed (1—200 wt.%) in org. plastic material, e.g., polyvinyl chloride, to provide a rodent repellent composition. Preferably the composition is employed in the form of a thin flexible film backed by fibrous material. F. R. BASFORD.

### 4.—APPARATUS AND UNCLASSIFIED

**How to build and use glass evaporation plant.** R. Scott (*Food Manuf.*, 1956, 31, 291—294, 296).—The construction, using glass, of a pilot-scale climbing film evaporator and a recirculating calandria evaporator are described. J. S. C.

**Three devices which aid crude fibre determination.** V. C. Midkiff (*J. Ass. off. agric. Chem. Wash.*, 1956, 39, 529—531).—(a) The apparatus is mounted on automobile valve springs so that it can be given a circular motion to rotate the liquid in a no. of beakers at the same time; (b) the capacity of the Gooch crucible is increased by attaching to it an inverted polyethylene bottle with the bottom removed, so that all the hot NaOH solution can be poured in one operation; (c) a wooden holder for tubes carrying hot NaOH solution and fitted with pinchcocks facilitates control of the flow. A. A. ELDRIDGE.

**Determination of insecticide residues in plant material.** I. Otter (*Mikrochemie*, 1956, [1—3], 125—133).—Methods are described for assessing the organophosphorus insecticides known as dimfox, schradan and mipafox at the 0.05—0.1 p.p.m. level. D. F. PHILLIPS.

**Microdetermination of calcium and magnesium in tissue ashes.** R. L. Griswold and N. Pace (*Analyt. Chem.*, 1956, 28, 1035—1037).—A procedure for separating micro-mol amounts of Ca and Mg from each other and from PO<sub>4</sub><sup>'''</sup> in biological tissues is described. The ashed sample is dissolved in water and passed through a cation-exchange column, after which Ca<sup>++</sup>, Mg<sup>++</sup> and PO<sub>4</sub><sup>'''</sup> are eluted one at a time. After evaporation to dryness and re-dissolution, Ca and Mg are each titrated with EDTA (disodium salt) at pH 10.5, using Eriochrome black T as indicator. Recoveries are ~100% for Ca and ~97% for Mg. W. J. BAKER.

**Determination of 20—100 μg. quantities of organic nitrogen.** D. Exley (*Biochem. J.*, 1956, 63, 496—501).—An ultramicro method is described. It involves digestion of the sample with H<sub>2</sub>SO<sub>4</sub> in presence of Se, and direct addition of Russel's reagent (Brit. Abstr. C, 1945, 129) (a phenol-hypochlorite reagent) after neutralization of the digest. The colour development is measured at 625 mμ. in specially-constructed capillary cells. The method is applicable to a range five times smaller than any previous method. The standard error of a single determination  $>6.5\%$  at 50 μg., and  $\pm 8\%$  for the range 5—20 μg. of N. The method is readily applicable to the micro scale (1.0—10 μg. of N) when no special apparatus is needed. J. N. ASHLEY.

**Determination of menadione and methods for identification of quinones.** W. J. Canady and J. H. Roe (*J. biol. Chem.*, 1956, 220, 563—570).—A colorimetric method is described for determination of 0.05—0.4 μmol. of menadione per ml. of solution. The method is based on formation of an intense blue colour when the 2:4-dinitrophenylhydrazine deriv. of menadione in ethanol is converted into an alkali salt. The NH<sub>4</sub> salt is used for the determination, and the optical density of the colour is read at 635 mμ. The method is very simple and extremely sensitive. The colour formation used in this procedure is employed as the basis of methods for identification of quinones by column and paper chromatography. (12 references.) J. N. ASHLEY.

**Colorimetric determination of magnesium and ammonium with sodium polyacrylate.** A. Mehlich (*J. Ass. off. agric. Chem. Wash.*, 1956, 39, 518—523).—Sodium polyacrylate is superior to any of the stabilizing agents previously used in the determination of Mg by means of Thiazole-yellow and of NH<sub>4</sub> by means of Nessler's reagent. For soils and plants a compensating solution containing CaCl<sub>2</sub>, AlCl<sub>3</sub>, hydroxylamine hydrochloride and triethanolamine is employed to avoid interference by Ca, Al, Mn and Fe. Procedure is detailed. A. A. ELDRIDGE.



## Journal of Applied Chemistry

The following papers are appearing in the February, 1957, issue of the *Journal of Applied Chemistry*

The decolorization of sugar liquor by bone charcoal

*By H. M. Thompson, G. J. Rayner, S. Hill  
and H. C. S. de Whalley*

The Pen Softening Point test (for bituminous materials)

*By A. L. Deadman*

The oxidation of monoethenoid fatty acids and esters.

The autoxidation products of erucic acid and its methyl and *n*-propyl ester

*By J. H. Skellon and P. E. Taylor*

The purification of plutonium by a thenoyltrifluoroacetone (T.T.A.) process

*By J. G. Cuminghame and G. L. Miles*

The volumetric determination of sulphate using a high-frequency conductimetric method

*By G. R. Jamieson*

Some characteristics of epoxide resin systems

*By F. J. Allen and W. M. Hunter*

Liquid-liquid equilibrium in systems containing nicotine

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