THE CORRELATION OF EASILY SOLUBLE PHOSPHORUS IN SOILS WITH RESPONSES OF CROPS TO DRESSINGS OF PHOSPHATE FERTILIZERS *

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When sufficient numbers of soils are examined empirical determination of easily soluble soil phosphate can be used to forecast crop responses to fertilizer dressings. Correlations between soil analyses and crop behaviour fail for individual soils and the causes have been examined.

Dilute acids dissolve relatively large quantities of phosphate from some soils where crops respond to phosphate fertilizers owing to (1) dissolution of iron and aluminium phosphates, which are useless to crops, (2) use of too much solvent, (3) use of an unsuitable solvent and (4) dissolution of phosphate from the interior of particles of calcium carbonate in calcareous soils.

Other soils contain very little phosphate soluble in dilute acid but, nevertheless, crops grown on them do not respond to phosphate fertilizers. In such cases crop growth may be limited by some other factor, such as drought. Phosphate dissolved by dilute acids is immediately reprecipitated by some soils; the extent of such reactions may be estimated by repeated extractions in the presence of added phosphate. Most mineral soils in eastern England fix very little phosphate during acid extraction, but fen soils and ferruginous soils may fix considerable amounts.

Changes in the amounts of soil phosphate that are soluble in dilute acid may occur when air-dried soils are stored. Such changes are accentuated by incubating moist soils with or without calcium hydroxide. Incubation experiments may be used to forecast increases in dilute-acid-soluble phosphate that occur during the growing period of a crop and changes which occur in easily soluble phosphate when acid soils are limed. Incubation with lime was used to measure reserves of soil phosphorus that were easily converted into forms soluble in dilute acids; such measurements were more satisfactory than simple extraction with dilute acid in relating crop responses and soil-phosphorus status in one group of field experiments.

Introduction

The results of soil analyses can be related to recommendations for manurial treatment only by means of field experiments that measure the responses of crops to dressings of phosphate fertilizers. There are few published accounts of work in England where the effects of fertilizers are related to analyses of the soils on which the tests were carried out. Such work is of little general value unless comparisons are made on a large number of soils and over several seasons; the experiments must be repeated for all important crops until some general pattern emerges.

Crowther[1] stated the results of a large number of comparisons of soil analyses and crop responses from 10 years' work on sugar-beet manuring. These data show that when sufficient numbers of soils are examined empirical determinations of easily soluble soil phosphate can be used to forecast responses to fertilizer dressings. Bondorff[2] summarized the results of 2000 Danish experiments and related the response of barley to superphosphate dressings with the analyses of soils taken from the experimental sites.

In such work relationships between soil analyses and crop responses become more reliable when the results of large numbers of field experiments are available. Inevitably there are exceptions where correlations fail. In the work described here some soil properties and conditions of acid extraction were examined which may lead to unsatisfactory values for easily soluble phosphate. Suggestions are made for using soil analyses to better advantage in guiding manuring on commercial farms.

There are two clear cases where correlations of acid-soluble soil phosphate with responses of crops to phosphate fertilizers are unsatisfactory. In one set of circumstances crops grown on soils with 'high' acid-soluble phosphate may nevertheless respond to phosphate fertilizers. On other soils, having 'low' acid-soluble phosphate, fertilizers may not increase crop yields. Some reasons for these two sets of circumstances were investigated separately in the present work. Changes in the equilibria between soil phosphorus compounds that occur during storage or after liming were also investigated.

Analytical methods

All determinations of phosphate were made by a colorimetric method described by Truog & Meyer.[3] Acetic acid extracts of mineral soils required no special precautions and phosphate determinations were made directly on the filtrates obtained after extraction.

It is not possible to determine soluble phosphate directly on soil extracts made with citric

* Read before the Agriculture Group on 16 December, 1952

acid since citrates interfere with reduction of phosphomolybdate complexes by stannous chloride (in forming the usual blue colour). The following method was therefore adopted when analysing solutions containing citric acid: 6 ml. of extract, together with 0·5 ml. of 0·5M-calcium chloride solution, was evaporated to dryness in a silica basin and ignited at 400–420° for 15 minutes. Hydrochloric acid (1 ml. of 6N) was added to the cool ignited residue and the liquid was evaporated to dryness on a water bath. Sodium hydroxide (6 ml. of 0·04N) was added and the residue was rubbed with a glass rod to dissolve potassium compounds. The solution was filtered through a dry paper and, when appropriate, potassium was determined in the filtrate. The filter paper and residue (which contained all the phosphate of the original extract) were returned to the basin, 10 ml. of N-sulphuric acid was added and the residue and filter paper were rubbed with a glass rod. After standing for 10 minutes the liquid was filtered and, after washing the residue, the filtrate was made up to 100 ml. A suitable aliquot was made neutral to phenolphthalein with ammonia and phosphate was determined by the method of Truog & Meyer. The amounts of phosphate dissolved from soils by citric acid and determined by this procedure agreed satisfactorily with values obtained by more tedious gravimetric methods. Organic matter, frequently present in citric acid extracts of soils, was destroyed during the ignition. On a few soils having less than 5 mg. of P_2O_5 per 100 g. of soil soluble in 1% citric acid there was slight interference due to ferric compounds and the blue colours developed had a greenish tint. The ammonia used in the neutralization enhanced the blue colour slightly, and all standard solutions used for calibrating the method were made up to contain the same amount of ammonia.

Experimental results

'High' acid-soluble phosphate in soils where crops respond to phosphate fertilizers

Iron and aluminium phosphates.—Phosphorus compounds that are useless to crops may be dissolved from soils by conventional acid extractants. Very acid soils usually contain iron and aluminium phosphates. Laboratory preparations of iron and aluminium phosphates 'age' and crystallize on storage, but remain appreciably soluble in dilute solutions of citric or hydrochloric acids. Presumably crystallized minerals of the type of wavellite ($3Al_2O_3,2P_2O_5,12H_2O$) and dufrenite ($2Fe_2O_3,P_2O_5,3H_2O$) are formed in soils by prolonged ageing; they have low solubilities in dilute acids.

Basic phosphates were prepared by cold precipitation from solutions of iron and aluminium chlorides by adding monobasic ammonium phosphate solution followed by ammonia until the pH was 8·0. The preparations were washed, air-dried and ground. Each phosphate (20 mg.) was shaken with 200 ml. of (*a*) water, (*b*) 0·5N-acetic acid, and (*c*) 1% citric acid for two hours. Dufrenite and wavellite were extracted similarly. The percentages of the total P_2O_5 in the phosphates tested which were dissolved by the three solvents are stated in Table I.

The crystalline minerals dufrenite and wavellite were only slightly soluble in any of the solvents used. The P_2O_5 in freshly prepared aluminium phosphate was almost completely soluble in all three solvents. Iron phosphate was very soluble in citric acid but very little P_2O_5 was dissolved by water or by 0·5N-acetic acid.

Table I

Extraction of iron and aluminium phosphates

Solvent	Percentage of total P_2O_5 dissolved by		
	water	acetic acid (0·5N)	citric acid (1%)
Dufrenite	—	1	1
Wavellite	—	3	2
Laboratory preparations of			
aluminium phosphate	100	98	90
iron phosphate	4	7	50

Soil : solvent ratios.—The proportion of soil phosphate dissolved by dilute acids may be unduly high when very low soil : solvent ratios are used. Varying quantities of a light soil derived from Bunter Sandstone were extracted by shaking for two hours with 200 ml. of 0·5N-acetic acid. The amounts of P_2O_5 extracted are given in Table II.

Duration of the extraction.—Very different periods of extraction have been recommended to dissolve easily soluble soil phosphate, ranging from one or two minutes when 'rapid' methods are used to one week in early methods in which citric acid was used (suggested by Dyer[4]).

Table II

Amounts of phosphate extracted from soil using varying soil/solvent ratios

Soil : solvent ratio	P_2O_5 extracted, mg. per 100 g. of soil
1 : 400	8·0
1 : 200	8·0
1 : 100	6·4
1 : 50	5·6
1 : 25	3·5
1 : 10	1·5

Extending the period usually increases the amount of phosphate extracted. A series of 30 soils taken from fields where sugar-beet manuring experiments had been carried out in 1939 by Rothamsted Experimental Station were extracted by shaking with dilute citric acid (2 g. of soil and 20 ml. of 1% citric acid solution were used). The soils were extracted in duplicate, one series being shaken for two hours and the other series for 24 hours. The amounts of P_2O_5 extracted during the two periods are plotted for each soil in Fig. 1. For practically all of these soils there were some increases in the amounts of phosphate dissolved when the period of extraction was increased twelve-fold.

Comparison of acid solvents.—When high soil : solvent ratios are used, the amounts of phosphate extracted by different acids from any one soil may vary widely. When low ratios are used the amounts of phosphate extracted by different acids become more comparable, provided that the pH values of the solutions are not widely different. A further series of soil samples taken from fields where sugar-beet manuring experiments were carried out in 1938[5] were extracted both with 1% citric acid and with 0·5N-acetic acid (0·5 g. of soil was shaken with 200 ml. of solvent for two hours). The amounts of phosphate dissolved by the two acids are plotted for each soil in Fig. 2. For the whole group, on the average, the two acids dissolved rather similar amounts of P_2O_5, but for individual soils there were some large differences between the amounts extracted by the two solvents.

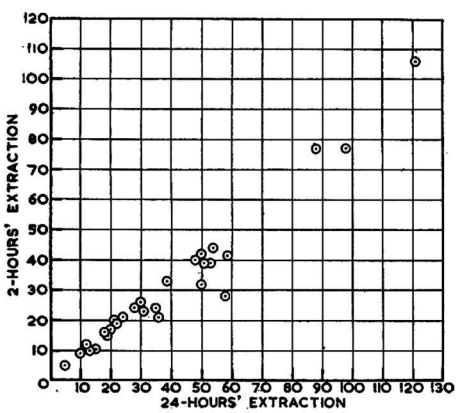

FIG. 1.—*The effect of duration of shaking on the amount of phosphate extracted by citric acid (mg. of P_2O_5/100 g. of soil)*

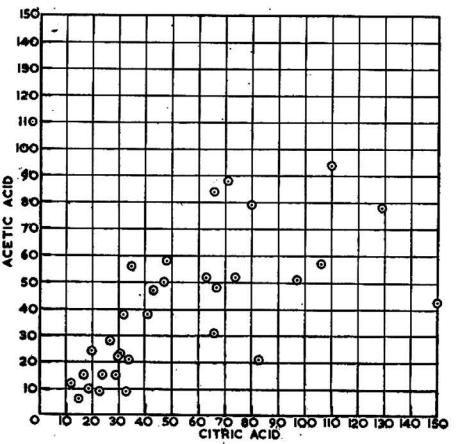

FIG. 2.—*Comparison of the amounts of phosphate extracted from soils by 1% citric acid and by 0·5N-acetic acid (mg. of P_2O_5/100 g. of soil)*

Calcareous soils.—Calcareous soils often contain large amounts of phosphate soluble in dilute acids, but crops grown on them may respond to phosphate fertilizers. Quite large particles of calcium carbonate containing relatively high proportions of phosphate are frequently present in such soils. In dilute acid extraction the particles are completely dissolved. Crops, however, may make use only of the phosphate on the surfaces of the particles.

A few samples of liming materials were taken from active quarries and total phosphate was determined by dissolving them in 5N-hydrochloric acid. All the samples examined contained appreciable amounts of phosphate (Table III).

Table III

Phosphate in liming materials

	mg. of total P_2O_5 per 100 g. of material
Cretaceous chalk	
Redbourn, Herts.	102
Hexton, Herts.	150
Leverstock Green, Herts.	200
Oolitic limestone	
Newport Pagnell (Sample 1)	75
„ „ (Sample 2)	134

In further experiments grains of calcium carbonate were picked out from a number of calcareous soils and total phosphate in the grains was determined. Some typical analyses are stated in Table IV. The amounts of phosphate in the particles selected varied greatly from soil to soil. On highly calcareous soils the quantities recorded may constitute a large proportion of the phosphate dissolved by dilute acids.

Table IV

Total phosphate in limestone particles picked from calcareous soils

	$CaCO_3$ in soil, %	mg. of P_2O_5 per 100 g. of $CaCO_3$ fragments
Soils derived from chalk		
1. Lincs.	1·7	494
2. Lincs.	3·0	269
3. Norfolk	3·7	312
4. Cambs.	7·9	597
5. Norfolk	24·0	60
6. Kent	30·8	180
Soils derived from oolitic limestone		
1. Lincs.	6·1	65
2. Northants	7·7	110
3. Rutland	10·0	900
4. Lincs.	10·2	85
5. Lincs.	13·4	107
6. Lincs.	28·9	39

Unresponsive crops grown on soils having little acid-soluble phosphate

Crop growth may be limited by other factors. Crops grown on soils where dilute acids dissolve very little phosphate may not respond to phosphate fertilizer. Deficiencies of some other element, as well as dry weather or poor physical soil-conditions, may limit crop growth. In very dry weather there may also be responses to phosphate dressings (particularly those placed near to the seed) due, in part at least, to stimulation of root development. Such effects may be independent of the amounts of acid-soluble phosphate in some soils.

When the results of soil analyses are being related to crop responses it is often legitimate to exclude poor crops. Manuring experiments on sugar beet were carried out in the dry season of 1938.[5] The general correlation of the responses of the crops (to 6 cwt. per acre of superphosphate) with the amounts of phosphate dissolved by acetic acid from soil samples (taken before the field experiments were laid down) was very poor (0·5 g. of each soil was shaken for two hours with 200 ml. of 0·5N-acetic acid). In Fig. 3*a* crop responses to phosphate dressings are plotted against acid-soluble soil phosphate for ten experiments where the mean yields were less than 20 cwt. of sugar per acre. In this small group of experiments crop responses were totally unrelated to soil analyses, although at five centres there were significant ($P = 0{\cdot}05$ or smaller) responses to superphosphate. For 22 other experiments where general yields were satisfactory the crop responses to superphosphate are plotted against acetic acid-soluble soil phosphate in Fig. 3*b*. There was a rough general relationship between soluble phosphate and the responses of the sugar-beet crops. There were also five experiments in this second group where superphosphate increased yields significantly; acetic acid dissolved only small quantities of phosphate from the corresponding soil samples taken from four of these experiments.

Fixation of phosphate during acid extraction.—If phosphate dissolved from soils by dilute acids is immediately precipitated, low values for acid-soluble phosphate are obtained. Such

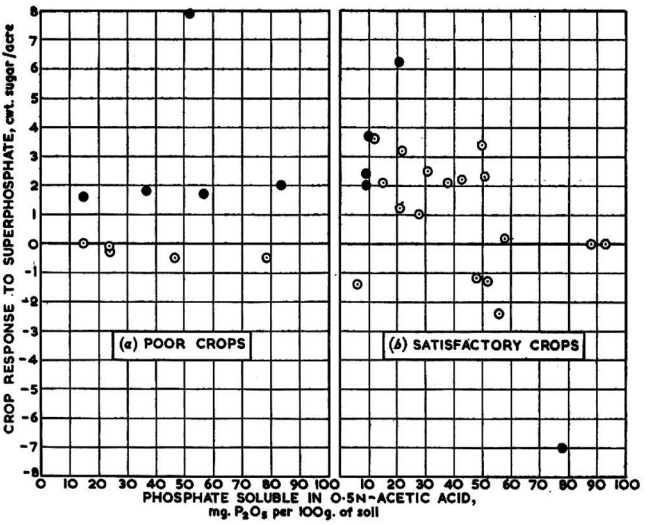

FIG. 3.—*Comparisons of responses of sugar-beet crops to superphosphate with amounts of acid-soluble soil phosphate for* (a) *poor crops and* (b) *satisfactory crops*

● = significant effect for $P < 0.05$

reactions were originally studied by Russell & Prescott.[6] Ghani[7] has suggested 8-hydroxyquinoline as a ' blocking ' reagent to prevent precipitation of phosphate, and the method has been applied by Williams.[8–10]

Cooke[11] found that a dilute solution of selenious and acetic acids prevented precipitation of phosphate by iron and aluminium oxides completely at pH 2·5. This mixture of acids was used to extract the series of soils taken from sugar-beet experiments carried out in 1938.[5] The relationship of the amounts of phosphate dissolved by this acid mixture with responses of the sugar-beet crops to dressings of superphosphate was not superior to the relationship obtained with conventional acid extractants. These results suggested that reprecipitation of phosphate after extraction was not important in the series of soils examined; the actual amounts of phosphate precipitated at pH 2·5 were therefore measured. Various quantities of KH_2PO_4 were added to 0·5N-acetic acid. Each soil (0·5 g.) was shaken with 200 ml. of these solutions for two hours. The amounts of recovered phosphate (b mg. of P_2O_5 per 100 g. of soil) were plotted against the amounts added (a mg. of P_2O_5 per 100 g. of soil) for each soil. There was a linear relationship (Fig. 4) between the amount of phosphate added and the amount extracted for all soils examined. The slope of the line measured arbitrarily the extent of precipitation of dissolved phosphate. Extrapolating the line back to cut the 'a' axis gave a value of 'x' mg. of P_2O_5 per 100 g. of soil, which was the amount of soluble phosphate unaffected by precipitation during extraction. Most of the soils in this series behaved similarly; the average percentages of added phosphate precipitated during extraction are set out in Table V, in which the soils are grouped into classes according to texture.

The amounts of precipitated phosphate were not related to any of the soil properties measured (such as pH) in the groups of sand and loam soils. Clay loams precipitated slightly more phosphate during extraction at pH 2·5 than lighter soils, and the small group of fen soils precipitated much more phosphate. Other very acid light soils from various parts of Britain were examined in the same way; none precipitated appreciable quantities of phosphate during extraction and the data are not presented here.

The amounts of added phosphate which were precipitated during extraction of a further series of fen soils were measured. The results are presented in Table VI. All soils except the sandy fen (3607) precipitated considerable quantities of phosphate during extraction. The amounts of phosphate dissolved (x mg. of P_2O_5 per 100 g. of soil) before precipitation occurred during the extraction were determined by the method demonstrated in Fig. 4. These amounts, together with the amounts of P_2O_5 extracted by the usual procedure

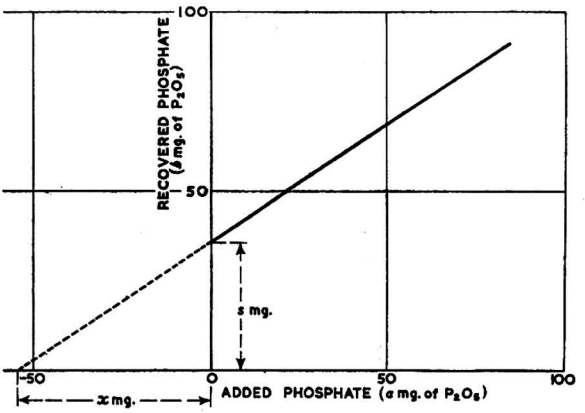

FIG. 4.—*Relationship between the amounts of added and recovered phosphate during acid extraction*

Table V

Percentages of added phosphate precipitated during extraction with 0·5N-acetic acid

Soil samples taken from 1938 manurial experiments on sugar beet [5]

Type of soil	No. of soils	Percentage of added P_2O_5 pptd. during extraction
Coarse sands	12	5
Fine sands	7	4
Light loams	6	3
Clay loams	4	12
Organic fen soils	3	27

Table VI

Percentages of added phosphate precipitated during extraction of fen soils with 0·5N-acetic acid

Soil No.	Type of soil	Percentage of added P_2O_5 pptd. during extraction	mg. of P_2O_5 extracted from 100 g. of soil	
			'Ordinary' value *s*	'Corrected' value *x*
4947	Clay fen	35	52	77
4924	,, ,,	24	22	29
3246	,, ,,	37	44	70
3607	Sandy fen	−2	60	59
3599	,, ,,	27	33	45
3299	Calcareous fen	16	62	74

(*s* mg.), are stated in Table VI. Such 'corrected' values are more reliable measures of acid-soluble P_2O_5 for soils which precipitate appreciable quantities of phosphate during extraction.

Changes in equilibria between phosphorus compounds in soil

Changes in storage.—The amounts of phosphate dissolved from a soil by dilute acid may depend on the length of time that the sample is stored. Changes in soluble phosphate depend on the moisture in the sample and on temperature of storage. The magnitude of such changes was determined by analysing a series of soil samples which had been stored at ordinary temperatures for 10 years. The samples were taken from the sites of a series of field experiments carried out in 1942 to compare alternative phosphate fertilizers.[12] The fields had been chosen by Advisory Soil Chemists and were low in acid-soluble phosphate. The soils were air-dried and ground to pass a 2-mm. sieve, and acid-soluble P_2O_5 was determined soon after the samples were taken in 1942 (2·0 g. of soil was shaken for two hours with 200 ml. of 0·5N-acetic acid). The stored soils were analysed again in 1952 by the same method. The differences between the amounts of P_2O_5 dissolved on the two occasions are plotted for each soil in Fig. 5 against

acid-soluble phosphate determined in 1942. Acetic acid dissolved more phosphate from most soils in 1952 than in 1942. In a few of the soils acid-soluble phosphate had decreased during storage. Generally the changes were of the order of 1 or 2 mg. of P_2O_5 per 100 g. of soil, but in some soils they were much larger.

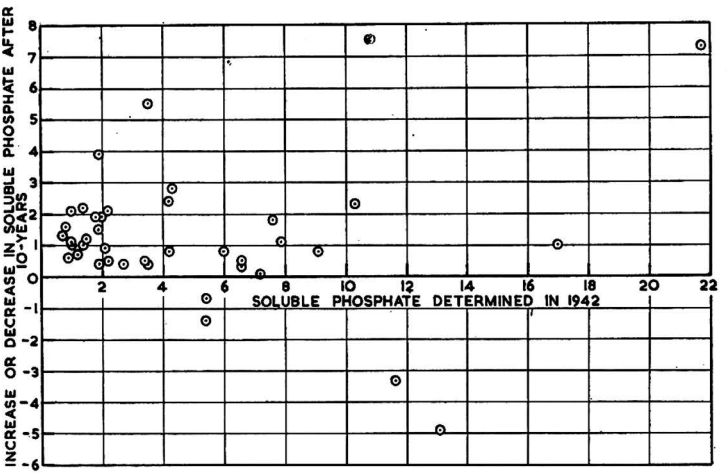

FIG. 5.—*Change in acetic acid-soluble phosphate during 10-year storage (mg. of P_2O_5/100 g. of soil)*

Changes in acid-soluble phosphate after incubating with lime.—Changes in the equilibria between phosphorus compounds proceed most rapidly when soils are moist and warm. Incubating at higher temperatures and normal moisture contents with additions of nitrogen and potassium gives full opportunities for both microbiological and chemical changes. By adding lime to acid soils, the equilibria between the inorganic forms of soil phosphorus may be altered completely. Many workers have reported changes in both acid-soluble phosphate and in the magnitude of crop responses to phosphate fertilizers after liming acid soils. Laboratory experiments were carried out on soils from a long-continued manurial experiment at Rothamsted to determine the effect of liming on acetic acid-soluble phosphate.

Soil samples were taken from the permanent-grass experiment in the Park at Rothamsted which began in 1856.[13] The samples were taken from unphosphated plots (Nos. 1 and 3), plots receiving regular dressings of superphosphate (Nos. 4_1 and 11_1), and the plot which receives regular dressings of farmyard manure (No. 13). Each of these plots is divided into two parts, one part being limed regularly. The limed and unlimed halves of each plot were sampled separately. The soil samples were air-dried and ground to pass a 2-mm. sieve. Soil (20 g.) was moistened with 3·4 ml. of a solution which contained 1·0 g. of NH_4NO_3, 2·5 g. of KCl and 1·5 g. of $MgSO_4,7H_2O$ per litre. The moistened soils were incubated in stoppered flasks at 28° for one month, the flasks being aerated on alternate days. A duplicate incubation was carried out on 20 g. of each soil moistened with the same solution and mixed with 0·15 g. of calcium hydroxide. At the end of the incubation the soils in the flasks were immediately sampled without drying, and 2·34 g. (equivalent to 2·0 g. of air-dry soil) was shaken with 200 ml. of 0·5N-acetic acid for two hours. The amounts of acid-soluble phosphate determined in this way were compared with the amounts dissolved from the unincubated soils; the results are summarized in Table VII.

The unmanured soils from plots 1 and 3 produced very little extra acid-soluble phosphate by incubating with lime. On the limed parts of the same plots incubation without lime had little effect, but, after incubating with lime, acid-soluble phosphate had increased to several times the amount extracted from the unincubated soils. Where superphosphate had been applied on very acid soil (plot 11_1), acid-soluble phosphate in the soil decreased on incubation without lime but increased slightly when the soil was incubated with lime. Where farmyard manure (plot 13) or superphosphate (plot 4_1) had been applied on less acid soils which were not limed regularly acid-soluble phosphate decreased on incubation, but increased markedly

Table VII

The effects of incubation, in the presence and absence of lime, on the phosphate dissolved by acetic acid from the soils of Rothamsted Park grass experiment

Plot		Treatment	Soil pH	Acid-soluble phosphate (mg. of P_2O_5 per 100 g. of soil)		
				Direct extraction	*Extra* P_2O_5 after incubation	
					Without lime	With lime
1	Unlimed	No phosphate	4·4	0·9	0·2	0·7
1	Limed		6·5	0·7	0·3	2·7
11_1	Unlimed	Superphosphate *	3·7	37·0	−11·0	2·0
11_1	Limed		4·6	44·0	−3·0	9·0
13	Unlimed	Farmyard manure	4·5	15·0	−4·0	6·0
13	Limed		6·9	38·0	12·0	18·0
3	Unlimed	No phosphate	5·0	0·9	0·1	0·7
3	Limed		6·5	0·6	0·8	3·4
4_1	Unlimed	Superphosphate *	5·0	62·0	−4·0	18·0
4_1	Limed		6·8	90·0	10·0	20·0

* Superphosphate is applied each year at 3·5 cwt. per acre.
In addition to the stated dressings the plots receive:
Plot 1: 206 lb. of sulphate of ammonia per acre each year
Plot 11_1: 618 lb. of sulphate of ammonia, 500 lb. of sulphate of potash, 100 lb. of sulphate of soda, 100 lb. of sulphate of magnesia per acre each year
Plot 13: receives 14 tons of dung per acre once in 4 years and 6 cwt. of fish meal per acre once in 4 years

when lime was added during incubation. Much larger increases were obtained when samples taken from the limed parts of these two plots were incubated.

Incubation experiments of this character may measure the ability of soils to release acid-soluble phosphate during the growing period of a crop. When lime is added during incubation the tests may also indicate the effect of liming acid soils on easily soluble soil phosphate. Soil samples taken from fields where phosphate fertilizer experiments were carried out in 1942[12] were incubated with calcium hydroxide in the way described above. Acid-soluble phosphate was determined before and after incubation. The relationship between the *extra* acid-soluble phosphate released by incubating the soils with lime and the responses of the swede crops to superphosphate was as satisfactory as the relationship between acid-soluble phosphate in the unincubated soils and crop responses. There were six experiments where the soils contained less than 2 mg. of P_2O_5 per 100 g. soluble in acetic acid (under the conditions described above). Of these, there were two sites where swedes gave responses of less than 10 tons per acre when manured with 5 cwt. of superphosphate per acre; both soils released appreciable amounts of acid-soluble phosphate when incubated with lime. There were also eight soils having more than 7 mg. of P_2O_5 per 100 g. soluble in acetic acid; the acid-soluble phosphate decreased after incubation with lime in only three of these soils, and in the three corresponding field experiments on swedes superphosphate gave large increases in yield.

Discussion

The methods used at present to determine the amounts of elements in soils that are useful to plants are essentially empirical. Since plant roots do not extract soil with dilute acids or buffered salt solutions and then absorb the dissolved ions, a perfect correlation between analytical data and crop performance cannot be expected. Although crops absorb ions from the soil solution, a liquid phase need not intervene between plant roots and the stock of undissolved fertilizer. Some workers have postulated that 'contact exchanges' of ions occur where the outer part of the root and soil colloids form a continuous system.

Relatively few critical comparisons of the results of soil analyses with the responses of crops to fertilizer dressings have been published. Burd & Murphy[14] stated that, to obtain satisfactory correlations of acid-soluble phosphate with crop performance, the degree of phosphate saturation of the absorbing minerals, the buffer capacity of the soil and the total phosphate present in the soil must be taken into account. Truog[15] has recommended a low soil/solvent ratio and a short period of extraction to prevent reprecipitation of phosphate after it has been dissolved. McGeorge[16] suggested that citric acid should be used to prevent reprecipitation

during extraction of Hawaiian soils. Davies[17] compared phosphate dissolved from a group of New Zealand soils by 1% citric acid, by 0·5N-acetic acid and by an acid calcium lactate solution. He found that for soils with low fixation powers the ' available ' phosphate values determined by the three methods agreed in general and there was a reasonable correlation with the results of field trials. The acetic acid and lactic acid methods extracted very little phosphate from soils with high fixation powers; citric acid extracted a considerable amount but was judged to displace ' absorbed ' phosphate. Davies stated that on such soils the lactic acid method gave the best correlation with field trials if the amounts of phosphate dissolved were corrected for the fixation occurring during extraction.

It is very difficult to choose a suitable acid solvent. On soils which contain high proportions of iron phosphate citric acid may dissolve phosphates that are useless to crops. Acetic acid is therefore likely to be more satisfactory than citric acid on very acid soils and on soils rich in iron oxides.

Satisfactory soil/solvent ratios are equally difficult to define. The amounts of phosphate dissolved from certain soils increase rapidly with increasing amounts of solvent, and chemists may wish to ensure stable extraction conditions by using low soil/solvent ratios. There is always a risk, however, of dissolving compounds that are useless to crops. When very small amounts of solvent are used it is easy to obtain comparable data from closely related soils, but much more difficult to use a common extractant on different kinds of soil. ' Rapid ' methods usually employ a small amount of acid or buffered solution and a short period of extraction. In experienced hands they have proved very valuable on certain classes of soil. Such methods need to be ' calibrated ' for each kind of soil and with some soils the results may be misleading.

There is no satisfactory method of determining easily soluble phosphate in English soils that contain a high proportion of calcium carbonate. Analytical methods can be ' calibrated ' only by intense local experience and from the results of many field experiments. When much soil is used the acid may be completely neutralized and little or no phosphate is dissolved. Excess of acid, used to overcome this difficulty, dissolves the whole of the calcium carbonate, including the large particles that commonly contain quite high proportions of phosphate. Phosphate contained in limestone particles does not vary regularly with the parent material of the soil or the locality. A satisfactory extractant for calcareous soils should not dissolve these particles completely. Citric acid forms a complex with calcium, and may be partially successful, but no reagent has been developed to dissolve only phosphate from the surfaces of the particles.

Reprecipitation of phosphate during acid extraction was not important in the soils examined here, and in eastern England there is no need to take special precautions when low soil/solvent ratios are used. Exceptions to this general statement are many fen soils and ferruginous soils (such as those derived from the Middle Lias Ironstone). Methods have been devised to measure acid-soluble phosphate without interference by reprecipitation. Ghani[7] suggested using 8-hydroxyquinoline to ' block ' precipitation by active iron and aluminium, and Cooke[11] showed that a mixture of selenious and acetic acids prevents precipitation of phosphate by iron and aluminium oxides. Unfortunately, rapid colorimetric analyses are complicated by the presence of reagents such as 8-hydroxyquinoline and selenious acid, and these methods are not very suitable for analyses of soils in advisory work. In routine laboratories it is probably better to use the method suggested in the present investigation and to carry out a number of extractions with several quantities of added phosphate. By plotting recovered phosphate against added phosphate the amount of phosphate extracted from the soil before reprecipitation occurred may be estimated. (Bondorff[18] reported that a similar method was used successfully in Denmark.)

Phosphate is often precipitated during acid extraction of lateritic and other tropical soils. Recently Birch[19] has described methods of relating soluble phosphate to crop responses on East African soils. He states that some method of preventing phosphate adsorption is needed to obtain a measure of the amount of phosphate associated with exchangeable bases.

The preliminary treatment of soils before analysis may influence the amounts of phosphate extracted. Samples taken for advisory purposes are usually dried and ground. Drying may alter the equilibria between various phosphorus compounds, and if soils retain free moisture further slow changes may occur. Soils must be ground to allow satisfactory sub-sampling. but fine grinding may expose surfaces within the soil particles which cannot be reached by plant roots. The duration and vigour of shaking may affect the dispersion of the soil. These factors have been discussed by Williams.[20]

The changes in acetic acid-soluble phosphate reported here, i.e. those that occurred when a number of air-dry soils were stored for 10 years, show that soils should be analysed soon after the samples are taken. Such changes may alter the advisor's arbitrary classifications and may vitiate critical work aimed at detecting only small differences in soluble phosphate.

The changes in acetic acid-soluble phosphate that occurred when limed soils were incubated have several implications in advisory work:

(1) Soils that have never been manured with phosphate and have had a long history of extreme acidity are not likely to give increases in acid-soluble phosphate for a considerable period after liming.

(2) In less-acid soils that have not received phosphate fertilizers, liming may release appreciable amounts of acid-soluble phosphate.

(3) On very acid soils that have received regular phosphate dressings, liming may release some acid-soluble phosphate.

(4) On less-acid soils that have been manured, liming may cause a considerable increase in acid-soluble phosphate. In further work (not described here) on reclaimed heaths and other very acid soils, very little acid-soluble phosphate was released by liming followed by incubation. On cultivated and manured arable soils which were acid, incubation with lime released amounts of acid-soluble phosphate fully equivalent to a normal dressing of superphosphate.

Measurements of changes in acid-soluble phosphate after incubation with lime may be useful on soils where it is difficult to relate soluble phosphate with the responses of crops to phosphate fertilizers. The technique may be used to measure reserves of soil phosphorus that can be converted easily into forms soluble in dilute acids. In soils where acid-soluble phosphate decreases on incubation with lime, phosphate fertilizers may be rapidly converted into useless phosphorus compounds. (Fixation under these conditions may be due largely to aluminosilicate minerals, since fixation by iron and aluminium hydroxides decreases with increasing soil pH.) Too few soils were examined here to provide a close relationship between soluble phosphate released by incubation and crop responses to phosphate dressings. The amounts of soluble phosphate released by incubating the soils with lime were, however, more successful in splitting the 1942 swede experiments[12] into a highly responsive group and a less responsive group than were the amounts of soluble phosphate determined by simple extraction with acetic acid.

Modifications in laboratory techniques alone will not provide more valid analytical data. Constant field experimentation is needed to recognize limits of deficiency or excess of soil phosphate. Improved correlation between easily soluble phosphate and crop responses on all types of soil will lead to more rational manuring and higher production at lower cost.

On all soils where attempts are being made to relate acid-soluble phosphate and crop responses different extractants at varying soil/solvent ratios should be used, together with measurements of the precipitation of added phosphate at various pH values. Such work should be carried out on very many soils and in such a way that trends in groups of soils can be determined by statistical methods. There is little doubt that in many areas correlations of soil analyses with crop responses are very far from being adequate.

Since present methods of extracting soils cannot be used to make fine distinctions in manurial recommendations and since effects of fertilizers vary with seasons, analyses for ordinary advisory purposes need have no great precision and for reporting analytical data only three categories are needed: (*a*) *low*, where all crops may suffer decreased yields without fertilizer and each crop of the rotation should be fertilized; (*b*) *medium*, where no losses in yield are expected by withholding fertilizer. Dressings may be recommended to replace phosphate removed by crops, provided that fertilizer supplies are adequate on a national scale and that crop prices show a sufficient profit to allow farmers to purchase fertilizer; (*c*) *high*, where no losses in yield can result from withholding fertilizer. Farmers should be advised not to apply dressings for a number of years.

Soil analysis will be most profitable when it is used to determine the manuring of unresponsive crops that need dressings of fertilizer only on very deficient soils. If the resources of soil chemists are concentrated on special crops and perhaps on delimiting special areas the value of the service to farmers will be greater. An immediate task is to map areas where soil phosphate is either generally deficient, or in general adequate. With such information plans could be made for manuring on a more rational regional basis, and it would be easier to determine national requirements of fertilizers.

One obstacle to better use of the results of soil analysis is the growing tendency of some farmers to purchase ready-mixed compound fertilizers and to apply a mixture to all fields irrespective of conditions on particular fields or the needs of individual crops. Complete fertilizers used for crops, such as cereals, which rarely respond to the phosphorus and potassium components, have little merit other than that they are physically convenient to apply, frequently they are wasteful. A fuller understanding by farmers of the principles of plant nutrition, together with adequate supplies of individual fertilizers in good physical condition, is needed before best use can be made of soil analysis in economic crop production.

Conclusions

Citric acid (1%) may dissolve iron and aluminium phosphates, which are useless to crops; acetic acid is more satisfactory for extracting acid soils than citric acid. Other dilute acids may be necessary for other types of soil.

Correct soil/solvent ratios must be chosen for each class of soil. Stable extraction conditions are obtained with a large volume of dilute acid.

Native limestones contain appreciable quantities of phosphate. Calcareous soils containing limestone fragments may therefore contain misleadingly high quantities of phosphate soluble in dilute acid. There is no satisfactory solvent for determining easily soluble phosphate in such soils. When correlations are made between acid-soluble phosphate and crop responses to fertilizer dressings all experiments where poor crops were grown should be treated separately.

Ordinary mineral soils in eastern England rarely precipitated appreciable quantities of phosphate during extraction with dilute acetic acid. Peat fen soils and ferruginous soils precipitated appreciable quantities of phosphate. When 'fixation' is suspected the amounts of phosphate precipitated during extractions with added phosphate should be measured. By graphical interpolation it is possible to estimate the amount of phosphate dissolved by dilute acid before precipitation occurs. Such measurements may be more useful in routine advisory work than special reagents used to prevent precipitation by iron and aluminium.

When air-dried soils are stored changes occur in the amounts of phosphate soluble in dilute acids. All soil samples should be analysed soon after they have been prepared.

Moistened soils were incubated alone and also with calcium hydroxide and the changes in dilute-acid-soluble phosphate were measured. With very acid and unmanured soils very little easily soluble phosphate was released by incubating with lime. On less acid unmanured soils and on very acid soils that had received dressings of phosphate fertilizers incubation after adding lime released appreciable amounts of dilute-acid-soluble phosphate. Such incubation techniques may be used to measure reserves of soil phosphorus which can be converted easily into soluble forms. The amounts of extra soluble phosphate released by incubating soils with lime were more successful in splitting a series of field experiments testing phosphate fertilizers into a highly responsive and a less responsive group than were the amounts of phosphate dissolved by direct extraction with dilute acid.

Determinations of dilute-acid-soluble phosphate made for advisory purposes need only be reported as 'low', 'medium' or 'high'. Many more field experiments and associated laboratory work are needed to improve correlations between easily soluble phosphate and crop responses to fertilizer dressings. Supplies of individual fertilizers in good physical condition are needed before farmers can make the fullest use of the results of soil analyses.

Acknowledgments

Thanks are due to Dr. E. M. Crowther and to Mr. R. G. Warren for their interest and advice during the course of the work described, and to Mr. J. K. Gasser and Mr. H. L. Nixon for help with some of the analyses.

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Received 19 February, 1953

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THROUGH-CIRCULATION DRYING OF SEAWEED

III.*—*Laminaria digitata* frond and stipe; *Laminaria saccharina* frond

By R. G. GARDNER and T. J. MITCHELL

The drying characteristics of *Laminaria digitata* frond and stipe have been studied and are compared with those of *L. cloustoni* frond and stipe.

A striking similarity was found in the shapes of the curves of time versus air velocity for the fronds and stipes respectively of the two species. The empirical equations for fronds were similar in type and those for the stipes were also similar. It was concluded that the physical differences between the two parts of the same plant had a greater influence on the drying time than the differences between the three common sublittoral species.

The close agreement between the species was most marked in the effect of bed depth on the drying time. This relationship was apparently independent of any particle-shape factor since fronds (either minced, shredded, pre-dried or wet) gave the same general type of curve, i.e. concave upwards. The bed-depth curves for minced or sliced stipe were in general agreement, but were in contrast with the frond curves.

These physical properties of stipes and fronds have been compared with those of other vegetable materials (hops, potatoes, carrots and brewer's spent grain) and it has been shown that the time versus bed-loading curves for all these materials can be classified into three groups, depending on whether the material is compressible or rigid.

The output versus bed-loading curve for the first group (materials similar to fronds) reaches an optimum value, and mathematical considerations have demonstrated that the optimum drying time is about twice the minimum time. The minimum time for through-circulation drying is the period required to dry a single layer of the material, and is represented by the intercept of the smooth curve (of time versus bed-loading) on the time axis.

The effect of agitation on the reduction of drying time was investigated for two-inch beds of *L. digitata* frond. Agitation was carried out at equal time-intervals and has been expressed as effective stages. In the first half of the run ($T = 7$ to $T = 3$, where T = total water content, lb. of water/lb. of bone-dry solid) three effective stages reduced the time to only 95% of the corresponding static time, whereas a reduction to about 40% was obtained in the range 3 to 0·1.

Bed-depth experiments suggest that *L. digitata* plants dry faster than, and *L. saccharina* slower than, *L. cloustoni*. Internal temperatures of a ½-in. slice of *L. cloustoni* stipe during a drying run have been measured. The effect on drying time of storage between harvesting and drying has been found for *L. digitata* frond.

Introduction

In a previous paper[1] the physical and chemical differences between the two parts of the plant, i.e. stipe and frond, were compared and contrasted. Physical differences between the three common sublittoral seaweeds used in the drying tests are described below.[2]

L. cloustoni has a sturdy stipe with a circular cross-section. The epidermis, or outer skin, of the stipe is rough and often has adherent parasitic growths. The stipe is surmounted by a flat palmate frond. The plant anchors itself firmly to rocks and is normally found in strong tideways, the buffeting of the water being apparently beneficial to its growth.

L. digitata has a smooth oval stipe which rarely has any parasitic growths. The frond is divided into ' fingers ' from which the plant derives its name. This species prefers medium tideways.

L. saccharina consists of a soft flexible stipe and a single crinkled frond. It usually attaches itself to shells or small stones and thus offers little resistance to dislodgment; it is consequently found only on sandy sea-beds in sheltered waters.

Black[3] measured the seasonal variation of the fresh weight of samples of these three species over a two-year period. Table I, taken from his paper, summarizes the average weight of the plants.

It would appear from Table I that the heaviest plant is *L. cloustoni* followed by *L. digitata*. The fronds of *L. digitata* and *L. saccharina* are here heavier than the corresponding stipes, whereas the reverse is true for *L. cloustoni*. Although these generalizations are not rigid and will vary with the habitat of the plant, they hold for the seaweed samples received for the drying tests.

Results

The through-circulation drier used in these experiments has been fully described,[4] and the experimental procedure used for stipe and frond is similar to that used for *L. cloustoni*.

* Part II: *J. Sci. Fd Agric.*, 1953, **4**, 237

Table I

Comparison of the weights of three common sublittoral seaweeds

		Maximum wt., lb.	Minimum wt., lb.	Average (450 plants) wt., lb.
L. cloustoni	Frond	2·06	1·12	1·50
(Luing Island)	Stipe	3·94	1·50	2·63
L. digitata	Frond	4·18	0·75	2·13
(Atlantic Bridge)	Stipe	0·94	0·31	0·56
L. saccharina	Frond	2·25	0·44	1·31
(Shuna Island)	Stipe	0·50	0·19	0·44

In all the reported tests on *L. digitata* frond, the raw material was minced before drying. Factors studied were: agitation of the frond bed, the effect of storage between harvesting and drying of the frond, and the effect of air velocity and bed depth on *L. digitata* frond and stipe. Seasonal variation of drying time was also observed for *L. digitata* frond, but there were insufficient data to allow this effect to be evaluated.

Agitation experiments

During drying, a bed of minced seaweed frond shrinks away from the basket sides allowing part of the air to short-circuit the bed. An experiment on stipe slices[4] verified that the upper layer of the bed did not dry appreciably until the later stages of the run. It follows that the upper central part of the bed will be the last to dry as it is remote from conduction from the walls, and this will prolong the drying operation as a whole. One possible remedy for this condition is to reverse the air flow about half way through the drying operation. This would be suitable for materials like stipe, which are non-adherent, but with frond the shortening of the drying time would not be so marked since the gaps in the bed would reduce the effectiveness of contact with the air. Agitation, on the other hand, if properly applied, is equivalent to inverting the bed and, in addition, larger masses containing wet knots are broken up and the spaces caused by edge shrinkage are closed. The following agitation tests were carried out to find if there was an optimum number of 'agitations' for a bed of seaweed frond.

A common basis of comparison for these tests is difficult to establish since the drying times (between the same limits of water content) for different degrees of agitation are not known until the end of the run. Consequently the agitations cannot be spaced out at equal time-intervals between the initial and final water-content limits. If a run is carried out with, say, three agitations at twenty-minute intervals, and the final water content is reached five minutes after the last agitation, then the operation is strictly a four-stage process but the number of 'effective' stages would probably be nearer 3·25. Although this concept of an effective stage is not wholly satisfactory in that it gives rise to fractional stages, it has been adopted in an attempt to proportion out the relative effect of mixings and to enable comparisons to be drawn. An effective stage is obtained by dividing the 'agitated' drying time (between specified limits) by the time interval between stirrings.

The drying times with different degrees of mixing cannot be directly compared because of variations in the dry loadings. This has been overcome by interpolating the corresponding time for a static bed at the same dry loading from Fig. 5, and expressing the effect of agitation as a percentage of the static-bed drying time. In this way, the series of tests were rendered independent of dry loading.

Agitation of minced *L. digitata* frond was carried out at regular time-intervals from the start of the run immediately after the basket had been weighed. Mixing was done by hand and required 80–100 seconds at the start (when the frond was sticky) and 40–50 seconds for the partly dried material. Intermediate weighings were also taken to enable the curves to be plotted accurately. Fig. 1 illustrates some typical runs with agitation, and shows clearly the kinks in the curves caused by the sudden increase of drying rate after the bed had been disturbed. As the initial water ratio of the runs in this series straddled 7·0, this value was selected as the upper limit of water content in preference to the value of 5·0 used in other tests.

A plot of the percentage reduction of drying time ($T = 7{\cdot}0$ to $T = 0{\cdot}15$, where T = total water content, lb. of water/lb. of bone-dry solid) for various effective stages is given in Fig. 2. The points are somewhat scattered, as would be expected for such variable material, but it appears that the effect of agitation falls off after three or four stages (i.e. two or three

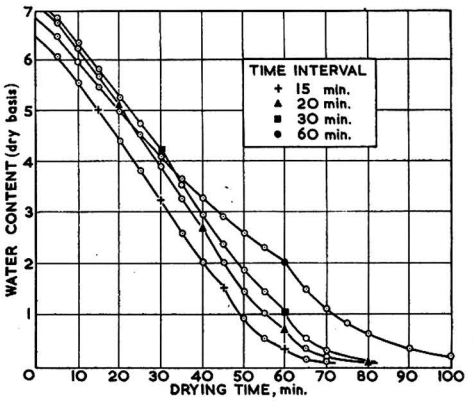

FIG. 1.—L. digitata *frond. Effect of agitation*

D.B.T. (dry-bulb temperature), 158° F; W.B.D. (wet-bulb depression), 75° F; L_d (dry loading, lb. of bone-dry solid/sq. ft.), 0·92; G (mass air flow, lb. of dry air/(min.) (sq. ft. of cross-sectional area of bed), 7·6

agitations). Inspection of Fig. 1 suggests that stirring is more effective in the later stages of drying, and this is borne out by Fig. 3 which shows the effect of agitation on the first and second parts of the drying runs. It is apparent that stirring brings about only a small time-reduction (5%) in the range $T = 7$ to $T = 3$ (which corresponds approximately to the constant drying-rate period), whereas the drying time in the latter part of the run ($T = 3$ to $T = 0{\cdot}1$) may be reduced to 40% of the time for a static bed. This may possibly be explained by the fact that at the start of the run the exhaust air is almost saturated and cannot pick up much more water even from freshly exposed surfaces. Towards the end of a run this condition does not hold, and the air can then dry the wet surfaces more readily. After stirring of the bed has been sufficient to expose all the wet fragments, and the air can penetrate easily, the controlling factor becomes the rate of release of water from the cells.

Figs. 2 and 3 show multistage drying with equal residence times in each stage, but it appears from Fig. 3 that better results would be obtained with unequal drying stages. This would mean that if a three-stage drying process were planned for frond, it would probably

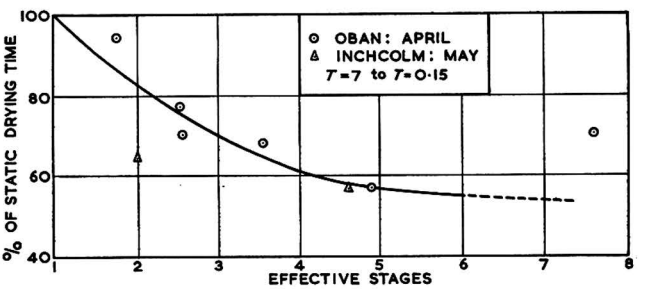

FIG. 2.—L. digitata *frond. Effect of agitation*

be better to agitate the bed at one-half and three-quarters of the drying time, rather than at one-third and two-thirds of the time.

If, as suggested previously,[1, 4] a continuous conveyor-belt through-circulation drier is used for seaweed, and the bed depth is progressively increased by reducing the speed of the lower belts, then the interval between agitations will increase towards the end of the run (assuming belts of equal length).

Tests on pre-dried minced frond[1] showed that much heavier loadings could be used in the later stages of drying (when the stickiness of the particles was reduced) with a resultant increase in output. This would indicate that the effect of increasing output by using deeper beds and by frequency of stirring are in opposition, and their relative importance will have to be assessed.

It should be noted, however, that the above agitation experiments were carried out with an average bed loading (L_d) of 0·92 lb. of B.D.S./sq. ft. (B.D.S. = bone-dry solid). Agitation will probably allow the optimum bed loading for frond to be substantially increased.

Time interval between harvesting and drying

As it was impossible to ensure that all drying tests were carried out at exactly the same time after harvesting, three tests were made in which wet *L. digitata* frond was kept in a loosely closed sack in the laboratory for varying periods before drying to find if this affected

the drying time. The seaweed was harvested at Inchcolm Island, Firth of Forth, on 2 April, 1952. Results of the tests are set out in Table II.

Table II

Effect of time interval between harvesting and drying L. digitata *frond*

Time between harvesting and drying, days	1	2	3
D.B.T.,* °F	160	159	157
W.B.T.,† °F	82	81	81
G, lb./(sq. ft.)(min.)	7·75	7·8	7·75
L_d, lb. of B.D.S./sq. ft.	0·644	0·640	0·668
Drying time ($T = 5{\cdot}0$ to $T = 0{\cdot}15$), min.	57	64·5	79·5

* Dry-bulb temperature † Wet-bulb temperature

A previous test on *L. cloustoni* stipe[4] did not reveal any marked alteration of drying time on standing, and it is possible that the behaviour of *L. digitata* frond may be related to the exudation of mucilage on the surface of the plants. By the third day the frond showed considerable evidence of decomposition. Comparatively little water appears to have been lost since the initial water content decreased only from 7·7 to 7·35 lb./lb. of B.D.S. over the three days.

Air velocity

L. digitata *frond.*—The curve of drying time ($T = 5$ to $T = 0{\cdot}15$) versus air mass velocity for *L. digitata* frond is shown in Fig. 4, and Table III lists the drying conditions used.

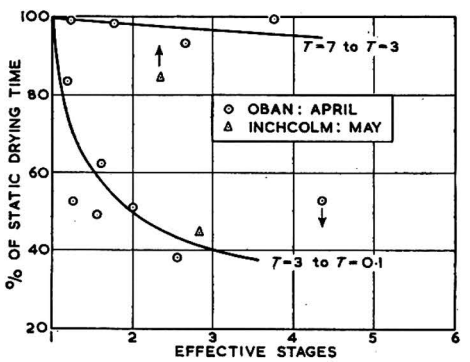

FIG. 3.—L. digitata *frond. Effect of agitation*

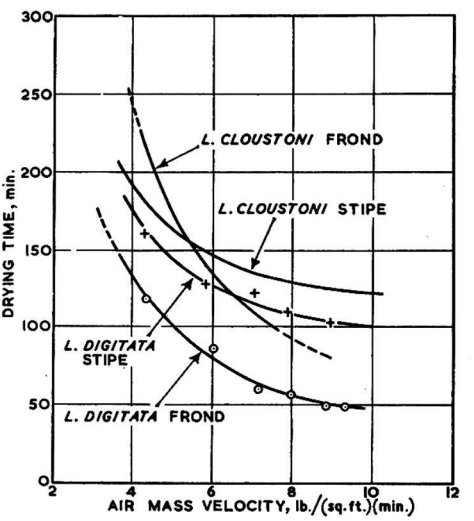

FIG. 4.—*Through-circulation drying of seaweed. Effect of air mass velocity on drying time* ($T = 5$ *to* $T = 0{\cdot}15$). *For other results refer to Table III*

The curve of drying time versus air mass velocity for *L. cloustoni* frond is drawn on the same graph for comparison. It is apparent that the curves are similar in shape and that the *L. digitata* frond dries in about 60% of the time required for *L. cloustoni*. It should be noted from Table III that the dry loading of the *L. digitata* was lower than that of *L. cloustoni* frond. The reduction in drying time with increasing air flow falls off after a rate of about 8 lb./(sq. ft.)(min.) is reached. If air recirculation is utilized, this air flow will probably have to be increased to compensate for the increased humidity.

The curve of drying time (θ) for *L. digitata* frond may be represented by the equation $\theta = 634G^{-1{\cdot}17}$, where G is the mass air flow, lb. of dry air/(sq. ft. of cross-sectional area of bed)(min.). The smooth curve for *L. cloustoni* frond is given by an equation of the same form: $\theta = 1620G^{-1{\cdot}4}$.

Table III

Effect of air mass velocity on seaweed drying

Species	Habitat	Month	D.B.T., °F	W.B.T., °F	L_d, lb. B.D.S./sq. ft.
L. cloustoni frond	Oban	May	156	83	0·75
L. digitata frond	Oban	May	159	83	0·71
L. cloustoni stipe	Oban	Mar.–May	157	84	1·20
L. digitata stipe	Oban and Inchcolm	May	159	83	0·97

L. digitata *stipe*.—Fig. 4 illustrates the curve of drying time versus air-flow rate for *L. digitata* stipe at an average D.B.T. of 157° F. The corresponding curve for *L. cloustoni* stipe is included for comparison (Table III). The marked similarity in appearance between the curves for stipe is further confirmed by their empirical equations which are both rectangular hyperbolae:

$$\theta = G/(0{\cdot}013G - 0{\cdot}03) \quad .. \quad .. \quad .. \quad L.\ digitata \text{ stipe}$$
$$\theta = G/(0{\cdot}0103G - 0{\cdot}02) \quad .. \quad .. \quad .. \quad L.\ cloustoni \text{ stipe}$$

All the curves shown in Fig. 4 are for samples harvested at Oban, but the curve for Inchcolm *L. cloustoni* stipe[4] is superimposable on the graph for *L. digitata* and the equations are therefore identical.

Bed depth

L. digitata *frond*.—The effect of bed depth on the drying time of minced *L. digitata* frond was investigated with an inlet-air temperature of 159° F. Drying conditions are listed in Table IV. This curve (Fig. 5) is concave upward and the output [lb. of C.D.S./(sq. ft.)(h.)] for $T = 5$ to $T = 0{\cdot}15$ reaches an optimum value at a dry loading of 0·53 lb. of B.D.S./sq. ft. or 1½-in. initial bed-depth.

The *L. digitata* frond particles forming the bed were sticky and flexible and the fresh *L. digitata* and *L. cloustoni* fronds were almost indistinguishable in appearance when minced. Curves for bed depth versus time for these two species of fronds are given on a composite graph (Fig. 5). The two curves should be reasonably comparable since the *L. digitata* was harvested at Oban in April, 1952, and the *L. cloustoni* at Oban in May, 1951. Black[3] found that the ash contents of fronds taken from the same locality in different years were reproducible to within a month.

Comparison of the two species suggests that slightly heavier bed-loadings may be practised with *L. digitata* (0·53 lb./sq. ft. as against 0·45 lb./sq. ft. for *L. cloustoni*). These optimum bed-loadings are applicable only if the bed is undisturbed during the drying run and if the frond is loaded when wet and sticky. If partly dried frond is loaded, the optimum loading can be increased as discussed previously in this paper. It is clear that the close similarity of the physical nature of the fronds results in the similarity of the effects of bed depth on the drying time of the two species.

L. saccharina *frond*.—Owing to difficulties of obtaining supplies of this species, only six tests were carried out. The fronds received were much thinner and more flexible than those of either *L. cloustoni* or *L. digitata*. As a result, the beds of *L. saccharina* were very compressible, and slight variations in packing appeared to cause wide fluctuations in drying time. The four points shown as circles in Fig. 5 represent the four bed-depth experiments and it can be seen that the drying times correspond roughly to those for *L. cloustoni* frond. Two pairs of tests, in which samples of *L. saccharina* and *L. cloustoni* fronds were harvested at the same time and place and dried under comparable conditions, indicated that *L. saccharina* required two to four times the drying time for *L. cloustoni*. This was attributed to a compression effect, which may have been exaggerated by the use of young fronds. *L. saccharina* stipes were not studied since they represented a very small fraction of the plant (Table I). It would be uneconomical to separate these stipes from the fronds, and in any case they would probably increase the porosity of the bed if they were minced with the fronds.

L. digitata *stipe*.—The drying-time versus bed-depth curve for *L. digitata* stipe is plotted on the composite graph Fig. 5. The equation for this curve is:

$$\theta = 296 \log_{10} (L_d + 2{\cdot}02) - 0{\cdot}102$$

and it is of the same type as the curve for sliced *L. cloustoni* stipe[4] also included in Fig. 5.

It is obvious that the *L. digitata* stipe dries in about 90% of the time required for *L. cloustoni* species. It should be stated, however, that the majority of the *L. digitata* stipes used were apparently from younger plants and the cross-section of the stipe was smaller than that of *L. cloustoni*. The largest *L. digitata* stipe section encountered in these tests was an ellipse about 1¼ in. × ⅝ in. but most were nearer ¾ in. × ⅜ in., with some even smaller. This smaller size may increase the drying rate to some extent but it is possible that the thickness rather than the diameter of the stipe slices is the controlling factor. The epidermis or outer skin of plants is often intended to prevent the loss of water. If the slice-thickness is kept constant then the skin will represent a higher percentage of the total surface area of the slice as the diameter is reduced. The skin will probably not lose water so readily as a cut surface, especially as the normal movement of water in the stipe will possibly be longitudinal rather than radial.

The striking similarities of drying characteristics existing between the stipes and fronds respectively of the two species suggests that the physical differences between the two parts of the same plant have a far greater effect than the differences between the two species. It should be noted, however, that the three plants belong to the same genus. The chemical and biological differences appear to govern the difference in time of drying between the three species.

If the physical differences of stipe and frond control the relationship of drying time to bed depth, and to a lesser extent air velocity, then these differences should be general and apply to vegetable material other than seaweed.

It has been suggested previously[1] that the optimum bed-loading value for through-circulation drying of frond was caused by compression of the lower layers of the bed preventing the ready access of air. As the bed depth is increased the lower strata are progressively compressed and consequently the drying time increases sharply. In addition, the frond particles are sticky and flat and they would be particularly susceptible to this effect. However, the test with shredded frond, which formed a much more porous bed, still gave the characteristic upward curvature. The stipe, on the other hand, was in the form of flat discs, but was more rigid and apparently was not markedly compressed even when minced.

The physical characteristics of materials in the two classes may be summarized:

Class I.—Rigid or granular, forming a porous bed.
Only slight compression of lower layers.
Examples: Stipe or stalks.

Class II.—Particles are soft, flexible, easily compressed. Bulk density increases markedly with increasing bed-depth.
Examples: Frond or leaves.

Other vegetable material

This theory has been tested for the through-circulation drying of other vegetable material. Through-circulation drying tests for potato, hops and carrot have been published[5–7] and tests on brewer's spent grain have been carried out on the present drier. The physical appearance of these materials is listed below and the drying conditions used are given in Table IV.

Table IV

Through-circulation drying of vegetable material

Material	D.B.T., °F	W.B.T., °F	*G*, lb./(sq. ft.)(min.) Average	Water content, lb./lb. of B.D.S. Initial	Final
L. cloustoni frond (minced)	156	81	7·1	5·0	0·15
L. digitata frond (minced)	159	83	7·8	5·0	0·15
L. cloustoni stipe (⅛-in. slices)	157	82	5·8	5·0	0·15
L. digitata stipe (⅛-in. slices)	157	82	7·8	5·0	0·15
Potato (⅜- × $\frac{5}{32}$-in. strips)	150	90	6·0	4·15	0·2
Brewer's spent grain	180	90	4·6	3·25	0·15
Hops	149	80	2·4	4·0	0·02
Carrot ($\frac{3}{16}$- × $\frac{5}{16}$-in. strips)	158	126	27·0	6·0	0·15

Potato.—This vegetable was cut into $\frac{5}{32}$-in. square strips which were then steam-scalded at atmospheric pressure for four minutes.[8] The curve was plotted from the bed-loading factors given in a U.S. Department of Agriculture publication.[5]

Hops.—The hop cones used were approximately ¾ in. diameter and 1 in. long. They

consist of a central axis or ' strig ' surrounded by leafy bracts. The bulk density of beds of hops varies with the hop variety, the ripeness of the cones, and the moisture content. Burgess[9] found that the bulk density of the hop beds increases with greater bed-depths (Table V) indicating that slight compression of the lower layers was taking place. The cones did not pack very tightly together, however, and offered only slight resistance to air flow.

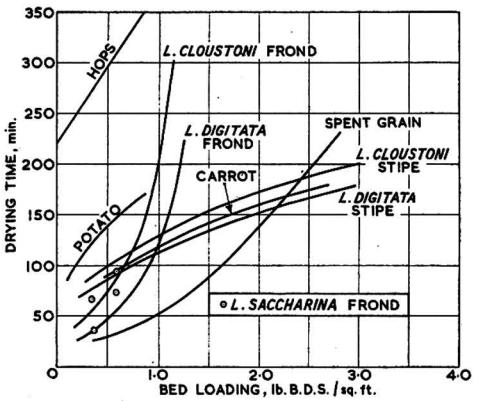

FIG. 5.—*Through-circulation drying of vegetable material. Effect of bed depth on drying time. Drying results are given in Table IV*

Table V

Bulk density of hop beds of different depths[9]

Bed depth, in.	Bulk density, lb./cu. ft.
1¼	4·20
8¼	5·04
11¾	5·40
18½	5·64
1¾	3·0
5¼	3·96
10½	4·20
13½	4·44

Carrot.—The carrots were cleaned, peeled, and cut into strips $\frac{3}{16}$ in. × $\frac{5}{16}$ in. cross-section, which were scalded before being dried. The air-flow rate used for the carrot tests was very much higher than that for the other vegetables.

Brewer's spent grain.—This is a waste product from the brewing industry and the samples used had a water content of 76–80%. In this condition the grains were sodden and tended to pack down in a mushy mass. Towards the end of the drying run the smaller husks became fluffy and were liable to become airborne, and consequently the air velocity had to be reduced.

The time versus bed-loading curves for the above vegetable materials and for seaweed are plotted on a composite graph (Fig. 5) with the experimental points omitted for clarity. Fig. 5 is intended to illustrate the shape of the curves for each material and is not a direct comparison of drying times. It shows that potato and carrot strips behave in a similar manner to stipe slices, whereas spent grain resembles the frond curves although the increase is not so sharp. Hops appear in a mid-way position as the effect of bed depth is linear. Possibly the larger material size produces a bed with a greater percentage of voids. Spheres may be the ideal particle shape for through-circulation drying and hops approximate to this shape. From a study of the time versus bed-depth curves, it is seen that none of them, on producing the smooth curve, would pass through the origin but would intercept the ordinate. This fact was observed for hops by Burgess[6] who called this time intercept the ' minimum time ', i.e. the time required for a single layer of the material to dry under the same air conditions used for drying the bed.

It is now possible to postulate three types of time versus bed-depth curves for through-circulation drying of vegetables: Type I, curve concave upward; Type II, curve concave downward; Type III, linear.

Type I, curve concave upward

Examination of the curves for frond in Fig. 5 suggests that they may be parabolic. The condition for this type of curve to reach a turning point is best considered by an example.

Let L = bed loading (lb. of B.D.S./sq. ft.)
θ = drying time (min.)
Let $\theta = aL^2 + b$ where b = minimum time, min., a = a constant

Now the output rate $R = L/\theta$, therefore $R = L/(aL^2 + b)$.

The condition for R to have a turning value (in this connexion it is a maximum) is that $dR/dL = 0$.

i.e. $$\frac{dR}{dL} = \frac{(aL^2 + b) - L(2aL)}{(aL^2 + b)^2} = \frac{b - aL^2}{(aL^2 + b)^2}$$

For dR/dL to be 0, aL^2 must equal b.

The value of θ to give the optimum output is therefore $\theta = 2b$, and the value of L when θ equals twice the minimum time gives the optimum bed-loading.

This result has been verified by an examination of the experimental curves for frond (dried, fresh and shredded) and for brewer's grain. In these instances the minimum time was obtained by extrapolation of the curve. This finding enables an estimate of the optimum drying time for this class of material under any conditions of temperature, humidity and air velocity to be calculated from one experiment—a test using a single layer under the same conditions. The actual value of L_d would still have to be determined by experiment but this should require only two or three further runs, e.g. pre-dried frond.[1] The preceding mathematical treatment applies strictly only to a parabolic function, but it is possible that most curves of a similar shape can be closely approximated to by a parabola.

The curves for the other vegetable materials of this type in Fig. 5 can be represented quite well by a parabola in the region near the optimum time, so that the factor of 2 appears to be sufficiently accurate for practical use.

Type II, curve concave downward

This type of curve cannot be so readily treated mathematically as there is some uncertainty as to the shape of the curve for values beyond the deepest beds used. The effect of bed depth on the output can, however, be predicted from consideration of the evaporation rates of beds of differing loadings. Fig. 6 is a plot of total evaporation rate in lb. of water/(sq. ft.)(h.) versus drying time between water ratios of $T = 5{\cdot}5$ and $T = 0{\cdot}15$ for *L. cloustoni* stipe slices. To enable the runs to be more easily compared, times are expressed as a percentage of the drying time between $T = 5{\cdot}5$ and $0{\cdot}15$ for each individual run.

A study of Fig. 6 reveals that for higher bed-loadings the initial constant evaporation rates are equal, due to the exhaust air approaching the limit of its water uptake. Evaporation is more efficient for the deepest beds because the constant rate extends over a larger time fraction of the drying run and also the evaporation rate is still at a reasonably high value when the material reaches the discharge water content.

The maximum evaporation that could take place for the conditions of the test would occur if the exhaust air was saturated throughout the run. It can be seen that the deepest bed approaches most closely to this state of affairs, and it may be postulated that as the depth is increased the area in the upper right side of the graph will become a smaller and smaller fraction of the whole. Clearly then the evaporation, and hence the output, will approach a limiting value, so that, within limits, the deeper the bed the higher the output becomes.

The areas under the curves in Fig. 6 give the quantity of water evaporated (in the run time) for each bed-depth. This average evaporation rate for a run is proportional to the output, and will have the same relation to bed loading as the curve of output versus bed depth given for *L. cloustoni* stipe.[4] This type of curve appears to be of the same general class as the linear type (e.g. hops) and the curves for stipe may in fact become linear in the region of higher bed-loadings.

These considerations are based on the assumption that the laws will hold for the greater depths, but it is obvious that compression must eventually occur even with comparatively rigid vegetable material, and the curves will then behave as Type I. As a result of air-pressure-drop measurements with beds of sugar-beet cossettes, Owen[10] concluded that compression was negligible for beds of this material up to 12 in. deep. It is probable that the physical characteristics of sugar-beet cossettes would not be widely different from those of stipe slices.

Type III, linear (upward)

Let $\theta = b + KL$, where b = minimum time, and K = constant.

Then $$R = \frac{L}{\theta} = \frac{L}{b + KL} = \frac{1}{b/L + K}.$$

As $L \rightarrow \infty$, $b/L \rightarrow 0$; therefore $R \rightarrow 1/K$ = a constant.

Therefore so long as the time versus bed-depth relationship remains linear, the output will approach a limiting value asymptotically. The output will not have an optimum value and so the bed depth will be determined by practical considerations, e.g. drier size and fan

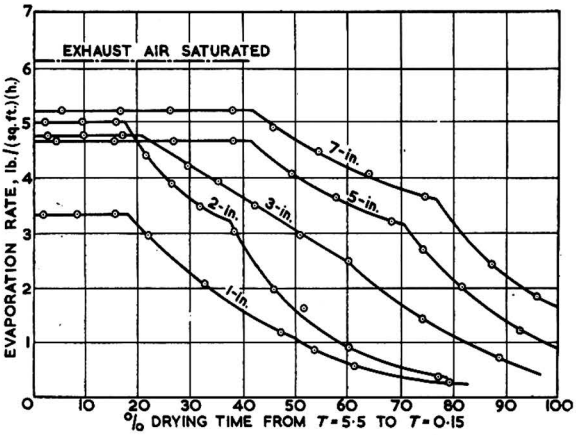

FIG. 6.—L. cloustoni *stipe. Effect of bed depth on evaporation rate. Results in Table IV*

power. If the effect of bed depth on the output rate is adopted as a criterion, it appears that the Type III curve is a special case of Type II. The physical characteristics of both types are defined in Class I.

Temperature of seaweed during drying

The internal temperature of a $\frac{1}{8}$-in. slice of *L. cloustoni* stipe was measured during drying by a copper–eureka thermocouple. A small radial hole was drilled through the outer skin of the slice and the couple pushed home so that the junction was embedded in the centre and did not break through the cut surface of the slice. A single layer of similar stipe slices (lying flat) was placed in the basket so that samples could be abstracted periodically for water-content determination as the customary procedure of weighing the basket and contents was impracticable.

The plot of material-temperature versus time (Fig. 8) shows that the seaweed temperature rose continuously during drying and did not remain at the W.B.T. for any appreciable length of time. At a water content of 0·25 the seaweed was 5° F below the D.B.T. and it reached the D.B.T. when at a water content of approximately 0·15. Ede & Hales[12] obtained substantially similar results for the internal temperature of a potato strip during drying.

Shrinkage of the individual slices is illustrated in Fig. 7, which was prepared from full-size photographs of the slices. The samples shown were selected as being reasonably typical of the batch. During the first ten minutes of drying, the slices contracted slightly in diameter and the surface water dried off. Slight curling became noticeable at a water ratio of 2–3,

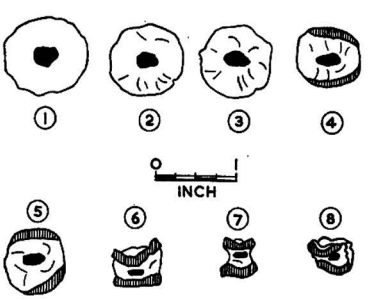

FIG. 7.—L. cloustoni *stipe. Shrinkage of $\frac{1}{8}$-in.-thick slices during drying. The medulla is shown in black and the epidermis is shaded*

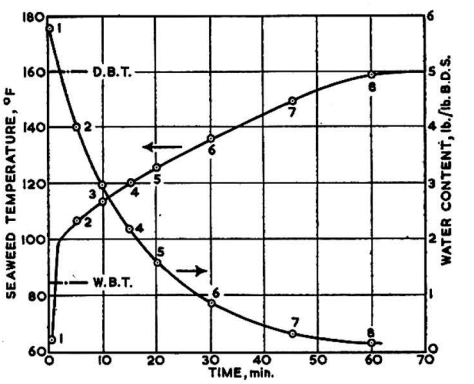

FIG. 8.—L. cloustoni *stipe. Internal temperature of a $\frac{1}{8}$-in.-thick slice during drying. Numbers on the curves refer to Fig. 7*

D.B.T., 160° F; W.B.T., 85° F; *G*, 7·6 lb./(sq. ft.)(min.)

and buckling became severe below 1, the material finally assuming the shape of a twisted saddle when dry. (Numbers on Fig. 8 correspond to the section numbers on Fig. 7.) This agrees with the observation[4] that the static pressure drop of a 3-in. bed of stipe slices becomes constant at an average water ratio of 1–1·5, when the bulk of the shrinkage will have occurred.

The initial drying period, when little shrinkage occurs, may correspond to the loss of water from the cell cavities, whereas severe shrinkage might ensue when the cell walls dry and change their shape. This water content, which would correspond to the fibre saturation point of wood, appears to be of the order of 2·0.

Constant drying rate

The initial drying rates of seaweed-stipe beds of three-inch depth or more are linear. Marshall[11] illustrated a water-content versus time curve for potato in which he suggested that the initial stages be approximated to by a straight line to simplify calculations for design purposes. For the seaweed tests the rates are linear (within small experimental error) and are not approximations. It was also observed that the exhaust air from the drier was unsaturated and further investigation revealed that in most cases the outlet air from the deepest beds was about 90% absolute humidity. It appears therefore that as the drying air approaches this value, its capacity for evaporating much more water falls away rapidly. This is in line with theory in that the air would require an infinitely deep bed to reach saturation under adiabatic conditions. It appears, then, that mass transfer for deeper seaweed-beds is of the order of a '90% equilibrium stage' for the air conditions specified.

Brown[8] did not observe constant-rate drying in tests with through-circulation drying of $\frac{5}{32}$-in. square potato strips when using inlet air conditions of 140° F D.B.T., 120° F W.B.T. and 5·6 lb./(sq. ft.)(min.) at a bed loading of 3 lb. of wet material/sq. ft. This may reasonably be attributed to the comparatively light loading used, since the constant drying rates for seaweed beds below 8·35 lb. of wet seaweed/sq. ft. had only a transient existence and, as shown in Fig. 8, a unit layer of stipe slices has no constant rate whatever. Constant drying rates have also been observed by the authors for through-circulation drying of three-inch beds of cloves, brewer's grain, sugar-beet and peat with air conditions approximating to those for stipe in Table IV.

Another factor that would tend to prolong the constant drying rate for vegetable material is shrinkage. As the bed contracts its resistance decreases and for a constant fan setting the air velocity should increase, so that the increased air flow would counteract the reduced water pick-up caused by the material becoming drier.

Acknowledgments

One of us (R. G. G.) is indebted to the Institute of Seaweed Research for an extra-mural research scholarship. This work forms part of a programme of research and development on seaweed undertaken by the Institute of Seaweed Research, and the authors are indebted to the Director for permission to publish. Acknowledgment is also made of practical assistance from James W. Simpson and Alistair S. Couper.

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Received 30 March, 1953

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STABILITY OF CONTACT INSECTICIDES.* IV.—Relationship between the Ultra-violet Absorption Spectrum and the Photolysis of DDT and the Pyrethrins†

By M. MAQSUD NASIR

The ultra-violet photolysis of DDT and the pyrethrins was investigated with selected wavebands in the middle and near ultra-violet. Severe decomposition is caused by light strongly absorbed by both insecticides at 2200–2400 Å. DDT is not affected by other parts of the spectrum, but the pyrethrins are slowly decomposed by radiation between 2400 Å and the visible region, probably by a light-catalysed oxidation rather than direct photolysis. DDT, like the pyrethrins, undergoes rapid photolysis in strong sunlight, suggesting that traces of light of the waveband 2200–2400 Å may penetrate to the earth's surface.

In previous papers in this series it has been reported that DDT[3] and the pyrethrins[1] are rapidly decomposed in ultra-violet light, that they are partly protected by benzeneazo-β-naphthol,[2, 3] and that all three absorb ultra-violet light strongly within the range[3] 2200–2400 Å.

These conclusions suggested that the rapid breakdown of the insecticides was associated with the comparatively narrow waveband strongly absorbed by them,[3] and the main purpose of this paper is to present the evidence which confirms this hypothesis. Arising from this work, further information is available about the part played by solar radiation in inactivating insecticidal deposits, and about methods of stabilizing DDT films.

Experimental

The procedure for each experiment falls into two parts, requiring description, first, of the production and isolation of the waveband used, and, secondly, of the analysis of the results of the biological estimation of the decomposition of the irradiated insecticide. The preparation of the insecticidal films, the methods of biological assay, and assessment of the results were in all material respects the same as those described in preceding Parts.

The same individual lamp was retained for this investigation. This was a high-pressure 'Osira' G.E.C. 125-w mercury-vapour lamp of a type similar to those whose spectra have been described by Garton[4] and by Radley & Grant.[5] These authors found that little light was emitted below 2350 Å, but direct comparison of reference emission lines in the ultra-violet was made with the emission spectrum of the lamp used in this work, and some emission was found down to 2180 Å, with appreciable intensity above 2200 Å.

Isolation of wavebands

The photolytic action of conveniently narrow wavebands was investigated by introducing into the optical system a series of cells containing liquids which cut off different fractions of the ultra-violet. Some Christiansen filters were also tried for this purpose, but their transmission was too low to allow the experiments reported to be completed in a reasonable time. All parts of the optical system were of quartz, including the lenses needed to ensure that light fell evenly and normally over the insecticidal films. The filters were kept cool by allowing the light leaving the lamp to pass through water, and by drawing air past them with a fan. Table I shows the liquids used in each filter. Their lower transmission limits have been recorded in the literature, and these values are given in Table I.

Table I

Filters used for isolating ultra-violet wavebands

Filter medium	Lower transmission limits (Å)		
	Observed	Recorded	Reference
Window glass (3 mm.)	3340	3130	Radley & Grant[5]
Benzyl alcohol (1 cm.)	2890	3370	Brode[6]
Decalin (1 cm.)	2850	2400	Sinsheimer & Loofbourow[7]
Acetic acid (1 cm.)	2530	2510	Brode[6]
Light petroleum (40–60°) (1 cm.)	2230	2920	Brode[6]
*cyclo*Hexane (1 cm.)	2200	2250	Sinsheimer & Loofbourow[7]
Water	2000 to visible		
No filter	1800 to visible		

* Part III: *J. Sci. Fd Agric.*, 1952, **3**, 482

† Part of a thesis approved for the Ph.D. degree by the University of London

Although all the organic liquids were of analytical-reagent quality, their spectral limits, when checked by a photospectrometer against standard emission lines, differed considerably from the published values, and these observed values were used in defining the wavebands employed.

Sets of filter papers were impregnated with one of the insecticides, and in each experiment two doses of the insecticide were used so that the reduced toxicity of the irradiated papers could be assessed by the usual relative-potency method,[8] employing unirradiated papers as a control. After a standard period of irradiation (20 minutes), the papers were tested and the fraction of the insecticide rendered inactive was computed. However, the different filters transmit different amounts of light, which is in any case not emitted in uniform intensity over the whole range, and these transmitted intensities were therefore measured in separate experiments. Two methods were used: the uranyl oxalate actinometer of Farkas & Melville,[9] which is sensitive to wavelengths between 2000 and 4500 Å; and the carbon tetrachloride actinometer of Gillam & Morton,[10] which is suitable for wavelengths lower than 2750 Å. The percentage of insecticide decomposed after the fixed irradiation period was adjusted to allow for the different intensities of irradiation at the various wavebands selected by the filters.

Results

When the filters listed in Table I were introduced successively into the optical system, the proportion of insecticide decomposed increased as the band passed by the filter reached further into the ultra-violet. To estimate the decomposition associated with any particular waveband, the difference was taken between the fraction decomposed when the filter transmitted the required waveband together with longer-wave light and the fraction decomposed when that filter was used which just excluded the waveband in question.

Fig. 1 shows the degree of decomposition of DDT when it was irradiated under standard conditions by light of the wavebands indicated. The decomposition associated with the most active region is set arbitrarily at 100% to provide a scale of reference for the other wavebands. On the same graph is shown a transcription of the ultra-violet absorption spectrum of DDT as recorded by Andrews, White, Gamov & Peterson.[11] It appears irrefutable that unless light is absorbed by the DDT no appreciable decomposition occurs, and that this decomposition is effected only by a waveband at about 2300 Å.

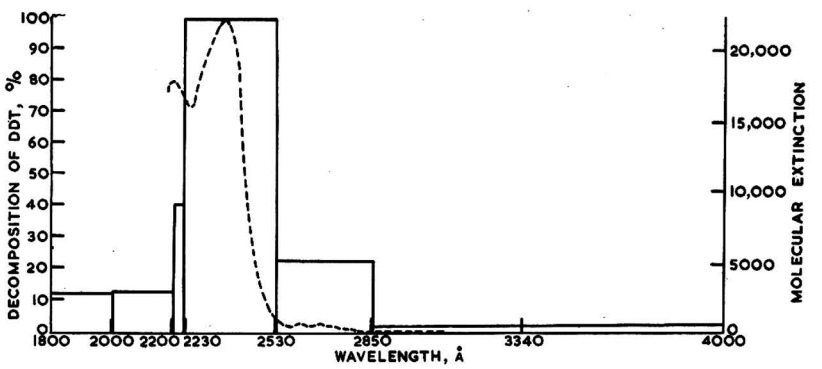

FIG. 1.—*Ultra-violet absorption and photolysis of DDT (absorption spectrum, shown as broken line, from Andrews* et al.[11])

Wichmann, Patterson, Clifford, Klein & Claborn[12] found that light of wavelengths above 2500 Å was slightly active in decomposing DDT, though much less so than the full spectrum from an ultra-violet lamp. This finding is consistent with Fig. 1, since some absorption occurs with light of up to 2800 Å. Decomposition of DDT by light of longer wavelengths was not detected in the experiments reported here, the toxicity of films irradiated with light excluding wavelengths below 2850 Å being not significantly different from that of the unirradiated control papers.

Lord[13] found that the decomposition of DDT by alkali was catalysed by metal ions, including ferric ions, and reviewed the evidence for the similar catalysis of thermal decomposition. This catalytic action of ferric chloride has been confirmed by Jonas.[14] As ferric

ions may occur on naturally irradiated substrates for DDT formulations an experiment was made in which the insecticidal film was prepared on filter papers previously wetted with 0·3 ml. of a 2% aqueous solution of ferric chloride of pH 4·7. The papers were dried at 60° before the insecticidal film was spread over them. When papers prepared in this way were irradiated, and tested as usual against similar but unirradiated films and against irradiated films prepared without the ferric chloride in the substrate, an unexpected result was obtained. Instead of an enhanced decomposition of the DDT a considerable degree of protection was afforded by the ferric chloride, 33% of the DDT which would have undergone photolysis being protected.[2] The dried deposit of ferric chloride was partly hydrolysed to the hydroxide. The extent to which reflection and absorption determine the protective action is not clear. Ferric oxide in the form of 'red mud' has been suggested as a protectant for pyrethrum films.

A graph, similar to that in Fig. 1, but showing the breakdown and absorption spectrum of pyrethrum films, is given in Fig. 2. Here the mixed natural esters were used, and the absorption spectrum shown is that of Gillam & West,[15] who worked with the natural mixture. Lord, Ward, Cornelius & Jarvis[16] have since determined the maxima for some of the fractionated constituents. These maxima show little difference, however, and no material change in the conclusions derives from the newer work.

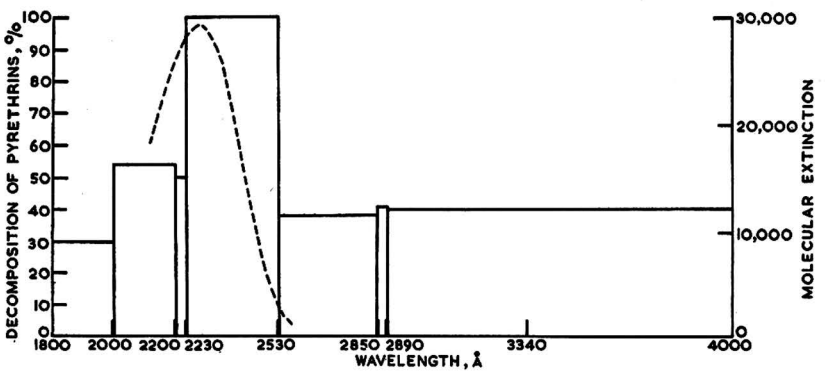

FIG. 2.—*Ultra-violet absorption and photolysis of pyrethrin (absorption spectrum, shown as broken line, from Gillam & West*[15])

The main conclusion from Fig. 2 is that, as with DDT, decomposition is most severe at about 2300 Å when the light is strongly absorbed. The most important difference between the two graphs is the considerable degree of decomposition suffered by the pyrethrins even when light is not strongly absorbed.

Decomposition of DDT films in sunlight

The results of this investigation confirm that the rapid photolysis of DDT and pyrethrum films is associated with the waveband 2200–2400 Å, but the occurrence of rapid breakdown of pyrethrum films on their exposure to direct sunlight[1] suggested that such irradiation included the critical waveband. This view was supported by the protective action of benzeneazo-β-naphthol in sunlight,[3] and by the reported decomposition of DDT films exposed to solar radiation.[12] The solar spectrum, however, is reported as terminating abruptly at about 2900 Å, so that shorter waves do not reach the earth's surface.[5, 17, 18]

A critical test of the presence of such shorter waves in sunlight was provided by an experiment in which three sets of DDT films, prepared as usual, were simultaneously exposed to direct solar radiation, to solar radiation filtered through thin (3-mm.) window glass, and to a control treatment in the dark. Two experiments were made, with irradiations lasting for 9 hours and 16 hours each. Throughout the experiments the plates on which the films were laid were tilted to preserve a normal incidence of the radiation. When the papers were removed and subjected to assay, the films exposed through window glass were as toxic as those preserved in the dark, so that changes other than those associated with light of wavelengths less than 3340 Å, which the glass removes, may be discounted.

In each experiment, however, the papers irradiated with unfiltered light were significantly ($P < 0{\cdot}01$) less toxic, corresponding to losses of about 50% of the initial weight of DDT after

9 hours' exposure, and about 70% after 16 hours' solar irradiation. These results are in broad agreement with those of Wichmann *et al.*[12]

The carbon tetrachloride actinometer was used to examine the solar radiation at the same time as the DDT films were being exposed. After 14 hours' exposure a measurable colour was developed in the solution, which Gillam & Morton[10] found only when the radiation extended below 2750 Å. A control actinometer under a glass plate was virtually colourless after a similar exposure period. These facts, taken together, constitute strong evidence that some radiation of the critical waveband for DDT and pyrethrum does reach the earth's surface. The photolytic activity of such radiation is so high that the intensity, compared with the visible component of solar radiation, may well have been considered negligible by other workers.

Discussion and conclusions

The results presented here establish that for DDT and the pyrethrins photolysis occurs when ultra-violet light is absorbed by the insecticide molecules. Clearly the mechanism by which ultra-violet photolysis of the pyrethrins is initiated is different from that by which the insecticide is decomposed when light is not absorbed, although, as Blackith[1] has shown, this slower reaction is also light-catalysed. Indeed, the results of these experiments provide convincing support for the views outlined in the preceding papers in this series, and, in particular, the division of the chemical processes leading to the inactivation of pyrethrum films into a rapid direct ultra-violet photolysis, a slower and more general light-catalysed decomposition (probably an oxidation) and a much slower atmospheric oxidation, taking place even in complete darkness.

The similarity between the absorption by, and reaction of, DDT and pyrethrum to the critical waveband lying between 2200 and 2400 Å supports the suggestion made in Part III that the protective action of benzeneazo-β-naphthol depends essentially on its known capacity to filter out light of the critical waveband. Although investigations of the solar spectrum have suggested that the ozone layer of the upper atmosphere completely filters out radiation lying between 2200 and 2930 Å,[5, 17, 18] the results of both exposing the insecticides to sunlight and direct measurements with a carbon tetrachloride actinometer point to the fact that such absorption is not complete for some bands within the critical region of the ultra-violet. According to Meyer[18] there is a minimum in the absorption spectrum of ozone at about 2200 Å and his figures suggest that the screening effect of ozone may be relatively weak for radiations of from 2000 to about 2300 Å.

The limited ranges for which absorption spectra are available preclude a discussion of the photolytic efficiency for different parts of the spectrum, and a further complication for the pyrethrins is their alternative decomposition processes. For DDT, however, Fig. 1 suggests that photolysis between 2530 and 2850 Å is greater, relative to the amount of light absorbed, than between 2230 and 2530 Å. Blackith showed that the rapid photolysis of pyrethrum films was independent of the presence of oxygen,[1] and that it proceeded, under standard conditions of illumination, at the same rate as the photolysis of DDT.[3] The work described in this paper emphasizes this parallel between the photolytic breakdown of two structurally dissimilar insecticides.

The protection of both insecticides against this ultra-violet photolysis depends on the addition of substances that absorb the critical waveband with sufficient intensity in thin films. Benzeneazo-β-naphthol[3] and, perhaps, ferric chloride, act in this way.

Acknowledgments

The writer is greatly indebted to Mr. W. R. S. Garton, of Imperial College Physics Department, who kindly made photospectrometric estimations of the emission spectrum of the lamp used in this work, and of the transmission limits of the ultra-violet filters. Grateful acknowledgment is due to the Government of Pakistan for the scholarship under which this work was done, and to Professor J. W. Munro for facilities to work in his department. The writer is indebted particularly to Dr. A. B. P. Page and Dr. R. E. Blackith for their valuable advice and constructive criticism.

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Received 2 March, 1953

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BEHAVIOUR OF FUMIGANTS DURING VACUUM FUMIGATION

II.*—Penetration of Methyl Bromide into Bagged Wheatfeed, and Related Diffusion Experiments

By W. BURNS BROWN and S. G. HEUSER

The penetration of methyl bromide into bags of wheatfeed has been investigated in experimental fumigations at atmospheric pressure and by various techniques in which reduced pressures are used. In a series of four-hour treatments the highest penetration factors were obtained when, after allowing an initial period of one hour at reduced pressure, the pressure was restored to atmospheric The effects of total pressure and of sorption on the diffusion of methyl bromide, carbon dioxide and hydrogen cyanide along a column of wheatfeed have been studied.

In Part I of this series[1] three techniques of vacuum fumigation in current use were outlined. In small experimental fumigations by these methods the penetration of methyl bromide into boxes of compressed dates was studied by the measurement of gas concentrations. The efficiency of penetration of fumigant to a particular point in a selected fumigation period was given by a penetration factor. This was defined as the concentration–time product obtained at the point expressed as a percentage of the product of the nominal concentration and the time. When testing the sustained-vacuum method, the method of vacuum fumigation with simultaneous admission of air and fumigant, and fumigation at atmospheric pressure, it was found that the sustained-vacuum method gave the highest penetration factors; these factors could be further increased by restoring the pressure to atmospheric some time before the end of the treatment. (The terms used in this paper to describe the various techniques of vacuum fumigation follow the recommendations of Page, Blackith, Brown & Heuser.[2]) It was clearly desirable to investigate this last point further. However, boxes of dates had proved to be unsuitable material for careful comparative tests since large differences in gas penetration could result from the large variations in their packing and condition. The investigations have therefore been continued with bags of wheatfeed.

For the diffusion of one gas into another (e.g. into air) the coefficient of diffusion is inversely proportional to the total number of molecules in unit volume. For a given partial pressure of the diffusing gas a large reduction in the partial pressure of the other gas will result in a large increase in the rate of diffusion. This effect should be sufficient to account for the more rapid penetration of fumigant into commodities in a sustained-vacuum treatment compared with that observed in a treatment at atmospheric pressure. The influence that sorption of the fumigant by the commodity may have upon the relationship between total pressure and rate of diffusion appears not to have been investigated. The diffusion of gases along a column of wheatfeed has therefore been studied at atmospheric and at reduced pressures. These tests

* Part I: *J. Sci. Fd Agric.*, 1953, **4**, 48

have been made with methyl bromide, the fumigant used in the main part of this work, with carbon dioxide, which shows negligible sorption by wheatfeed, and with hydrogen cyanide, which is sorbed to a greater extent than methyl bromide.

Experimental

The experimental fumigations of single bags of wheatfeed were carried out in a 1700-l. steel chamber in a room maintained at 15° and 70% relative humidity. Each bag weighed 112 lb. and the least depth to the centre of a bag was about 9 in. The moisture content of the wheatfeed was approximately 11%.

In all the tests a measured volume of methyl bromide was drawn from a counterpoised gas-holder. The procedure in the vacuum-fumigation tests was exactly as described in the corresponding tests on boxes of dates. For the tests at atmospheric pressure the pressure in the chamber was first reduced by about 3 cm. The required volume of methyl bromide vapour was introduced from the gas-holder and the pressure then adjusted to atmospheric. A fan, providing only very gentle stirring, was kept running in the chamber for ten minutes. In all the vacuum-fumigation tests the initial pressure before introduction of fumigant was 5 cm. In Expt. 10, in which the pressure was restored to atmospheric immediately after introduction of the fumigant, the final pressure was attained in about one minute. During the rapid release of air into the chamber in this test the temperature rose to about 20° and cooling during the subsequent ten minutes produced a slightly reduced pressure. This was again adjusted to atmospheric pressure. Similar adjustments were made after restoring the pressure to atmospheric in other tests.

Gas samples were drawn from the centre of the bag in all the tests and also, in some tests, from a point 3 in. below the surface. The techniques of gas-sampling and determination of methyl bromide have been described.[1]

For the diffusion experiments use was made of a glass tube 26 in. long and 1·5 in. in diameter, closed at one end by a cap fitted with a flat-flanged joint. This was filled with wheatfeed by gently tapping down to a constant volume to within 2 in. of the open end. The wheatfeed was held in place within the tube by a piece of fairly coarse wire gauze secured by a clip. Glass capillary sampling-tubes were fitted in the diffusion tube at distances of 2 in., 10 in. and 18 in. from the exposed face of the wheatfeed, with their ends at the centre of the bore.

For the tests at atmospheric pressure an even concentration of fumigant was established in the 1700-l. chamber at 15°, then the open end of the diffusion tube was inserted into a port in the side of the chamber. The tube was supported externally in a horizontal position and held tightly in the port by a rubber ring. For the tests at reduced pressure the whole tube was placed in a horizontal position inside the chamber. The pressure was first reduced to 5 cm., the fumigant was introduced, and the pressure was then adjusted to 7 cm. It was found that at this pressure an even distribution of fumigant throughout the chamber was quickly attained. In all the experiments a constant concentration of fumigant at the exposed surface of the wheatfeed could be assumed with very little error. Each test was continued for four hours.

In the experiments with methyl bromide the required dose of fumigant was drawn from the counterpoised gas-holder. Carbon dioxide was drawn directly from a cylinder through a reducing valve. The required concentration of hydrogen cyanide was obtained by boiling off the liquid from a small flask. Provision was made for collecting gas samples in 20-ml. evacuated flasks from the three points in the diffusion tube. The capillary tubes from these points were kept closed except when attached to sampling flasks. The free-space concentration was also determined. Concentrations of methyl bromide were determined as described in Part I. Carbon dioxide was absorbed in 0·05N-sodium hydroxide, and, after precipitation of carbonate with barium chloride solution, the excess of sodium hydroxide was titrated with 0·05N-hydrochloric acid, using phenolphthalein–thymol blue as indicator. Hydrogen cyanide was absorbed in sodium hydroxide solution and determined iodometrically as described by Lubatti.[3]

Results

Curves showing the probable variation of concentration with time have been drawn for each experiment on a bag of wheatfeed. Those for Expts. 1–3 and 7–10 are reproduced in Figs. 1–5. Areas below the curves have been measured to provide estimates of the concentration–time products in mg. h./l. obtained in selected fumigation periods. These are shown in Table I, together with the corresponding penetration factors.

The rates of diffusion of methyl bromide, carbon dioxide and hydrogen cyanide along a

Table I

Summary of results on bags of wheatfeed

Expt. No.	Method	Sampling position	Nominal concn., mg./l.	Period of treatment, h.	Concn.–time product, mg. h./l.	Penetration factor	Estimated penetration factor for sustained-vacuum method
1	Atmospheric fumigation ..	Centre	40	3	16	13	
				4	29	18	
		3 in. deep		3	43	36	
				4	67	42	
2	Vacuum fumigation with simultaneous admission of air and fumigant	Centre	48	3	20	14	
		3 in. deep		3	40	28	
3	Vacuum fumigation with atmos. pressure restored after 3 h. 10 min.	Centre	40	3	83	69	
				4	127	79	70
		3 in. deep		3	91	76	
				4	133	83	75
4	Atmospheric fumigation ..	Centre	95	3	27	9	
				4	59	16	
5	Vacuum fumigation with simultaneous admission of air and fumigant	Centre	95	3	40	13	
6	Vacuum fumigation with atmos. pressure restored after 3 h. 15 min.	Centre	95	3	186	65	
				4	302	79	69
7	Vacuum fumigation with atmos. pressure restored after 3 h.	Centre	70	4	206	74	61
		3 in. deep		4	200	71	60
8	Vacuum fumigation with atmos. pressure restored after 2 h. 10 min.	Centre	70	4	253	90	
		3 in. deep		4	234	84	
9	Vacuum fumigation with atmos. pressure restored after 1 h.	Centre	80	4	299	94	
		3 in. deep		4	284	89	
10	Vacuum fumigation with atmos. pressure restored immediately after dosage	Centre	65	3	81	42	
				4	133	51	
		3 in. deep		3	147	75	
				4	195	75	

column of wheatfeed are illustrated by curves showing the increase of concentration with time at each sampling point (Fig. 6), the concentrations found being expressed as percentages of the free-space concentration, which differed in each test (Table II).

Discussion

Tests on bags of wheatfeed

The first three experiments were carried out at nominal concentrations of 40 to 48 mg./l. The results of Expt. 1 (Fig. 1) illustrate the slow penetration of methyl bromide to the centre of a bag of wheatfeed in a treatment at atmospheric pressure. Penetration is far from complete in the four-hour period, the penetration factor at the centre being only 18.

Vacuum fumigation with simultaneous admission of air and fumigant was tested in Expt. 2 (Fig. 2), but the rate of penetration was not greater than that found with treatment at atmospheric pressure. The initial peak concentration which was observed at the centre of the package at the beginning of the fumigation of boxed dates[1] did not occur in the centre of the bag of wheatfeed. This difference is undoubtedly due to the greater and more rapid sorption of methyl bromide by the finely divided wheatfeed, so that all the fumigant forced into the bag during the application of the fumigant–air mixture is sorbed by the outermost layer in the bag.

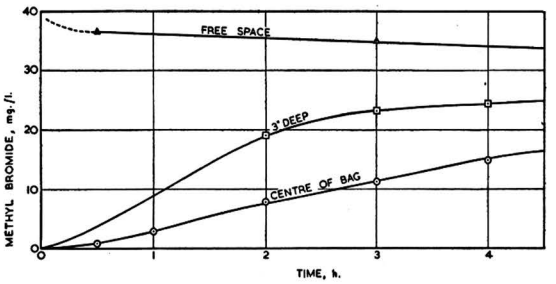

FIG. 1.—*Fumigation at atmospheric pressure. Expt.* 1

In Expt. 3 the reduced pressure was maintained for 3 h. 10 min. Steeply rising curves were produced (Fig. 3), showing very good penetration throughout the bag within one hour. On restoring the pressure to atmospheric a characteristic peak concentration was obtained in the bag, similar to that produced in experiments on boxed dates.[1] The increase at the centre was not as great as in the tests with dates, and the subsequent fall in concentration towards the level of the free-space concentration was more rapid. The penetration factor obtained at the centre in a period of four hours was 79, as compared with an estimated figure of 70 which would have been obtained had the reduced pressure been sustained throughout this period (Table I, final column).

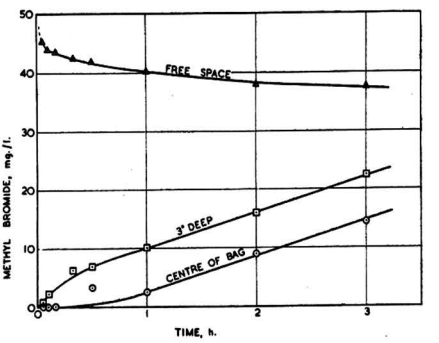

FIG. 2.—*Vacuum fumigation with simultaneous admission of air and fumigant. Expt.* 2

The treatments given in Expts. 1, 2 and 3 were repeated at a higher nominal concentration in Expts. 4, 5 and 6. For each technique the curves obtained were similar in shape to those obtained at the lower concentration and are not shown. The concentration–time products were correspondingly higher (Table I) but the penetration factors were almost the same. Again, in Expt. 6, a useful increase in the penetration factor at the centre over that obtained by the sustained-vacuum method resulted from the restoration of atmospheric pressure after 3 h. 15 min.

With wheatfeed, penetration of methyl bromide under the conditions of a sustained-vacuum treatment was so rapid that it seemed likely that it would be advantageous to restore the pressure to atmospheric before a lapse of three hours. The sharp rise in concentration should be obtained provided there has been adequate penetration before the pressure is raised, and the earlier this peak concentration is produced the greater should be the benefit to the penetration factor. This was tested in Expts. 7, 8 and 9, all based on a four-hour period of treatment, the pressure being restored to atmospheric after 3 h., 2 h. 10 min. and 1 h. In a final experiment (Expt. 10) the pressure was restored to atmospheric immediately after introduction of the dose of methyl bromide. This test corresponds to a method which has been practised to some extent in the U.S.A.

The increase in concentration on raising the pressure to atmospheric was approximately the same in Expts. 7, 8 and 9 (Fig. 4), and the penetration factors at the centre and at the 3-in. depth were greatest in Expt. 9 in which the period following the rise in pressure was longest. The result of raising the pressure to atmospheric before an adequate penetration of methyl bromide into the bag has taken place was investigated in Expt. 10 (Fig. 5). A high concentration was attained almost immediately at the sampling point 3 in. deep, but there was no sudden increase in concentration at the centre. Evidently the entry of air to the chamber forced fumigant into the bag but this fumigant was very largely sorbed in the outer layers. There was a small initial fall in concentration at the 3-in. depth due to sorption, but the amount of gas sorbed by the wheatfeed in these outer layers quickly approached equilibrium with the free-space concentration and thereafter sorption caused little hindrance to

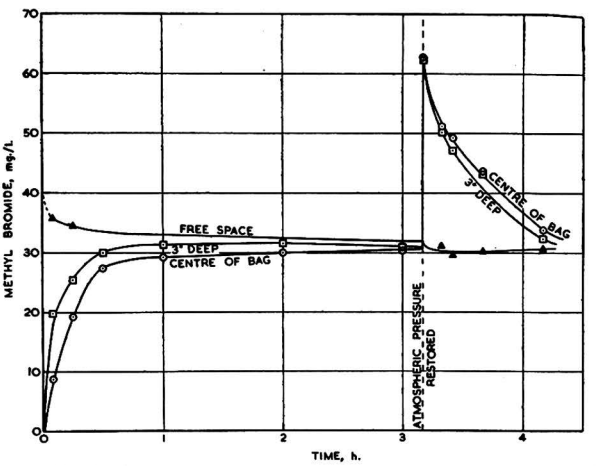

FIG. 3.—*Vacuum fumigation with atmospheric pressure restored after* 3 *h.* 10 *min. Expt.* 3

the diffusion of gas through these layers. Thus the concentration at the 3-in. depth was maintained at a level little below the free-space concentration. Further, the more rapid diffusion through the outer layers of the bag allowed the concentration at the centre to rise more rapidly than in a treatment entirely at atmospheric pressure (e.g. Fig. 1). Similar effects were not obtained in the vacuum-fumigation tests with simultaneous admission of air and fumigant (e.g. Fig. 2), presumably because in this method the proportion of methyl bromide to air in the free space remains constant throughout the operation of raising the pressure to atmospheric, whereas when the air is introduced after the fumigant the proportion of fumigant to air is initially very much higher. In this second case a much larger amount of fumigant is forced into the bag during the rise in pressure.

Although in these tests with wheatfeed the method of vacuum fumigation with atmospheric pressure restored immediately after dosage gave a higher penetration factor at the centre of the bag than either the normal method at atmospheric pressure or the method with simultaneous admission of air and fumigant, this factor was less than would be obtained with the usual sustained-vacuum method. Since in the treatment of this product under the particular conditions of these tests restoration of the pressure to atmospheric one hour after introduction of fumigant gives a penetration factor greater than that obtained by the usual sustained-vacuum method it follows that, at one particular time during this first hour, restoration of the pressure to atmospheric would produce a penetration factor equal to that given by the sustained-vacuum method. Raising the pressure before this particular time will give inferior penetration and at any time afterwards superior penetration. In general this initial period will be affected by a number of factors including the nature of the product and the package, the loading of the chamber, the reduced pressure employed, and the speeds of introduction of fumigant and air. The effect is probably responsible for the widely divergent views which have been expressed, after comparative tests, on the relative merits of the sustained-vacuum method and of vacuum fumigation with atmospheric pressure restored immediately after dosage.

The time at which restoration of the pressure to atmospheric will give the highest penetration factor will also be affected by the factors just given. Some further investigation of variations arising from the nature of the product has been undertaken and will be the subject of a further communication.

The insecticidal efficiency of a treatment depends not only upon the efficiency of penetration but upon the resistance of the insects under the particular conditions of test. Until more information is available on the results of pressure changes and the shape of the concentration–time curves on the minimum concentration–time product effective against different kinds of insects, we cannot be certain which technique of fumigation employing reduced pressures will be most efficient. However, unless the biological effects run very markedly counter to the results on the physicochemical behaviour of the fumigant, it can be expected that the most efficient treatment will consist of a period at reduced pressure followed by a period at atmospheric pressure. This procedure merits trials on a full commercial scale.

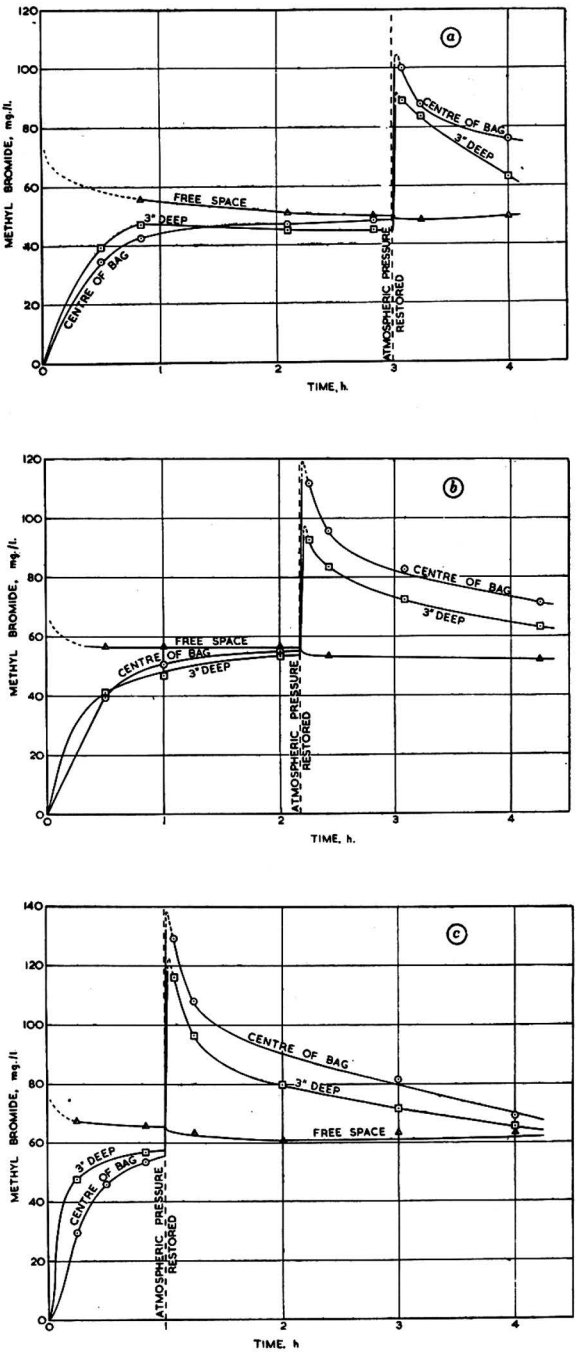

FIG. 4.—*Vacuum fumigation with atmospheric pressure restored after* (a) 3 *h.*, (b) 2 *h.* 10 *min. and* (c) 1 *h. Expts.* 7–9

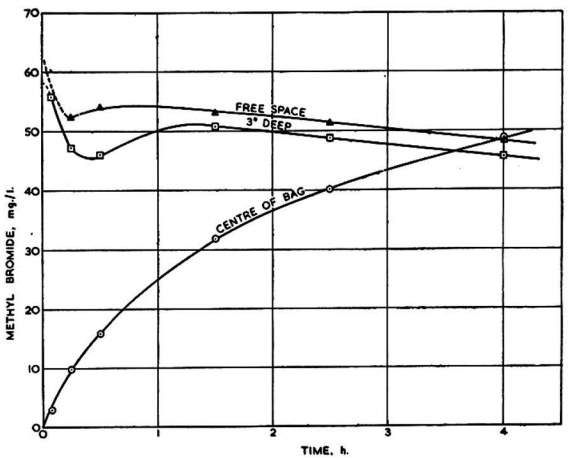

FIG. 5.—*Vacuum fumigation with atmospheric pressure restored immediately after dosage. Expt.* 10

Diffusion experiments

The diffusion experiments in the column of wheatfeed were designed to elucidate some of the effects observed in the experiments on bags. The period of each test was limited to four hours and it was not possible in this time or with this apparatus to determine coefficients under conditions of steady flow. The experimental techniques introduced certain errors into the determination of the true rate of penetration along the column. Small variations in pressure during establishment of the working free-space concentration occurred in the experiments at reduced pressure. In all the experiments the removal of gas samples from the column would artificially accelerate the penetration. For one gas the effect should be the same at the two pressures tested, but the errors would diminish with increasing sorption and would therefore be greatest for carbon dioxide and least for hydrogen cyanide. These errors may limit the accuracy of any calculations which are attempted but nevertheless some useful comparisons can be made.

The three gases tested show widely different rates of penetration along the column corresponding to their different degrees of sorption on wheatfeed. Carbon dioxide, which is not sorbed to any practical extent, penetrated most rapidly, whereas with hydrogen cyanide, which is most strongly sorbed, there was no penetration in four hours to the 18-in. point at reduced pressure or to the 10-in. point at atmospheric pressure.

The effect of total pressure upon the rate of penetration of each gas along the tube can be seen by comparing the times required to obtain at a selected point a concentration equal to 20% of the free-space concentration to which the free surface of the wheatfeed was exposed. Points at different distances from the free surface must be chosen for each gas. These times are shown in Table II, together with the ratios of the times taken by each gas at atmospheric

Table II

Comparison of rates of diffusion at atmospheric and reduced pressures

Gas	Approx. total pressure, cm.	Free-space concn., mg./l.	Depth of penetration, in.	Time to reach 20% of free-space concn., min.	Ratio of times taken at pressures of 76 and 7 cm.
CH_3Br	76	37	10	260	8
CH_3Br	7	37	10	33	
CO_2	76	50	18	118	10
CO_2	7	40	18	12	
HCN	76	20	2	106	9
HCN	7	18	2	12	

pressure and at 7 cm. In spite of the wide differences in rate of penetration shown by the three gases these ratios lie close together and do not differ widely from the ratio of the total pressures. The rate of diffusion of each gas in free air would be expected to be inversely proportional to the total pressure.

Assuming that carbon dioxide is not sorbed by wheatfeed the results obtained with this gas can be used to calculate by Graham's law the rates of penetration for the other two gases if there had been no sorption. These estimated figures can then be compared with the observed rates of penetration. This has been done in Table III for the times taken to obtain at the

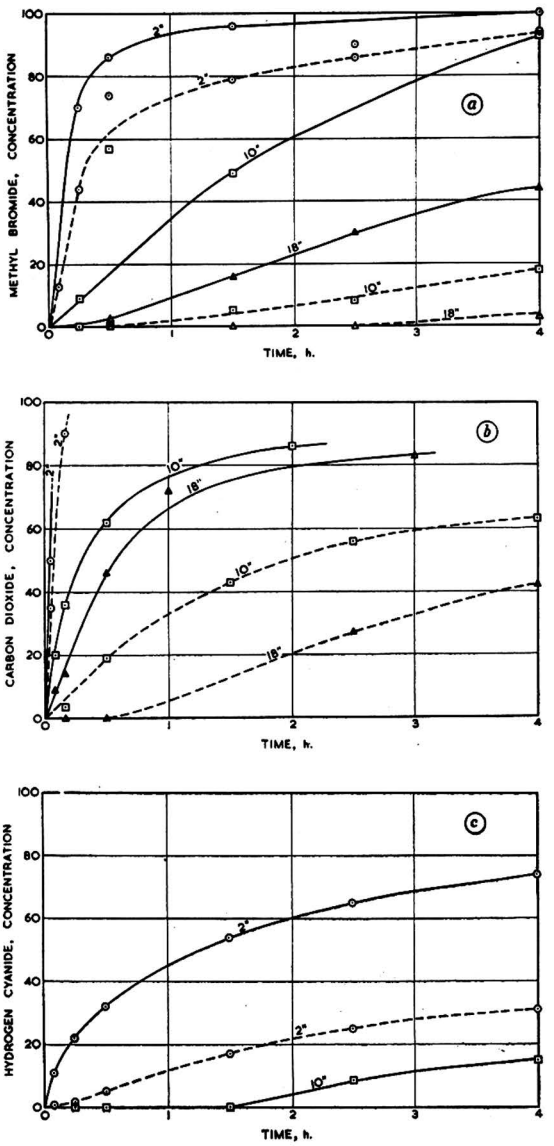

FIG. 6.—*Penetration of gases through a column of wheatfeed at atmospheric pressure (broken lines) and at a pressure of 7 cm. (solid lines): (a) methyl bromide, (b) carbon dioxide, (c) hydrogen cyanide. Concentrations are expressed as percentages of the free-space concentration*

10-in. point a concentration equal to 15% of the free-space concentration. According to these estimates sorption by the wheatfeed has reduced the rate of penetration of methyl bromide to about one-sixth and of hydrogen cyanide to one-hundredth of that to be expected if there were no sorption. With methyl bromide the same reduction was obtained at atmospheric pressure and at a pressure of 7 cm. This supports the evidence of the results in Table II that the effect of sorption on the rate of penetration of a gas into a bulk of material is unaffected by the total pressure. The sorption of methyl bromide and hydrogen cyanide by wheatfeed at different total pressures is being studied and will be the subject of a later communication.

Table III

Effect of sorption on rates of penetration

Columns (*a*) to (*e*) show times in minutes to reach 15% of the free-space concentration at a depth of 10 in.

Approx. total pressure, cm.	Carbon dioxide measured	Methyl bromide			Hydrogen cyanide		
		estimated for no sorption	measured	ratio (*c*)/(*b*)	estimated for no sorption	measured	ratio (*e*)/(*d*)
	(*a*)	(*b*)	(*c*)		(*d*)	(*e*)	
76	24	35	210	6	—	—	—
7	3	4·4	25	6	2·4	240	100

The results of these tests emphasize the great influence of the total pressure upon the rate of penetration of fumigant during the period of treatment under reduced pressure. Thus a reduction of this working pressure by one-half (e.g. from 10 cm. to 5 cm.) can be expected to produce double the rate of penetration. It can also be deduced that the lower this working pressure the greater will be the rise in concentration within the package when the pressure is restored to atmospheric.

Acknowledgment

This work has been carried out as part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research.

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Received 25 March, 1953

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THE EFFECT OF VARIETY, STORAGE AND LOCALITY ON THE ASCORBIC ACID CONTENT OF THE POTATO TUBER

By R. M. ALLISON and C. M. DRIVER

Tubers of 20 varieties of potato grown in New Zealand have been analysed for ascorbic acid content at harvest and at monthly intervals during storage in a commercial-type clamp, in two successive years. Variety was a significant factor in causing differences in the ascorbic acid content of potatoes, and these differences were reflected in the response of the different varieties to storage. An increase in ascorbic acid after prolonged storage has been found and this is discussed with reference to temperature variations, sampling methods and metabolism during storage.

Introduction

During the last two decades, the potato as a source of vitamin C has received considerable attention from nutritionists. Although not a very rich source of this vitamin, the potato is important because its appetite value, cheapness, ready availability and keeping quality make it a daily contributor to the normal Western European dietary. Thus the two or three medium-sized potatoes eaten daily by most Europeans may, if suitably cooked, contribute at least half of the daily vitamin-C requirement of 75 mg. Indeed, Salaman[1] has emphasized that during the two world wars of the twentieth century the potato has been the main defence against scurvy in the European countries involved. Notwithstanding its obvious importance, the systematic study of the effect of variety, environment, storage and cooking on the vitamin-C content of potatoes received meagre attention until the commencement of the second World War. This is shown by the fact that Lampitt & Goldenberg,[2] in their review of the literature on the potato as a food, cite only 20 papers dealing with vitamin-C content. However, during the past 12 years the published work on vitamin-C content has shown a more systematic approach, and there can be little doubt that a great deal of this work received its main stimulus from the nutritional problems associated with the war. Later investigations have been concerned with sampling techniques and the distribution of this vitamin within the tuber.[3–5] That such studies must precede any attempt to define differences due to environment, variety or storage is emphasized by the considerable divergence of opinion expressed by earlier workers who have attempted to study these factors. The effect of variety, storage and soil-type have been studied by Julén,[6] Lampitt, Baker & Parkinson[7] and Murphy, Dove & Akerley,[8] and the effects of storage have been investigated in more detail by Esselen, Lyons & Fellers,[9] Baker, Parkinson & Lampitt[10] and Wokes & Nunn.[11] A recent study of such factors affecting the nutritive value of potatoes is that of Leichsenring *et al.*[12] The physiology of ascorbic acid loss during storage has been the subject of a study by Barker,[13] who, on the basis of close correlation between ascorbic acid and sucrose changes in one variety only, postulates a close relationship between ascorbic acid and respiration.

Most of these investigators have considered relatively few varieties, and the desire seems to have been to secure representative results rather than to study varietal differences in behaviour. So far, the most comprehensive study of varieties is that of Murphy *et al.*,[8] who tested 54 varieties, consisting of 22 table varieties, 15 promising seedlings, 14 German varieties and two wild types. Most of these varieties were grown in replicated trials in Maine, U.S.A. These workers came to the conclusion that there are considerable varietal differences in vitamin-C content, and that these differences are strongly inherited. Most of the commercial varieties they tested are not grown in New Zealand, and only three, Katahdin, Chippewa and Sebago, are included in the present study. Of the 19 varieties investigated by Smith & Gillies[3] seven are subjects of this study, namely Arran Banner, Arran Chief, Arran Consul, Arran Pilot, Majestic, King Edward VII and Up-to-Date.

Although losses in ascorbic acid during storage are well known, no adequate consideration appears to have been given to the differential behaviour of varieties during storage. The choice of suitable varieties for supplying the winter demand for potatoes should take cognizance of this differential loss of ascorbic acid if the nutritionally desirable level of vitamin C is to be maintained during the whole of the storage period. Even if such traditionally rich sources as citrus fruits and tomatoes are available, the cost of their vitamin C is usually several times the cost of the vitamin from potatoes.[14] Leichsenring *et al.*,[12] in an extensive collaborative study covering 11 States in the U.S.A., have demonstrated a variety : storage interaction for a small number of varieties stored at different temperatures, but in general their storage conditions differed from those commonly encountered here.

The present paper is concerned mainly with varietal differences and the different behaviour in storage of varieties grown in New Zealand.

Materials and methods

The seed tubers used were the highest grade available under the New Zealand certification scheme. The actual virus content of the stocks from which the samples were drawn is shown in Table I.

The first trial was grown in 1948–49 on a silt loam at Lincoln, Canterbury, New Zealand, and consisted of 19 commercial varieties and one locally bred seedling, R.G.R. 64/7, grown in a randomized block of six replications. The trial was harvested on 24 May, 1949, and yield results were taken. The first analyses for ascorbic acid were made on 28 June when the tubers were placed in storage, and analyses of material from storage were made on 2 August, 13 September, 19 October and 10 November, giving five series of tests in all. For analyses,

replications one, two and three from the yield trial were bulked to give replication A, and replications four, five and six to give replication B. For the storage experiment, 20 tuber samples (table size) were taken at random from replications A and B and placed separately in small bags. Each series of 20 varieties from each replication was placed in a large sack and the process was repeated to provide two replications of all varieties for each proposed sampling. The sacks were placed in the centre of a commercial-type clamp and two replications were withdrawn at each sampling date, analysed separately, and the results averaged.

Table I

Virus-disease content of potato varieties

Healthy	40–70% virus X	100% virus X	100% X + Y	Not known
Arran Chief	Chippewa (60)	Arran Banner	Aucklander Short Top	Arran Pilot
Epicure	Cliff's Kidney (70)	,, Consul	Aucklander Tall Top	Hardy Ilam
Jersey Bennes	Dunbar Standard (65)	Dakota Red		Up-to-Date
King Edward	Glen Ilam (50)	Inverness Favourite		R.G.R. 64/7
	Iron Duke (50) (= President)	Majestic		
	Katahdin (40)			
	Sebago (70)			

For the 1949–50 trial, certain varieties were substituted and replicated trials were grown at Pukekohe (Lat. 37° 12′ S.), Palmerston North (Lat. 40° 25′ S.), Lincoln (Lat. 43° 38′ S.), and Gore (Lat. 46° 7′ S.). Those away from Lincoln were harvested at maturity, the replications were bulked and a large random sample was sent to Lincoln for testing. The remainder of this sample was stored and analysed again at the conclusion of the experiment. The Lincoln trial was analysed a total of eight times, twice before harvest, and six times after harvest. For the first two the trial was sampled *in situ* on 28 February and 20 April, 82 and 31 days respectively before harvest. The trial was analysed again at harvest (1 June), at the beginning of storage (16 June), and thereafter at intervals of a month to six weeks (25 July, 22 August, 27 September and 8 November). For the first sampling each of the six replications was analysed separately, but for all subsequent samplings replications one to three and four to six were bulked as in the previous year's trials. The design of the storage experiment was the same as that of the previous year, except that the clamp was larger and provision was made for observing temperature changes at the centre of the clamp by means of a recording thermometer.

The sampling method used was similar to that of Murphy *et al.*,[8] except that the sectors were cut at right angles to the line joining the stem and bud ends of the tuber, midway between the two ends. One sector was taken for ascorbic acid analysis and the other for dry-matter determination. The total weight of six such sectors was between 30 and 40 g. Details of the analytical method are reported here as it enables large numbers of samples to be analysed rapidly. The combined sectors from six tubers were quickly weighed and transferred immediately to 25 ml. of 20% (w/v) metaphosphoric acid. The sample was extracted by maceration in a Waring Blendor for 2½ minutes with the addition of 50 ml. of glass-distilled water and 50 g. of crushed ice. Capryl alcohol (2–3 drops) was added to the macerate to break the foam. The macerate was transferred quantitatively to a stoppered measuring cylinder and made up to 250 ml. After thorough mixing the macerate was filtered, the first turbid portion of the filtrate was used for rinsing the flask, and suitable aliquots of the clear filtrate were rapidly titrated in duplicate with 2 : 6-dichlorophenolindophenol solution. No analyses for dehydroascorbic acid were performed. Dry-matter samples were chopped finely, quickly weighed, and dried for 20 hours at 105°. Results are expressed as mg. of ascorbic acid per 100 g. of fresh tissue.

As a preliminary to the ascorbic acid analyses it was necessary to determine the minimum number of tubers to be sampled in order to obtain significant differences of a satisfactory order. Samples were taken from a number of tubers, each was analysed separately as described above and the reversed t test (Snedecor[15]) applied to the results. For a significant difference ($P = 0{\cdot}01$) of 2 mg. per 100 g., five tubers were sufficient.

Results

From the 1948–49 experiment, only the initial and final ascorbic acid and dry-matter figures are given in Table II, as the ascorbic acid decay curve in that year was similar to that

of 1949–50, and the later experiment covered a longer period. However, the results from the five sample dates are included in the statistical analysis. Table III gives the harvest and final ascorbic acid and dry-matter figures for the four localities in 1949–50, and Fig. 1 shows the changes in ascorbic acid content of the 20 varieties grown at Lincoln and used in the 1950 storage experiment. Results from the first sampling at Lincoln in 1950 are excluded from the statistical analysis in Table IV as the whole six replications were sampled compared with two bulk replications (bulks of replications one to three and of four to six) from the remaining seven samplings.

Table II

Initial and final ascorbic acid and dry-matter contents for 1949 storage experiment

Variety	Ascorbic acid, mg./100 g., wet basis		Dry matter, %	
	Initial 28/6/49	Final 10/11/49	Initial 28/6/49	Final 10/11/49
Arran Banner	18·2	10·0	21·3	23·7
,, Chief	13·8	9·9	22·6	22·9
,, Consul	13·1	10·3	23·5	23·4
,, Pilot	14·4	9·6	20·0	21·6
Aucklander Short Top	19·1	9·9	24·0	21·9
,, Tall Top	19·5	10·5	22·7	22·1
Chippewa	19·2	8·4	22·4	23·8
Cliff's Kidney	15·4	8·5	21·0	24·0
Dakota Red	18·2	10·5	24·6	25·0
Dunbar Standard	18·2	11·0	22·6	25·0
Glen Ilam	16·6	10·2	22·6	22·9
Hardy Ilam	12·0	7·4	20·8	22·2
Inverness Favourite	20·3	10·8	24·4	20·4
Iron Duke	14·5	10·6	21·4	25·1
Jersey Bennes	15·8	9·0	23·7	23·0
King Edward VII	14·7	8·8	23·4	23·0
Majestic	12·3	9·7	24·2	25·3
Sebago	13·6	10·0	20·8	21·3
Up-to-Date	18·5	12·9	24·2	25·2
R.G.R. 64/7	11·7	11·1	19·5	19.6
Average	16·0	10·0	22·49	23·07

Discussion

The limits of ascorbic acid content at harvest were 40·6 mg. per 100 g. for Inverness Favourite grown at Pukekohe, and 11·1 mg. per 100 g. for Arran Chief grown at Lincoln in the same year (1949–50), a range of 29 mg. per 100 g. After 5½ months' storage this range had decreased to approximately 6 mg. per 100 g. with the lowest and highest figures 7 and 13 mg. per 100 g. respectively. For tubers grown at Lincoln in successive years, the ranking of varieties with respect to ascorbic acid content at harvest was very similar, ranking coefficient, $r = 0{\cdot}76^{**}$, although the 1950 results were significantly higher than those for 1949; $F = 11{\cdot}7$ ($F = 8{\cdot}40$ for $P = 0{\cdot}01$). (The symbol ** here and subsequently denotes significance greater than $P = 0{\cdot}01$.)

General analysis of experimental results

Differences between varieties were shown by analysis of variance to be highly significant in both years. For 1949 the variance ratio was $F = 6{\cdot}72^{**}$, and for 1950 including all four localities and the 17 varieties common to all trials $F = 8{\cdot}13^{**}$. These findings confirm those of Murphy *et al.*,[8] who have reviewed the widely divergent views expressed by earlier workers. However, it should be pointed out that in the experiments reported here the replication of ascorbic acid analyses was applied only to varieties grown at Lincoln, and all results on these varieties are the means of two replications except with the first pre-harvest sampling of 1950. In this instance all replications were sampled as indicated previously, and tested separately. Variance analysis gave $F = 21{\cdot}47$ for variety differences, the corresponding F value for $P = 0{\cdot}01$ being 2·11. Significant differences between the means for two varieties were 2·6 mg. per 100 g. at $P = 0{\cdot}05$ and 3·5 mg. per 100 g. at $P = 0{\cdot}01$.

Analysis of the two storage experiments for varietal differences in ascorbic acid loss gave highly significant variance ratios (Table IV). On the basis of these values differential behaviour can be considered as established, but consideration must be given to the fact that no analyses for dehydroascorbic acid were performed. An increase in the proportion of the oxidized to the reduced form during storage could nullify the strong interaction already established for the

Table III

Initial and final ascorbic acid and dry-matter contents of tubers grown in four localities and stored at Lincoln in 1950

Variety	Ascorbic acid, mg./100 g., wet basis							
	Lincoln		Pukekohe		Palmerston N.		Gore	
	Initial 16/6/50	Final 8/11/50	Initial 22/5/50	Final 8/11/50	Initial 16/5/50	Final 9/11/50	Initial 16/6/50	Final 9/11/50
Arran Banner	18·7	10·5	27·8	9·1	17·0	10·7	20·6	—
,, Chief	11·1	10·6	17·4	8·9	12·7	10·6	13·3	10·8
,, Consul	13·5	10·6	17·3	8·9	14·6	12·2	15·2	10·3
,, Pilot	16·1	8·4	25·7	8·8	16·4	11·4	15·8	11·3
Auckland Short Top	19·3	10·8	27·2	9·5	22·0	10·3	21·6	10·7
,, Tall Top	22·9	11·3	—	—	21·9	12·9	24·6	10·9
Cliff's Kidney	15·8	11·6	25·5	8·5	19·9	10·2	21·9	9·1
Dakota Red	18·6	11·2	18·4	9·8	17·5	9·8	18·9	10·9
Dunbar Standard	18·5	11·6	—	—	19·8	8·5	25·5	9·4
Epicure	16·7	11·8	22·0	8·7	18·2	9·2	19·3	9·4
Glen Ilam	18·6	11·1	25·0	10·0	20·3	13·7	20·2	10·5
Hardy Ilam	15·4	9·7	19·3	7·3	13·9	—	17·0	7·6
Inverness Favourite	22·5	10·8	40·6	10·1	25·8	10·6	28·6	10·8
Iron Duke	14·4	11·5	19·0	10·1	20·2	12·2	15·0	9·3
Jersey Bennes	16·1	11·2	29·7	10·9	19·0	9·9	20·6	9·3
Katahdin	18·0	10·9	25·3	10·8	17·1	10·9	22·2	10·6
King Edward VII	18·5	9·4	37·3	9·5	26·6	13·7	25·2	9·2
Majestic	16·5	10·5	—	—	16·8	—	16·2	7·6
Sebago	15·2	11·1	23·6	8·2	18·7	11·1	16·0	9·0
Up-to-Date	17·4	12·2	27·7	10·6	18·0	11·5	18·8	13·2
Average	17·2	10·8	25·2	9·4	18·8	10·8	19·8	9·9

Variety	Dry matter, %							
	Lincoln		Pukekohe		Palmerston N.		Gore	
	Initial 16/6/50	Final 8/11/50	Initial 22/5/50	Final 8/11/50	Initial 16/5/50	Final 9/11/50	Initial 16/6/50	Final 9/11/50
Arran Banner	19·5	17·6	21·5	21·9	22·6	18·9	24·4	—
,, Chief	20·7	18·3	24·3	19·8	24·6	24·3	25·4	28·0
,, Consul	21·7	19·2	22·8	20·7	26·4	22·9	26·7	25·4
,, Pilot	19·4	17·8	22·2	19·8	22·2	21·8	22·0	22·6
Auckland Short Top	20·6	18·6	21·4	24·9	22·9	19·3	25·2	26·3
,, Tall Top	21·9	21·4	—	—	20·9	20·1	27·9	25·6
Cliff's Kidney	22·1	15·9	20·0	20·4	24·6	22·5	26·4	20·9
Dakota Red	22·2	19·4	19·5	20·7	25·8	27·0	28·8	24·4
Dunbar Standard	20·9	20·7	—	—	24·9	22·8	24·8	26·9
Epicure	22·6	18·6	20·9	20·0	22·7	26·1	22·9	21·9
Glen Ilam	20·1	17·5	22·2	18·0	22·5	18·9	23·5	22·5
Hardy Ilam	21·9	16·3	20·5	19·1	21·0	—	24·4	22·2
Inverness Favourite	21·9	19·9	23·3	17·9	24·9	26·4	27·3	28·1
Iron Duke	21·4	22·6	24·8	25·3	24·1	19·5	28·0	—
Jersey Bennes	19·9	19·1	20·1	20·8	21·0	26·7	26·3	28·9
Katahdin	20·8	19·6	20·1	19·4	23·5	18·8	23·7	21·3
King Edward VII	19·8	19·7	22·4	21·3	22·3	23·1	25·3	25·6
Majestic	22·5	20·6	—	—	24·0	—	24·5	24·1
Sebago	21·5	20·1	23·6	18·3	25·4	21·0	25·3	26·1
Up-to-Date	20·0	19·3	21·6	24·0	24·7	20·8	26·3	27·0
Average	21·0	19·1	21·9	20·7	23·6	22·3	25·5	24·9

reduced form, especially if varieties low in ascorbic acid are proportionally high in dehydroascorbic acid. Lampitt *et al.*[5] have shown that in freshly harvested tubers dehydroascorbic acid contributes approximately 10% of the total vitamin-C content. Emillson[16] found even less, but noted that the proportion tends to increase up to the breaking of dormancy and may reach 20–30% of the antiscorbutic activity, thereafter remaining constant. The two varieties he investigated showed no difference in behaviour in this respect. Lampitt *et al.*[7] on the other hand obtained much smaller differences during storage over a period of six months, and no differences are apparent in the behaviour of the nine varieties which they analysed. Murphy *et al.*[8] do not discuss dehydroascorbic acid at all, whereas Barker & Mapson,[17] who considered a wide range of storage conditions over an extended period, state that for the variety King Edward VII dehydroascorbic acid rarely exceeded 10–15%. Leichsenring *et al.*[12] have shown,

Table IV

Statistical analysis of results from 1949 and 1950 experiments

		Degrees of freedom	Mean Square	F calc.	F for significance P = 0·01
1949	Ascorbic acid				
	Varieties (V)	19		6·72*	2·13
	Sampling dates (S D)	4		86*	3·58
	(Dates 19/10 & 10/11)	1		3·87*	3·97 (P = 0·05)
	V × S D	76		4·55	1·70
	Error	80	53·625		
1950	Ascorbic acid (Lincoln)				
	Varieties	19		10·10*	2·05
	Sampling dates	6		19·44*	2·97
	(Dates 27/9 & 8/11)	1		18·89*	8·18
	V × S D	114		2·14	1·52
	(V × dates 27/9 & 8/11)	19		0·30	—
	Error	139	2·121		
	Dry matter (Lincoln)				
	Varieties	19		4·91	2·02
	Sampling dates	6		15·1	2·94
	(Dates 27/9 & 8/11)	1		13·1	6·83
	V × S D	114		1·29	1·34 (P = 0·05)
	Error	139	1·413		
	Ascorbic acid (all localities)				
	Varieties	16		8·13†	2·40
	Localities (L)	3		17·49†	4·22
	Sampling dates	1		> 1000	7·23
	V × L	48		1·38	1·63 (P = 0·05)
	V × S D	16		8·66	2·43
	L × S D	3		49·2	4·25
	Error (V × L × S D)	45‡	3·164		
	Ascorbic acid (excluding Pukekohe)				
	Varieties	19		6·00†	2·40
	Localities	2		2·83†	3·25 (P = 0·05)
	Sampling dates	1		1106	7·44
	V × L	38		1·80	1·76 (P = 0·05)
	V × S D	19		7·80	2·47
	L × S D	2		17·72	5·29
	Error (V × L × S D)	34§	1·780		
	Dry matter (all localities)				
	Varieties	16		3·09	2·43
	Localities	3		55·3	4·25
	(Gore & the rest)	1		114	7·23
	Sampling dates	1		18·9	7·23
	V × L	48		1·06	—
	V × S D	16		1·13	—
	L × S D	3		1·11	—
	Error (V × L × S D)	45‡	3·10		

* Tested against V × S D interaction
† Tested against V × L interaction
‡ Three missing plots
§ Four missing plots

however, that varietal differences do exist with respect to dehydroascorbic acid content and further, that with the three varieties they tested a strong variety : storage interaction was apparent. Notwithstanding these facts the interaction for the reduced form was still highly significant and the inclusion of the oxidized form in the analyses seems merely to have increased the significance of the interaction for total ascorbic acid.

The likelihood of dehydroascorbic acid materially affecting the variety : storage interaction in the present work would thus appear to be remote, although it is desirable that further work should attempt to confirm this point.

Comparisons between localities may be made in Table III. The most outstanding feature of the results is the very much higher ascorbic acid content of tubers grown at Pukekohe, the northernmost locality, and the closely similar results for the other three localities. Variance analysis of the results from localities excluding Pukekohe gives a non-significant variance ratio for localities, whereas the inclusion of Pukekohe in the analysis gives a highly significant

variance ratio. Excluding Pukekohe there is a significant variety : locality interaction, while the inclusion of the Pukekohe results renders the interaction non-significant. The Pukekohe figures must be regarded with suspicion, however, as drought conditions prevailed during the maturing period and the yields of all varieties in the trial were consequently very much depressed. There is the possibility that physiological maturity had not been reached when the trial was harvested, and the high results may be a consequence of the known high ascorbic acid content of immature tubers. Comparisons between localities are further complicated by the fact that analyses were performed as the material came to hand from the other centres, and there was no control over the method of storage during transport. Harvest dates were widely different, varying from 21 April in Palmerston North to 1 June in Lincoln. Further, transport difficulties prevented the trial at Pukekohe being analysed till six weeks after harvest; three weeks elapsed with Palmerston North potatoes and five weeks with tubers grown at Gore. The Pukekohe soil was a volcanic loam in contrast with the silt loam and alluvial silts of the other three localities. However, Idjo,[18] Murphy *et al.*,[8] and Baker *et al.*[10] conclude that other environmental conditions greatly overshadow the effect of soil type. Seasonal differences between the trials grown at Lincoln in successive years were statistically significant; on comparing the harvest figures it is found that $F = 11{\cdot}7$ ($F = 8{\cdot}4$ for $P = 0{\cdot}01$). Information extending over many more seasons and detailed meteorological observations are necessary before any attempt can be made to analyse environmental effects. Such a study of the interaction of heredity and environment must await the elaboration and application of field techniques described by Hamner,[19] or the use of controlled environments.

In contrast with the higher ascorbic acid content exhibited by varieties grown at Pukekohe, those grown at the southernmost station, Gore, had significantly higher dry-matter contents. Reduction of the results in Table III referring to dry matter showed that, with respect to the 17 varieties common to all four trials, variety and locality differences and the effect of storage were highly significant. However, none of the interactions proved to be statistically significant, all having variance ratios approximating to unity. It is beyond the scope of this paper to consider dry-matter content otherwise than in relation to ascorbic acid content. A perusal of the results contained in Table III, however, failed to reveal any such relationship.

Detailed examination of the response of varieties to storage

The ascorbic acid decay curves for each of the 20 varieties grown and stored at Lincoln in 1949–50 are shown in Fig. 1. The varietal results have been divided arbitrarily into three

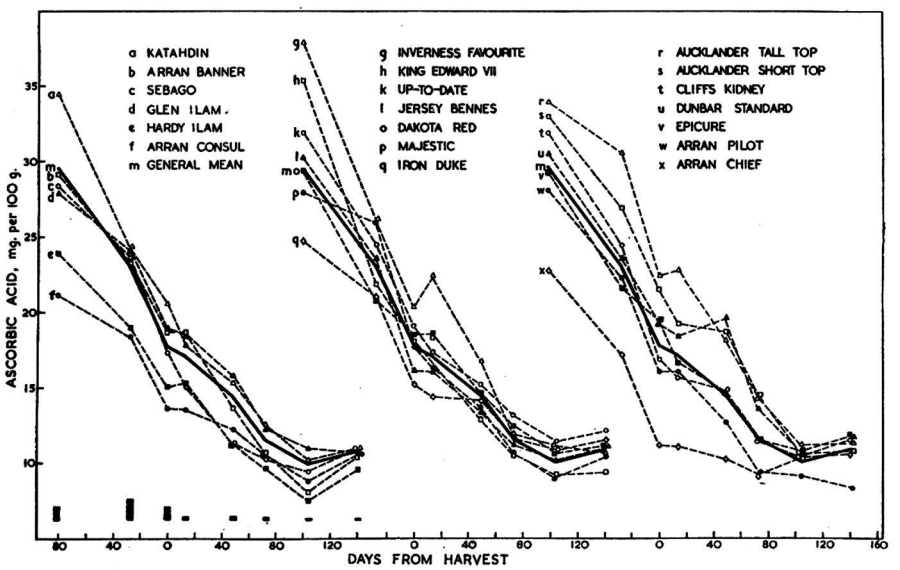

FIG. 1.—*Changes in ascorbic acid content of 20 varieties of potato grown and stored at Lincoln in* 1949/50

sections for clarity of presentation, and the general variety mean is indicated in each by an unbroken line. The harvest date was 16 June, 1950, and other testing dates are shown as the number of days before or after this date. The standard error of the general variety mean is given for each sampling date as a block below each series of determinations in the left-hand portion of the figure, a block being used instead of the usual line because of the smallness of some of the values.

A detailed examination of these curves reveals consistent behaviour by most varieties in several respects, with some interesting exceptions. The rate of ascorbic acid loss before the harvest appears to increase in most cases with the death of the haulm, which had begun to show senescence by the second pre-harvest sampling. This increased rate of loss is especially obvious in the variety Majestic, but King Edward VII, Dakota and Epicure are exceptions to the rule in showing a decreased rate of loss. During the period between harvest and placing the tubers in storage, ascorbic acid appears to remain relatively constant in 11 of the 20 varieties. Noteworthy exceptions are Glen Ilam, Katahdin, Sebago, Inverness Favourite, Majestic, Up-to-Date, Dakota, Dunbar Standard, Epicure and Aucklander Short Top. Of these, Katahdin, Sebago, Majestic, Up-to-Date and Epicure have a decay curve of practically constant slope until approximately half way through the storage period, after which the rate of loss slackens considerably in common with the behaviour of most varieties. The rise in ascorbic acid shown by Inverness Favourite between harvesting and storage should be considered as an extreme of the relatively normal behaviour of the other 11 varieties. In four cases, namely Iron Duke, Cliff's Kidney, Dunbar Standard and Aucklander Short Top, the period of relatively constant ascorbic acid content occurred after the tubers had been placed in storage.

The explanation of these changes in rate of ascorbic acid loss is difficult without parallel data on temperature changes, as it seems likely that they may be accounted for on this latter basis. Barker & Mapson[17] have shown that the transfer of tubers previously stored at a constant temperature to storage at temperatures above and below this conditioning temperature was followed by an increase in ascorbic acid which persisted longer the greater the temperature change. That such temperature changes occur when the tubers are harvested and stored temporarily in bags in an open shed, as in the present work, is highly probable. Further, these changes are likely to be rapid, following diurnal variation and changing weather conditions. However, the exceptional behaviour of some varieties has still to be explained. Without further experiments, the only suggestion that can be made is that a certain physiological state is necessary within the tuber before response to temperature changes occurs. Evidence of physiological differences between varieties with respect to dormancy is given by Emillson,[16] and in this laboratory differences in amino-acid metabolism have been found.[20]

An examination of the late storage results reveals a remarkably consistent rise in ascorbic acid content after approximately five months' storage. Two exceptions are Arran Pilot which, although showing a levelling-off in ascorbic acid loss, continued with a downward trend, and Arran Chief, which began to increase in ascorbic acid content approximately one month earlier. This phenomenon has not been reported by other workers for normal storage conditions such as have been used in the present work. Wolf[21] is quoted by Emillson[16] as having observed an increase in ascorbic acid content after storage for some months at refrigeration temperatures. There is the probability that the phenomenon he observed is similar to that noted by Barker & Mapson[17] and Leichsenring *et al.*,[12] who found that there was an increase in ascorbic acid content when tubers stored at a low temperature were transferred to a higher temperature, the effect being greatest when the difference between initial and final storage temperatures was greatest. In the experiments reported here, all varieties had commenced sprouting by 1 October, and at the final sampling all tubers carried sprouts two to five inches long. Variance analyses showed that the increase in ascorbic acid was statistically significant. Although a similar trend was noted in the 1949 storage trial, the odds against such a result being due to chance were lower and the differences not quite significant at the 5% probability level. In the 1949 trial the time between the last two sampling dates was three weeks less than in 1950, and it is possible that with a later sampling the increase may have been significant. In 1950 a simultaneous highly significant decrease in dry matter was noted, indicating a considerable increase in the metabolic activity within the tuber. The increase in ascorbic acid would thus seem to be closely associated with metabolism.

Recordings at the centre of the clamp showed that the temperature remained fairly constant at 5° from the end of June to mid-September, when there was a sudden rise to the range 7–10° for about two weeks, after which there was a fall to 5–7° until mid-October, when a fault in the thermograph caused recording to be discontinued. Warmer weather conditions prevailed

from then on so that the temperature inside the clamp must also have risen before the final sample was tested on 8 November. Further, it is likely that the temperature inside the clamp rose more quickly than the outside temperature at the end of the storage period because of the onset of vigorous sprouting. Barker & Mapson[17] showed that the ascorbic acid increase which they obtained, on transferring potatoes that had been stored for some months from a lower to a higher storage temperature, did not occur until seven days after the transfer. Thus it is possible that thermal change within the potato clamp during the month of October is sufficient to explain the observed rise in ascorbic acid content and, further, that the occurrence of this increase in 18 of the 20 varieties is merely a reflection of a common physiological condition.

It should be pointed out that the sampling method used in the present study, while similar to that used by Murphy *et al.*[8] and Leichsenring *et al.*,[12] differed in one important respect from that used by these and other workers. The sector cut from the median portion of the tuber undoubtedly sampled the medullary and cortical regions in a more representative manner than did the cylindrical longitudinal borings used by Barker[13] and Barker & Mapson.[17] Further, Lampitt *et al.*[5] and other workers have shown that the concentration of ascorbic acid increases at the bud end of the tuber during early storage and incipient sprouting, and Julén demonstrated a concentration gradient with a minimum in the central pith and a maximum in the inner cortex. Thus the increase noted in the present study may have been due to the translocation of ascorbic acid from the medulla to the cortical layers with the onset of vigorous sprouting. As has been previously pointed out, no analyses for dehydroascorbic acid were performed in the present work, and it is consequently possible that the oxidation of some or all of the reduced form may have been overlooked. Julén[6] indicates that the analysis of sectors cut in the manner used in this investigation is more likely to give correct results; but the choice between these two hypotheses, namely translocation and oxidation, or a combination of them, and that based upon thermal change for an explanation of the final increase in ascorbic acid, cannot be decided from the present work. There is the likelihood that all three factors contribute.

Appendix

The production and utilization of ascorbic acid from potatoes in New Zealand

An estimate of the production of ascorbic acid from potatoes in New Zealand in the year 1949–50 is given in Table V. The figures for yield and production of varieties are extended from information covering approximately half the commercial area supplied by the New Zealand Government Statistician, to whom thanks are due. The ascorbic acid value used for calculation for each variety is an approximation based upon the tests of material grown in the four

Table V

The estimated production of ascorbic acid from potatoes in New Zealand

Varieties	Area, acres	Total yield, tons	Yield per acre, tons	Ascorbic acid, mg. per 100 g.	Ascorbic acid (lb. per acre), at harvest	Total yield of ascorbic acid, lb.
Aucklander Short Top ..	6,672	44,447	6·66	19·3	2·88	19,200
Dakota Red	2,933	21,380	7·29	18·6	3·04	8,900
Arran Chief	2,533	27,204	10·74	13·2	3·18	8,000
,, Banner	1,730	10,376	6·00	18·7	2·49	4,300
Aucklander Tall Top ..	524	4,830	9·22	22·9	4·73	2,500
Inverness Favourite ..	426	4,362	10·24	24·5	5·62	2,400
King Edward	422	3,058	7·25	25·2	4·09	1,700
Sebago	294	3,010	10·24	16·9	3·88	1,100
Epicure	230	1,206	5·24	19·0	2·23	500
Iron Duke	220	2,656	12·07	17·3	4·68	1,000
Other varieties	1,801	12,898	7·16	16·0	2·57	4,600
Total farm production ..	17,785	135,427	7·61	—		54,200
Home gardens	5,000	25,000	5	20		11,200
Gross total	22,785	160,427	—	—		65,400
Ascorbic acid from oranges						
(*a*) Australian			2·71	40	2·43	
				60	3·64	
(*b*) U.S.A.			7·35	40	6·58	
				60	9·88	

localities, and takes into account the area in which each variety is grown. The production from home gardens is an estimate only, since the information on the quantity of seed potatoes sold to home gardeners is incomplete. For comparison the yield per acre of ascorbic acid from oranges is given for two countries, Australia and the U.S.A., calculated from data in the FAO Yearbook of Food and Agriculture Statistics, 1948,[22] with values of ascorbic acid of 40 and 60 mg. per 100 g. fresh weight.

The production of potatoes in 1949–50 was below the full requirements of New Zealand, but even so, at 75 mg. daily requirement 65,400 lb. of ascorbic acid will supply the full requirements for one year of 1,080,000 people. Allowance must be made for the fact that a portion of the total crop is used for seed each year, and for some losses in storage, though the loss during storage is counterbalanced to some extent by the fact that during many months of the year potatoes are eaten immature when ascorbic acid content is higher than in potatoes used for calculation. Although losses occur also in cooking, it is nevertheless obvious that the potato supplies a very large portion of the ascorbic acid requirements of New Zealand (population two million approximately).

Notes on varieties

The utilization of the main commercial varieties in New Zealand is illustrated in Table VI.

Table VI

The utilization of the main commercial varieties in New Zealand

Variety	Proportion of production, %	Where grown	Maturity	Period eaten	Stage eaten	Remarks
Aucklander Short Top	33	(*a*) Hawkes Bay, Wellington, Nelson	2nd Early	Dec.–Mar.	Immature	Eaten while ascorbic acid content is high
		(*b*) Canterbury	Early Main Crop	Feb.–July	Immature to mature	
Arran Chief ..	20	(*a*) Auckland	Early	Sept.–Dec.	Immature	Differs little when used
		(*b*) Canterbury, Otago, Southland	Late	June–Nov.	Mainly after storage	
Dakota Red ..	16	Canterbury	Late	July–Nov.	Mature and after storage	A good keeper, utilized late winter when differs little from others
Arran Banner ..	7½	Throughout	2nd Early	Nov.–Mar.	Immature	Used when ascorbic acid content is high
Aucklander Tall Top	3½	N. Canterbury	Late	May–Oct.	Mature	Above average in ascorbic acid
Inverness Favourite	3	Cant., Otago, Southland	Main Crop ,, ,,	May–Oct.	,,	Above average in ascorbic acid
Sebago	2	(*a*) Wellington	,, ,,	Apr.–Aug.	,,	Compares unfavourably with others
		(*b*) N. Canterbury	,, ,,	May–Sept.	,,	
King Edward VII	2	Otago, S'land	,, ,,	Mar.–Dec.	,,	Above average in ascorbic acid
Iron Duke (President)	2	Wellington N. Canterbury	,, ,, ,, ,,	May–Sept.	,,	Ascorbic acid average, but yield per acre high
Epicure	1	Throughout	Early	Aug.–Mar.	Immature	Used when ascorbic acid content is high

It would appear from the Table that, with the possible exception of Sebago, we are utilizing varieties that are above average in ascorbic acid content during summer, autumn and early winter, and reserving for late winter and spring those varieties which, though somewhat lower initially, are little different at the end of the storage period. It will be noted that even at the end of the storage period, values obtained for ascorbic acid in New Zealand have been consistently much higher than values reported from overseas, so that although consumption of potatoes per head of population may not be as high as elsewhere, it would still appear that a high proportion of the ascorbic acid requirement is met from this source.

Acknowledgments

The authors wish to express grateful acknowledgments to Mr. G. M. Wright of the Applied Mathematical Laboratory, Department of Scientific & Industrial Research, Lincoln, New

Zealand, for statistical analysis of the results, and to Miss D. T. P. Hopkins, Mr. T. J. Walter and Mr. J. P. B. Chapple for technical assistance.

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Received 10 February, 1953

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THE BACTERIOSTATIC EFFECTS OF EXTRACTS OF CHICKEN DUODENUM ON CERTAIN MICRO-ORGANISMS

II.*—Preparation and Properties of Concentrates

By J. BARRETT and N. J. BERRIDGE

The chromatographic fractionation of concentrates prepared from chicken duodenum by solvent treatment led to preparations having the properties of unsaturated fatty acids. The most active fractions had a bacteriostatic power roughly equal to that of linoleic acid, but their chemical properties suggested acids of greater molecular weight.

The bacteriostatic properties of extracts of the duodenal contents of chickens, together with some general properties of the active material in the contents, have already been described.[1, 2] Most of the properties so far observed are those of a fatty acid. Thus Laser[3, 4] has recently studied the inhibition of the respiration of *Bacillus subtilis* by various fatty acids and he has shown the increase of the effect with increasing unsaturation. The subject has

* Part I: *J. Sci. Fd Agric.*, 1953, **4**, 81

been reviewed by Pollock,[5] and, from a somewhat different angle, by Kodicek.[6] One of the most striking phenomena is the suppression by some colloids of the inhibition due to fatty acids. This was also found to occur with the inhibitory substance from the chickens, and the results of various other experiments on partially purified extracts suggested moreover that the responsible fatty acids were unsaturated. With this exegesis the purification and examination of the material were attempted.

Methods

Biological assay and units

The technique of assay was identical with that already described[2] but, for reasons mentioned in the introduction, linoleic acid was chosen as a standard instead of penicillin. In this paper therefore one unit is the amount of material that gives the same inhibition under the conditions of the test as 1 μg. of linoleic acid. The growth rate of the test organism (*Streptococcus agalactiae*) was decreased by approximately 50% in a medium containing 3 μg./ml. of linoleic acid.

Chemical determinations and tests

These were made by the usual methods (see e.g. Hilditch[7]).

Experimental

Paper chromatography

The fatty nature of the bacteriostatic substance was first noticed during paper chromatography. The location of the inhibitory substance after chromatography was determined in the usual way by placing the dried strips of paper on agar seeded with *Strep. agalactiae* (see e.g. Winsten & Eigen[8]). Diffusion was allowed to occur during 12 hours at 5° and the paper was left in position during incubation. Development with distilled water, phosphate buffer or salt solutions gave elongated streaks. Light petroleum, butanol–acetic acid, propanol–water and ethyl methyl ketone–water each gave a spot at the solvent front. Ethanol (60–80%) and acetone (80%) gave results with R_F values ranging from 0·7 to 0·9. After running chromatograms in an atmosphere of ethylamine, as used by Hiscox & Berridge[9] for identifying volatile fatty acids, the bacteriostatic properties could no longer be detected, although the acid could still be observed on spraying with indicator. It is probable that oxidation had occurred (see e.g. Bergström, Blomstrand & Laurell[10]).

Preparations

Although it was suspected that the material sought was a fatty acid, solvent extraction under alkaline conditions could not be used as a process of purification because it led to a loss of bacteriostatic activity. This, however, did not apply to later preparations (since May, 1952).[2] The following is a typical example from a number of preparations that differed in minor details. Duodena from 60 chicks were minced coarsely, covered with distilled water and stirred for about 20 minutes. Removal of the tissue by straining through gauze yielded 400 ml. of suspension. To this was added 1600 ml. of alcohol, and the mixture was boiled under reflux for half an hour, cooled at −16° overnight and filtered. The highly active filtrate was concentrated by boiling off the alcohol *in vacuo* and the active material was separated from the resulting slurry by extraction under nitrogen with light petroleum (A.R., b.p. < 40°). Three extractions were made with a total volume of 500 ml., and the extracts were concentrated to an oil by distillation at atmospheric pressure. The addition of 100 ml. of acetone to this oil produced a copious precipitate. The mixture was boiled for 5 minutes to ensure complete extraction of acetone-soluble material from the precipitate, which was then filtered off at 2°; the filtrate, together with washings from the precipitate, was concentrated by evaporation *in vacuo* until no more acetone would distil. There remained 1·6 g. of brown oil having a bacteriostatic activity equal to that of pure linoleic acid and an iodine value (Wijs) of 163. (The theoretical iodine value of linoleic acid is 182.) Further fractionation of this oil was attempted by chromatography on silica (see below).

In another preparation the final acetone solution was cooled at − 16° and filtered from the precipitate at this temperature. In this case also the oily concentrated filtrate had the same bacteriostatic activity as linoleic acid; its iodine value was 178 and its equivalent weight (by titration) was 292. (The equivalent weight of linoleic acid is 280.) The precipitate filtered from the acetone solution at − 16° remained solid at room temperature. It had no bacteriostatic activity. Its iodine value was 85.

Chromatography on silica

Silica gel was the last of several materials tried for chromatography. Alumina (Brockman) retained the material too tenaciously, even after various de-activating procedures, including the addition of 15% of water. Charcoal was also too strongly adsorbent, and talc and kieselguhr were too weak. In spite of the results with paper strips, partition chromatography with columns of paper pulp or of cellulose powder was not successful.

Preliminary experiments showed that oxidation occurred readily during chromatography. Therefore not only was oxygen excluded during the experiment, but an attempt was made to remove oxygen from the silica as well as to purify it in other ways. The ' silica gel for chromatography ' (L. Light & Co. Ltd.) was washed by stirring three times with alcohol and dried. It was then thoroughly washed with benzene, dried at 150° and allowed to cool *in vacuo* over phosphorus pentoxide. Nitrogen was used to release the vacuum. This silica was poured as a suspension in light petroleum (through which nitrogen had been bubbled) into a column 2 cm. × 20 cm., suction being used to remove entrapped bubbles.

The oil, prepared as already described, was applied as a solution of 1·6 g. in 10 ml. of light petroleum and washed in with a few ml. of light petroleum. Elution was then effected as described in Table I. Nitrogen was bubbled slowly through the solvent layer at the top

Table I

Chromatography of fatty substances from the duodenal contents of chickens

Fraction No.	Eluent	Volume, ml.	Colour	Wt. after evaporation of solvent, mg.
1	Light petroleum, b.p. < 40°	40	Colourless	16
2	Light petroleum : benzene, 2 : 1	70	,,	—
3	,, ,, ,, 1 : 2	60	,,	—
4	Benzene	100	,,	209
5	,,	125	,,	323
6	Benzene : ethyl ether, 1 : 1	46	Trace of green	128
7	,, ,, ,, ,,	10	Green band	573
8	,, ,, ,, ,,	45	Trace of green	39
9	,, ,, ,, ,,	40	Deep yellow	42
10	,, ,, ,, ,,	30	,, ,,	10
11	,, ,, ,, ,,	50	,, ,,	34
12	,, ,, ,, ,,	80	Pale yellow	24
13	Ethyl ether	90	,, ,,	8
14	,, ,,	90	Pale green	2
15	,, ,,	100	Pale yellowish-green	58
16	Ethanol	160		51
			Total weight recovered	1517

Weight of product added to column, g.	1·65
Weight of fractions recovered, g.	1·52
Iodine value of product added to column	163
Mean iodine value of fractions recovered $\left[\frac{\Sigma\,(\text{Iodine value} \times \text{wt.})}{\Sigma\,(\text{wt.})}\right]$	158
Bacteriostatic activity added to column	1,650,000 units
Bacteriostatic activity recovered	830,000 ,,

of the column and the solvent outflow was regulated to between 12 and 25 ml./h. Fractions were taken, sometimes arbitrarily, sometimes according to the appearance of the column, any bands observed (e.g. the green band, fraction No. 7) being separated. From the figures in Table I it is clear that virtually all the material applied to the column was recovered and that, according to the mean iodine value of the recovered material, oxidation had been prevented. There was, however, a 50% loss in bacteriostatic activity. Pure linoleic acid run under similar conditions was not extracted unless the eluent contained benzene; complete recovery was made after eluting with 100% benzene (corresponding to fraction 5 of Table I). In a control experiment with a mixture of unsaturated fatty acids (linoleic acid, technical) loss in bacteriostatic power did not occur. Further, full recovery of activity was obtained when another extract from chickens was run on a silica column and eluted with benzene only. In this case the equivalent weights of the fractions were determined by titration. They varied from 294 to 396 and were thus considerably in excess of that of linoleic acid.

The iodine values and the specific activities of fractions 4–13 from the experiment in Table I are plotted in Fig. 1. The bacteriostatic activities for fractions 13 and 14 must be accepted with reserve since so little material remained after evaporating so much solvent. It is clear from the other values, however, that the bacteriostatic activity in these extracts is not solely a function of the degree of unsaturation. This is confirmed by the results in Table II, obtained after the further fractionation of eluate No. 7. After dissolving in acetone, this material yielded crystals at room temperature, fraction (*a*), and a further crop on cooling the filtrate to — 16°, fraction (*b*). Fraction (*c*) was the solution remaining at — 16° and (*d*) represents the washings of the crystals that separated at room temperature. The substance (*a*) had the same melting point as cholesterol and also gave a green colour in the

Table II

Fractionation of eluate No. 7 (see Table I)

Fraction (see text)	Weight, mg.	Specific activity units/mg.	Iodine value (Wijs), g. I_2/100 g. of product
(*a*)	119	0·013	175
(*b*)	146	0·23	238
(*c*)	164	0·54	213
(*d*)	144	0·53	236

Liebermann reaction. Eluate No. 5 (of Table I) was subjected to countercurrent distribution in 10 tubes according to the technique of Bush & Densen ;[11] as solvents a mixture of 15 ml. of 0·1N-acetate buffer at pH 4·0, 68 ml. of acetone, 68 ml. of ethanol and 150 ml. of light petroleum (b.p. 80–120°) were used. No evidence of impurity was obtained, but further experiments were not made because a large number of transfers would have been needed to analyse mixtures of closely related fatty acids.

Other chemical tests

Fraction No. 5 from the chromatographic separation (Table I) gave a bromination product having a melting point of 112°, suggesting a dienoic acid (cf. Hilditch[7]).

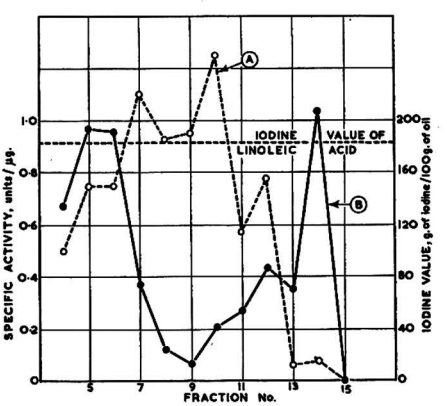

FIG. 1.—*The iodine values (A) and specific bacteriostatic activity (B) of fractions eluted from a silica column*

The oil soluble in acetone at — 16° was used without further purification and the products of bromination were found to include a precipitate that was insoluble in ether and melted with decomposition at 230°. It seems probable therefore that polyethylenic acids were also present. This was confirmed by oxidation with permanganate in acetone according to the technique of Armstrong & Hilditch,[12] except that the free acid, not its methyl ester, was used. Although the volatile acids were almost identical in kind and quantity with those produced from linoleic acid, as shown by paper chromatography, the non-volatile fraction

contained a mixture in which azelaic acid, m.p. 106°, could not be demonstrated, but an acid with a melting point of 73–75° was obtained by crystallization from water.

Discussion

The method of preparation that has been outlined was developed step by step to secure maximum bacteriostatic activity. That this method would also concentrate the unsaturated fatty acids is in itself evidence for the view that the bacteriostasis observed was in fact due to unsaturated fatty acids. The behaviour on chromatography and the few chemical tests confirm this view. The equivalent weights, iodine values and bromine derivatives indicate that in some of the fractions dienoic acids were probably present. The lower iodine values and higher equivalent weights of the active fractions, if pure, are such as would be given by dienoic acids containing 22–24 carbon atoms. Fig. 1 shows that other bacteriostatic substances were also present and the inactivation of some extracts by solvents under alkaline conditions distinguished them sharply from solutions of pure linoleic acid.

These extracts prepared from duodenal contents were, however, not only complex but variable. In such a mixture there are many possibilities of changes. Kodicek,[6] for example, has shown that the bacteriostatic properties of fatty acids are very easily modified by the presence of other substances and a variety of changes may thus take place during purification. When to these are added the various chemical changes such as oxidation and isomerization, which may occur, it is the overall consistency of the values for bacteriostatic power, rather than the occasional variations, which appears surprising, and it is clear that speculation about the losses of activity cannot be fruitful without further evidence.

When the antibacterial properties of the contents of chicken duodenum were first observed it seemed important to discover the nature of the responsible substances, particularly in view of the work now in progress on the effect of feeding antibiotics to animals, an effect that is thought to be the result of the action of the antibiotic on the gut flora. The present work has demonstrated the existence in the gut of bacteriostatic substances originating from the animal itself, and the experiments have indicated that the substances are probably chiefly unsaturated fatty acids. The picture is therefore complicated, because the bacteriostatic effect of fatty acids is not constant, changing even to a stimulatory effect under certain conditions. In the gut itself, or in simple aqueous extracts, sensitive bacteria may be either stimulated or inhibited. These opposite effects have, in fact, been observed on different occasions.[2] This does not of course exclude other antibiotics which may, on occasion, be produced by the gut flora, but it does emphasize the complexity and variation of the environment and should be remembered when interpreting experiments on the bacteriology of the gut.

Acknowledgments

The authors wish to thank Dr. M. E. Coates and Dr. J. W. G. Porter for their assistance in obtaining chickens, and to record their gratitude to Dr. M. L. Meara for kindly providing samples of pure fatty acids.

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Received 25 March, 1953

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Printed in Great Britain by Butler & Tanner Ltd., Frome and London

J. Sci. Food Agric., 4, August, 1953

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

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