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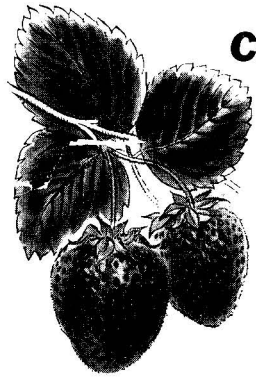

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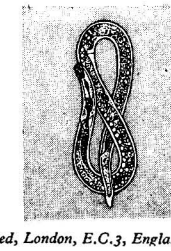

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LIPIDS AND THE PROBLEM OF ATHEROSCLEROSIS: A SURVEY*

By R. A. MORTON

Dept. of Biochemistry, The University, Liverpool

Introduction

THE average expectancy of life for males in England and America is about 60 years and for females about 64 years[1] and is rising. In ancient Rome it was probably between 20 and 30 years. In this country it was as low as 34 years in the decade 1879–89 and by 1911 it was 46·6 years. Between 1850 and 1930 the percentage of the population over 50 years of age in the U.S.A. rose from 8·9 to 17·2. In 1940 the U.S.A. had 24 million people between 50 and 74 years and by 1980 the predicted number is 42,000,000.

This tendency is to some extent world-wide and is due in part to improved sanitation, as well as to the spectacular successes of chemotherapeutic agents and antibiotics in the treatment of infectious diseases. It is also due in part to improved social and economic conditions and to increased agricultural and industrial productivity. Progress in research and its application to the general welfare has brought within sight the virtual abolition of deficiency diseases. Emphasis has shifted from the relief of overt deficiency to food fortification as an 'insurance' against possible hidden inadequacies of a border-line nature.

As more and more people survive beyond middle age so must interest increase in geriatics and gerontology, to use words which are now in vogue.

In the U.S.A.[2] in 1950–51 cardiovascular disease was responsible for more than half the total deaths (1·5 million p.a.): arteriosclerosis and hypertension accounted for 9 out of 10 deaths from cardiovascular disease.

It must be recognized that some bodily changes are aspects of physiological ageing; thus the incidence of cardiovascular disease in women increases after the menopause. But on the whole, knowledge concerning the biochemistry and physiology of senescence is meagre, and the assumption that the lesions so commonly seen in the arteries of the old are due simply and wholly to chronological age is both unwarranted and unnecessarily discouraging. Nevertheless the statement that there must be a biochemical aspect of senility cannot be brushed aside.

The subject of cardiovascular disease is today attracting a great deal of attention from investigators trained in a wide range of disciplines (medical statisticians and epidemiologists, clinicians, endocrinologists, histologists and anatomists, nutritionists, biochemists, etc.). It may be doubted whether in fact any single person can properly assess all the work in its entirety. The evidence is very complicated and some seems contradictory. The rising incidence of coronary disease is widely accepted as a fact both established and disturbing, but among biochemists and nutritionists a note of hopefulness can be sensed. There is a cautious feeling that it may prove possible in a relatively short time to reverse the trend.

As this review is written mainly for chemists, the following notes may be useful.

Elastic arteries such as the aorta and the pulmonary artery contain elastic tissue, which tends to change with advancing years. The inner layer (*tunica intima*) facing the blood is made up of a single layer of cells like a mosaic, whereas the thicker *tunica media* is made of elastic tissue or muscle tissue, depending on the type of artery. The *tunica media* is thinner in veins.

Arteriosclerosis, a term first used by Lobstein in 1833, covers several conditions which produce a thickening of the vessel wall. Since then the word has been applied to all scleroses or hardening of arteries. *Atherosclerosis* was first used by Marchard in 1904 for the amorphous lipid accumulation in the *intima* (Greek *athero* for mush), in particular the lipid-laden intimal plaques shown characteristically in the aorta. In many aged persons post-mortem examination reveals extensive calcification of aortal lesions, almost always of long standing. The words atheroma and atherogenesis apply to the lipid infiltration and its causation. In international medical statistics arteriosclerosis includes (*a*) diseases of the coronary arteries and angina pectoris, (*b*) intercranial diseases of vascular origin such as cerebral atherosclerosis and (*c*) the renal atherosclerosis of chronic nephritis. A thickening

* Read before a joint meeting of Oils and Fats Group and Nutrition Panel of the Food Group, 15 March, 1957

Table II

Changing dietary pattern in U.S.A.

(*a*)

Year	Per person/year 1910	1952	Difference, %
Sugar, syrup, lb.	87	107	+23
Potatoes, lb.	213	103	−51·6
Cereal products, lb.	292	163	−44·2
Milk, pints	366	498	+36
Eggs*	293	395	+34·8
Fats and oils, lb.	59	67	+13·6
Fruit, vegetables, lb.	368	433	+18
Meat, fish, poultry,	little overall change		
Total fat intake contributed	30% of calories 16% of weight	45% of calories 26·6% of weight	

* Contain about 5 g. fat each
(After Katz, Stamler & Pick[20])

(*b*) Food availability in g. and in cal./person/day.

Year	1935 g.	1935 cal.	1955 g.	1955 cal.
Protein	90	360	97	388
Fat	134	1206	148	1332
Carbohydrate	440	1760	384	1536
	664	3326	629	3256
Fat contribution as percentage of caloric value		36		41
Fat as percentage by wt.		20·2		23·5

(Stare,[21] based on Department of Agriculture statistics)

(*c*)

	Year	% of calories provided by fat
College men	1891	44
College women	1894	36
College athletes	1898	39
Adult women	1953	36–46

(After Stare[21])

Table III

Average fat intake in different countries as percentage of total food calories

		%
Japan and parts of Africa		8–10
Italy, Spain, Portugal	about	20
United Kingdom, France, Germany, Scandinavia	,,	35
Canada, Australia, New Zealand		37–38
U.S.A.	,,	40

A number of studies have been directed towards a more rigorous test of the relationship between diet and the incidence of atherosclerosis. One investigation was on groups of healthy adult urban males in Naples and in St. Paul, Minneapolis. The Italians were on diets providing some 20% only of the calorie value in the form of fat whereas for the Americans the proportion was 40%. From about 18 to 35 years of age the higher fat intake of the Minnesotans did not influence the serum-cholesterol levels, which were the same in both groups. After the age of 35 the values for the Neapolitans became practically steady, whereas those for the Americans continued to rise until the difference was 40–50 mg. per 100 ml. for the age group 50–60. There was no difference in relative obesity between the two groups.[24, 25]

A similar investigation in Madrid showed that working-class men on a diet low in both calories and fat had low serum-cholesterol levels in middle age. If, however, the comparison was made between Minnesotans and wealthier Spaniards on a full diet rich in fat, no differences were found in the age-distribution of serum cholesterol. Over the age of 30 it was evident that the social group in Madrid on the high-fat diet exhibited a significant elevation of serum-cholesterol

compared with the group on a low-fat diet.[26] Keys & Anderson[22] later compared groups of men in Naples with a group in Bologna where the diet ' contains more fat than in any other part of Italy '. The results supported the claim for a significant correlation between fat intake, serum concentrations of cholesterol and lipoprotein and the incidence of coronary heart disease in populations. Englishmen tend to be somewhat thinner than wealthy Minnesotans or Spaniards, but the fat intake (35·4% of calorie intake) is high and so also is the serum-cholesterol in middle age.[27]

It may be concluded that there is now an impressive case for the view that a high fat intake results in a change in the blood serum that increases the risk of secondarily induced or ' triggered ' clinical disease.

There is, however, evidence to suggest that the incidence of cardiovascular disease continues to increase after the fat intake has ceased to rise rapidly. It may be that in relatively wealthy populations a substantial part of the fat used in cooking and reckoned to be dietary is not actually eaten. The proportion of waste is not easy to assess, and it is uncertain whether ' nutritional damage ' is done to lipids in cooking or repeated use for deep frying. It is improbable that trends in the incidence of atherosclerosis can be tied down to differences in the relative proportions of fats of animal and vegetable origin consumed in the past 25 years (see Stare[17]). During that period, however, there has been in many countries a substantial increase in the consumption of hydrogenated fat. According to some speculations this could diminish the intake of essential fatty acids, linoleic and linolenic, or it could introduce some harmful artifact into the diet. Claims have been made that the essential fatty acids tend to counteract tendencies to hypercholesterolaemia in experimental animals.

Rôle of cholesterol

The epidemiological studies indicate then that in man, the elevated serum-cholesterol (which often favours cholesterol deposition in atheromata) is a sequel to high fat intake. To procure experimental atherosclerosis, however, it is necessary to feed cholesterol to animals, and even so there are great differences between species.[28] Atheromatous lesions can be produced by cholesterol feeding in the rabbit, the chicken, the hamster and the guinea pig[3] and in the thiouracil-fed dog (induced hypothyroidism) given cholesterol.[29] The experimental animals show a syndrome similar to that observed in man.[5, 30] Some species are resistant even to cholesterol feeding, but according to Gould, man is ' the only mammal known to be prone to develop atherosclerosis spontaneously. His average plasma-cholesterol concentration is uniquely high.' [31]

It is perhaps possible to exaggerate the uniqueness of man in this respect. The two-carbon fragment produced in the metabolism of fatty acids is the normal starting point for the endogenous synthesis of cholesterol, and there is little reason to suppose man to be an abnormal producer of cholesterol. He may be unusual in that his normal plasma-cholesterol concentration is high compared with his safe upper limit of plasma-cholesterol.

A relationship between cholesterol metabolism and atherosclerosis is firmly established but somewhat complicated.[32] The lipid present in early atheromatous deposits contains neutral fat, phospholipid, free cholesterol and cholesterol esters[33, 34] in proportions agreeing closely with those of blood plasma but not with those characteristic of other tissues[35, 36] (see Table IV). This is the main reason for regarding the plasma lipid as the source of the deposit in the arterial intima, in spite of the fact that cholesterol can be synthesized in aortal tissue.[37] When plasma cholesterol levels are consistently low (about 160 mg./100 ml.), the incidence of cardiovascular disease is low,[38] by contrast, in certain nephritic conditions accompanied by high blood cholesterol, atherosclerosis often appears, although many persons develop overt atherosclerotic signs without marked hyper-cholesterolaemia.[39] This may merely indicate that the currently accepted figures for ' normality ' are too high and embrace too wide a range. Doubts have arisen whether simple statements about total serum cholesterol possess much direct significance. Serum levels represent a balance between a number of interrelated processes. There is first a circulation of cholesterol (Fig. 1) and a disappearance of cholesterol by faecal excretion of coprosterol formed from it in the large intestine. Cholesterol is synthesized, mainly in the liver[31, 40, 41] from small

Table IV

Lipid composition of blood plasma and of intimal tissue

	Total[a] lipid	Cholesterol[b] Free	Cholesterol[b] Esterfied	Phospholipid[b] (total)	Glycerides[b]
Blood plasma		14·1	38·3	22·8	23·3
Normal media	8·31	17·3	16·7	34·1	31·9
Normal intima	14·4	14·2	38·6	20·1	19·1
Early plaques	25·9	16·2	38·5	19·0	20·5
Fibrous plaques	27·2	18·1	47·5	14·9	15·0
Calcified tissues	12·8	21·9	47·2	13·2	13·1
Atheromas	36·0	27·2	42·1	16·0	10·4

[a] as percentage of wet tissue
[b] as percentage of total lipid

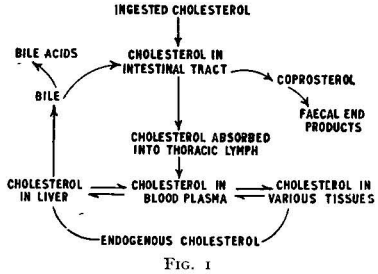

FIG. 1

molecules, but some synthesis undoubtedly occurs at other sites.[42] Squalene,[43, 44] an unsaturated hydrocarbon ($C_{30}H_{50}$) occurring in liver oils from certain Elasmobranch fishes, is an intermediate in the biosynthesis of cholesterol,[45] probably via lanosterol. ^{14}C-labelled acetate has been used to prove that both carbon atoms are used for biosynthesis in mammalian liver *in vivo* and *in vitro*.[46–48] The evidence that squalene is a true intermediate rests mainly on the high efficiency of the synthesis of cholesterol from labelled squalene.*

In the living animal oral administration of cholesterol results in substantial absorption, but liver synthesis from acetate is depressed.[49, 50]

Although the homeostasis may be imperfect, it is clear that some adjustment occurs continually. The fasting animal exhibits diminished capacity for endogenous liver synthesis of cholesterol, but after breaking fast synthesis proceeds for a time at a rate greatly exceeding the normal.[51]

In man the amount of cholesterol secreted into the lumen of the gut with the bile is between 0·5 and 1·0 g. per day, the total biosynthesis being of the order 2 g. per day.[31] Much of this biliary free cholesterol is re-absorbed. Both neutral fat and bile salts are required for the absorption of cholesterol[52] (labelled with ^{14}C). The cholesterol must, however, be esterified[53] by a pancreatic esterase. From this situation the hypocholesteraemic action of a diet low in neutral fat may be plausibly rationalized.

The whole problem of lipid transport is relevant here; naturally enough, other variables such as the phospholipid/cholesterol ratio[54] in plasma and protein–lipid interrelationships[55] have been considered.

Lipoproteins

Nothing is more important concerning lipids in the animal than the fact that they circulate in blood at relatively high concentrations in spite of the fact that they are not in themselves appreciably soluble in aqueous media. The problem of conferring such mobility on lipids has

* Further evidence was presented to the Biochemical Society, 28 March, 1957, and a summary is in the press (Cornforth, J. W., Cornforth, R. H., Popjak, G., & Youhotsky-Gore, *Biochem. Soc. Proc.*, 1957

been solved by the evolution of lipoproteins in which the hydrophilic properties of soluble 'globular' proteins allow transport, as if they were soluble, of the bound lipids, which are sometimes major constituents of plasma lipoproteins.

It has been suggested[56, 57] that almost 'all lipids normally found in tissues and body fluids exist in the form of lipoproteins or lipid–protein complexes'. Gofman and his colleagues,[58–60] have applied the method of ultracentrifugal flotation analysis to human plasma and have found a range of lipoprotein molecules of varying size and density. This procedure is as follows:

The density of the plasma is first adjusted to 1·063 by addition of sodium chloride. The next step is to bring to the surface, in a preparative ultracentrifuge, lipids and lipoproteins of density less than 1·063. The top layer is removed by means of a pipette and then fractionated in an analytical ultracentrifuge. The unit of migration (1 Svedberg, S) follows from the statement that a molecule which undergoes sedimentation at a rate of 5×10^{13} cm./sec./unit field of force has an S value of 5, and a molecule which undergoes flotation at a rate of 5×10^{-13} cm./sec./unit field of force has an S value of 5.

The work of Gofman and his group has revealed the existence of perhaps ten distinct types of lipoproteins in human blood sera.

The compounds with S_f values over 75 are of low density and are very rich in fat. They appear particularly strongly in sera obtained after a fat-rich meal. When the S_f values are between 30 and 75, the densities are not far from unity, and the neutral fat content of the molecules is high. At least three lipoproteins, with flotation rates S_f 10–20, are rich in cholesterol (about 30%) and occur to an unusual extent in plasma from atherosclerotic subjects. The normal cholesterol carriers are β_1-lipoproteins with S_f values 3–8. The pattern of lipoprotein distribution is characteristic for the atherosclerotic[61, 62] (see Tables V and VI), but not perhaps very simply.

Table V

Ultracentrifugal classification of serum lipoproteins

Approx. density	S_f units	% Protein	% Phospholipid	% Cholesterol	% Glycerides
0·96–0·99	40,000–400	~5	~5	~5 (all free)	85–75
	(includes chylomicrons and most of the lipid of alimentary lipaemia)				
	30–20	glyceride content falls steadily			
0·99–1·029	*20–10	at least three types, each with mol. wt. ~3,000,000 and about 30% cholesterol			
	4	25	25	30 (¾ esterified)	practically none
1·03–1·05	3–8	correspond with β_1-lipoprotein and carry cholesterol and phospholipid			

* Amounts present are high in sera from atherosclerotic subjects

Table VI

(a) Lipoproteins in human blood sera

'Normal'	S_f 4, S_f 6 or both present
	S_f 40,000–400: present after fatty meals but transiently
Minimal to minor metabolic defect	S_f 4, 6 and 8 at increased concentrations
More severe defects	S_f 4, 6, 8 followed successively by
	S_f 10, 13, 17, 20 and 20–40
	S_f 40,000–400: rises after meals may be less transient
Very severe defect	S_f 4 and S_f 6 now decreased as part of shift towards higher S_f values

(b) Plasma concentrations of lipoproteins with S_f *10–20 in humans*

(Means in mg./100 ml.)

	Males 20–40	Males 40–70	Females 20–40	Females 40–70 years
'Normal'	15	16	7	13
Diabetes	19	18	22	21
Hypothyroid	31	55	—	27
Coronary disease (myocardial infarction and coronary insufficiency)	—	33	—	49
Hypertension	30	25	—	25

The problem of extracellular lipoproteins[63] can also be studied by more chemical methods of separation applicable to fairly large volumes of plasma. Two groups of lipoproteins (α- and β-) account for $\frac{9}{10}$ of the plasma lipid. The method of fractionation (Cohn, Method 10)[64] is based on ethanol precipitation at $-5°$[65–67] and the products can be purified on a small scale by electrophoretic or ultracentrifugal methods (Table VII). The concentration of α-lipoprotein is strikingly constant even in diseases characterized by hyperlipaemia. The β-lipoprotein is rich in lipid—both phospholipid and free and combined cholesterol.

Surgenor[63] concludes that ' the analytical fractionation procedure, which yields a β-lipoprotein fraction that includes the whole range of flotation components, may provide the simplest index of predisposition to or actual existence of atherosclerotic lesions '

Table VII

(*a*) *Plasma proteins fractionated* (*Cohn*,[64] *method* 10)

Fraction	Constituents
A	*Albumin, α_1-*lipoprotein*, 2_2-glycoprotein, β_1-metalloprotein
B	γ-globulins
C	*β_1-*lipoprotein*, β_1-euglobin, ceruloplasmin
D	Fibrinogen, prothrombin, globulin, etc.

* Contain nearly all the plasma cholesterol

β-lipoprotein tends to increase in nephritis and atherosclerosis. It has approx. 2 molecules of cholesterol for one of phospholipid, whereas in α-lipoprotein cholesterol and phospholipid are nearly in equimolecular amounts.

(*b*) *Plasma lipoproteins*

	Lipoprotein α	Lipoprotein **β
Molecular weight (excluding water)	200,000	1,300,000
(including water)	250,000	2,100,000
Molecular shape and size	oval, 300 × 50 Å	spherical, d = 185 Å
Density† (hydrated)	1·16	1·032
Amount present (as % of plasma protein)	3	5
Protein content, %	60	23
Lipid content, %	39·3	76·7
Phospholipids and glycerides	21	29·3
Cholesterol (esterified)	15	39·1
Cholesterol (free)	3·3	8·3

** Ultracentrifugal fractions S_f 4 and S_f 6 have physical properties agreeing closely with those of isolated β-lipoprotein and contain most of the cholesterol of plasma

† Most plasma proteins have density 1·33–1·34

Lipoproteins and lipase

The structure of β-lipoprotein[68, 69] is such that about 39% is water integral to the molecule. The molecule is hydrophilic in behaviour, although there is not enough protein to provide a monolayer on the outside of more than half the spherical structure. The charged groups of the phospholipid molecules must also face outwards, with cholesterol and cholesterol esters tucked inside the sphere with the water. It seems likely that the formation of atheromatous deposits, and a possible stage at which biochemical failure or abnormality could occur, is in the build-up or breakdown of lipoprotein. This leads naturally to the ' lipaemia clearing factor ',[70–72] first considered by Hahn[73] and later by Anderson & Fawcett.[74] The former noticed that chylomicrons quickly disappeared from the plasma of post-absorptive dogs after an injection of heparin. This led eventually to the idea that a heparin-activated enzyme existed that could hydrolyse the triglycerides of lipoprotein. The enzyme differed from pancreatic lipase in that its activity was limited to lipoprotein lipids and in fact, as the term ' clearing factor ' implies, the substrates are the lowest-density lipoproteins of alimentary lipaemia. Various tissues can supply the co-protein, which after heparin interaction makes up the enzyme. This lipoprotein lipase[75–77] quickly attacks the neutral fat of low density lipoproteins and the liberated fatty acids enter into combination with serum albumin.[78, 79]

The lipoprotein lipase seems to require heparin. It is certainly antagonized by protamine in an expected manner.

Activated glyceride in lipoprotein of low density	*Lipoprotein lipase* albumin or Ca^{++} →	complexed fatty acids + mono- and di-glycerides + glycerol + lipoprotein of higher density

The importance of this work is that it reveals a process whereby lipoproteins of very high lipid content could shed the products of triglyceride hydrolysis. If the clearing factor mechanism plays a substantial part in regulating the distribution of lipoproteins in plasma, then any failure of normal heparin production might conceivably set in motion a train of events leading to atheromata.

To anyone who has dissected a well-calcified aorta from an aged atherosclerotic subject, lipid biochemistry does not promise a full answer. Lansing[80] has advanced cogently the view that arteriosclerosis is really two diseases, one a defect in cholesterol metabolism or circulation and the other a breakdown of elastic elements in the media of arteries, accompanied by calcification of the elastic substance. In Lansing's view the second precedes atheromatosis and becomes more severe with chronological age; it may occur without atheromatous deposits. If the two 'diseases' co-exist, the intimal accumulation of cholesterol is consequent on the breakdown of elastic tissue in the supporting layer.

Rôle of elastin

The elasticity of arteries is due to a protein elastin which can be prepared[81] from defatted fresh *tunica media* from human aortas, digested at 98° in 0·1N-NaOH for 40–45 minutes. Other proteins (e.g. collagen) are preferentially hydrolysed, and the elastin is left substantially intact. The elastin content of aortas[80] (medial tissue) remains nearly constant over the human life-span, and any loss of elasticity is not due to a fall in the amount present. Lansing and his colleagues, however, find that the amino-acid composition of young and old arterial elastin is not the same. Finely ground elastin is heterogeneous and can be separated into 'light' and 'heavy' fractions by flotation in sucrose solution of density 1·3. The heavy elastin is greatly preponderant in the material from aortas of aged persons, and the light elastin preponderates in the aortas of young persons. The 'old heavy' elastin showed a considerable increase in free carboxyl groups, compared with the 'young light' elastin, and had 6·39% calcium compared with 1·14%. Even when the apparently normal areas of aortas are separated and studied, there is a steady increase in the calcium content of medial elastin with age, to a maximum of about 5%.

Lansing regards the enzyme elastase[82, 83] as a possible means of linking lipid metabolism and elastic tissue change.

Elastase is obtained from pancreas and occurs only in islet tissue; it acts on elastin to convert the fibrous protein to globular protein with no apparent fission of peptide links. It is thus essentially a solubilizing enzyme. Elastase is itself an unusual enzyme, in that it can be administered orally without loss of efficacy.

Balo & Banga[82, 83] found the elastase content of the pancreas of human atherosclerotic subjects to be substantially less than normal. Lansing points out, however, that the differences recorded might simply be a reflexion of age.

	Average age	Elastase units/g.
Arteriosclerotics	61	9
Other diseases	34	155
Violent death	31	208

When rabbits on a diet containing cholesterol (0·3%), which normally produces fatty liver and atheromatosis in six weeks, were given elastase daily, the following observations were made:

hypercholesterolaemia:	no effect of elastase
fatty livers:	inhibition almost complete
atheromatosis:	reduced incidence

Further work on the elastase preparations will be awaited eagerly. The fact that the enzyme comes from the pancreas is of special interest, because it is generally agreed that diabetics

tend to develop atherosclerosis as well as other forms of arteriosclerosis at an accelerated rate, but Mann & Stare[84] make it clear that regulated diabetics have no necessary hypercholesterolaemia or hyperlipaemia.

Cardiovascular disease and hormones

There can be no doubt that various hormones have important effects on lipid metabolism and on atherosclerosis.[85] Oliver & Boyd[86] found that administration of ethynyloestradiol to survivors of myocardial infarction corrected the abnormal concentrations of plasma lipid and lipoprotein, but they were cautious about reporting beneficial long-term effects. The same authors discussed[87] the interesting miscellaneous evidence that coronary atherogenesis is, at least in part, an endocrine problem. They present an interesting Table (Table VIII) showing the sex and age distribution of coronary disease in Edinburgh.

Table VIII

Sex and age distribution of 1000 consecutive cases of coronary disease attending Edinburgh Royal Infirmary

	No. of cases		Ratio of men to women
	Men	Women	
Younger than 35	19	1	19 : 1
35–39	44	3	15 : 1
40–49	188	26	7 : 1
50–59	256	53	5 : 1
60–69	195	86	2 : 1
Older than 70	68	61	1 : 1

If it be accepted that there is evidence for some kind of major fat–lipoprotein–sterol influence in cardiovascular disease, two reasonable points remain to be considered here.

Effect of composition of fat

The first is the question whether the absolute daily intake of essential fatty acids (linoleic and linolenic) is in any sense crucial. Stare fed a series of fats at a 20% level to rats on an atherogenic diet. Eight fats, ranging from coconut and butterfat (fairly saturated) to maize, sardine and tung oils (much more unsaturated), were tried. The first two oils resulted in plasma-cholesterol values of 597 and 548 mg./100 ml., whereas with cottonseed oil and maize oil the values were 340 and 384 mg./100 ml., respectively, but tung oil, with its triethylenic (conjugated) elaeostearic acid, resulted in the highest blood cholesterol of all. Partially hydrogenated cottonseed oil had practically the same effect as the non-hydrogenated oil.

Against this there is evidence[88–90] that relatively high intakes of some vegetable fats, especially the more unsaturated ones, are effective in lowering-plasma cholesterol levels. There is also evidence[91] that vegetarians tend to show low blood-cholesterol levels despite a high fat intake. In cholesterol-fed chicks, no differences seem to have emerged when comparison was made of animal with vegetable fats or more unsaturated with less unsaturated vegetable fats.

Keys & Anderson report briefly on a well-controlled experiment on 24 men, in which olive oil and cottonseed oil were compared. ' These fats were chosen because among common food fats of vegetable origin they have the greatest chemical dissimilarity. . . . No difference between the effects of the two oils on serum-cholesterol concentration could be found.'

Investigations on the essentiality of linoleic and linolenic acids for different species reveal considerable differences and, although the acids are very probably indispensable for man, the deficiency syndrome has not been produced experimentally.

The administration of a fat-free diet to rats presumably produces essential fatty-acid deficiency. There results a marked lowering of serum cholesterol, but the concentration in liver and adrenal glands increases to as much as twice the normal.[92, 93] With weanling animals the changes develop within a week on the fat-free diet and are enhanced with time.[94]

The significance of essential fatty acids in relation to experimental atherosclerosis in the rabbit[95] seems to have been definitely established. In this species 9% of fat was not atherogenic, but maize oil, containing essential fatty acids, had a clear inhibitory action on cholesterol-induced atherosclerosis (Table IX).

Table IX

Degree of atherosclerosis in rabbits on different diets for 2 months

Oil	Fat, %	Dietary cholesterol, %	Degree of atherosclerosis*
—	0	0	0·06
Maize oil, I val. 130	9	0	0·10
Shortening (hydrogenated vegetable fat, I val. 72)	9	0	0·10
—	0	3	3·8
Shortening	9	3	3·71
Maize oil	9	3	2·71

(Based on Kritschevsky *et al.*[95])

It must now be agreed that serum-cholesterol levels tend to fall when fat intake is reduced to less than 50 g. per day and that the replacement of typical animal fats by certain vegetable fats result also in a decrease.

A recent experiment[96] on medical students given a high-fat diet (58·5% of calorie value as fat) showed that maize oil significantly lowered plasma cholesterol in 8 days with a further small decrease during the next 8 days; replacement of maize oil by butter fat or lard after 8 days significantly increased the plasma cholesterol, but dripping (beef) or chicken fat was less effective. On a diet containing 0·7% of total calories as fat, plasma-cholesterol levels fell by about 22%. If maize oil was then given to provide 20% or 60% of the calorie value of the diet, the levels fell still further, by 7% and 15% respectively. On the other hand, butterfat at the same levels raised the plasma-cholesterol by 7% and 22%. Mixtures of butterfat and maize oil gave results consistent with the idea that the animal fat contained a substance (or substances) which increases plasma cholesterol and that the maize oil contains a substance (or substances) acting in the reverse way. The hypotheses most in favour are that the shorter-chain fatty acids are in the main responsible for the first effect and the essential fatty acids for the second.

On the hypothesis advanced by Sinclair[97] and others, that hypercholesterolaemia is the result of a *relative* essential fatty acid deficiency, it might be expected that the intake of essential fatty acids will have fallen appreciably along with the widespread increase in atherosclerosis. It appears, however, according to McCann & Trulson,[98] that half a century ago about 14·6% of the dietary fat in the U.S.A. was linoleic acid whereas it is now 12·7%. Sinclair[97] and others fear that hydrogenation of fat may not only greatly reduce the amount of ' essential fatty acid ' but may also produce ' unnatural ' isomers, which might possibly be harmful. There is, however, experimental evidence against this,[99, 100] in the sense that hydrogenated fat does not necessarily elevate cholesterol levels.

Suspicion has been directed against hydrogenated vegetable oil used for deep-fat frying, but it seems clear that there is little basis for condemnation.[101] Lard and cottonseed oil that had been aerated at 95° for 200–300 hours were molecularly distilled at 280° and gave respectively 17% and 40% of polymeric residue derived apparently from polyunsaturated acids. A hydrogenated shortening which had been used for 80 hours at 190° for frying chipped potatoes similarly yielded 7% polymeric residue.

Rats given diets containing 20% of autoxidatively produced polymeric residue soon died, but 10% could be tolerated. The residue from the hydrogenated vegetable oil was much less toxic. The distillates were not harmful in short-term experiments. The tests used in this work were severe; in normal human diets the case against hydrogenated fat in relation to cardiovascular disease is not a strong one.

Conclusion

There are many aspects of the lipid–liprotein–cholesterol–essential fatty acid–atherosclerosis complex of problems, in seeking to isolate the more significant variables, and the conscientious student of the literature is often tempted to seize upon one aspect as the answer to the problem.

If the sombre realities of cardiovascular disease make it necessary to reach an interim verdict, the following is suggested:

(1) that given more research, a rational preventive approach may emerge before long;

(2) that a significant reduction in fat intake is probably desirable for *most* men and for older women in this country; (3) that the food industry should without delay explore how best to use animal and vegetable fats separately or together.

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FACTORS INFLUENCING THE LEAD CONTENT OF WINE

By B. C. RANKINE

Winemaking procedures, materials and equipment were examined for their influence on the lead content of wine.

Unwashed grapes contained from less than 0·01 to 0·98 p.p.m. of lead (mean 0·13 p.p.m.), depending on the extent of surface contamination which was partly due to lead arsenate spray residue. Grapes, washed with acid to remove surface contamination, contained from less than 0·01 p.p.m. to 0·10 p.p.m. (mean 0·05 p.p.m.). Fermentation removed from solution 11 to 45% (mean 30%) of the lead present in the grape juice.

Fining agents, fortifying spirit and acids normally added to wine did not significantly alter the lead content, nor was it lowered by the Möeslinger blue fining, which is used in some countries to remove copper and iron. Brass winery equipment and rubber wine hoses were found to be sources of contamination, but stainless steel, plastic, and glass equipment and a filter pad examined were not.

Maturation of young wine produced a slight decrease but refrigeration and ion-exchange treatment produced no significant alteration in lead content. Contact with various types of cask wood, paraffin wax, bottle corks and also wine analysed before and after shipment to England led to no significant change.

The influence of wine type and composition on the extent of lead contamination was examined. Contamination was not found to be related to sulphur dioxide content, titratable acid or pH but was higher in sweet fortified wines than in dry wines, and in these wines added sulphur dioxide and tartaric acid produced a further increase.

The influence of lead bottle capsules was not investigated as these are known to be a major source of contamination.

The method of determination of lead is given.

Introduction

The lead content of foods and beverages has recently been the subject of an enquiry by the Food Standards Committee of the British Ministry of Food, and new recommendations for the limit of lead in foods have been gazetted.[1] The limit for wine has been fixed at 1 p.p.m., with a reduction when possible. The co-operation of wine-producing countries has been invited to assist in fixing the lowest practicable limit.

The Australian Wine Board realized the importance of this limit and its proposed reduction, and has expressed its desire to co-operate through the Australian Wine Research Institute with the British Ministry of Food. The lead content of 55 Australian wines has already been determined and the results are recorded elsewhere;[2] the content varied from 0·04 to 0·86 p.p.m. (mean 0·23 p.p.m.).

These results were viewed with satisfaction as all the wines contained less than the legal limit of 1 p.p.m., and only two out of the 55 samples examined contained over 0·5 p.p.m.

Should, however, the limit be reduced to 0·5 p.p.m. or below, it is likely that some Australian wines would not conform with United Kingdom requirements, and in view of the desire to expand this export trade, a more detailed investigation of the factors influencing the lead content of wines has been carried out.

Experimental

Methods of analysis

Lead

It is appropriate to record the method of lead analysis employed in this investigation, because it was not included in the previous paper[2] and there is no standard method published for determining lead in wine.

The method is based on that described by Sandell[3] for determination of lead in biological materials and has been modified by the South Australian State Department of Chemistry, and subsequently by the author. The organic matter in the sample is destroyed by wet digestion with nitric and sulphuric acids and the lead is extracted and estimated spectrophotometrically using mixed-colour dithizone. The grapes were sampled after thorough blending in a high speed macerator.

a. *Reagents*

Water, nitric acid A.R. and chloroform B.P.—redistilled through Pyrex glass.

Sulphuric acid A.R.—distilled through a silica retort.

Dithizone in chloroform—a 0·1% solution in chloroform was purified by extraction with dilute ammonia in the usual way and the purified solution diluted with chloroform to a desirable dark green colour for use. The most suitable concentration for the estimation was that which gave about 50% transmission in the spectrophotometer compared with distilled water at 510 mμ. If the transmission exceeds about 60% the reading for the higher lead concentrations is not a true indication of the lead content because all the dithizone is used up and a mixed colour is not obtained.

Ammonium hydroxide, sp. gr. 0·9—gaseous ammonia from a cylinder was passed into distilled water until saturated.

Ammonium citrate 50% (w/v)—adjusted to pH 8·5–9·0, with aqueous ammonia, extracted with 0·1% dithizone solution and the excess dithizone removed with chloroform. (All samples of ammonium citrate available were heavily contaminated with lead.)

Hydroxylamine hydrochloride A.R.—20 g. dissolved in 100 ml. of water and purified, if necessary, by extracting with dithizone and washing with chloroform.

Thymol blue indicator—0·2 g. dissolved in 4·3 ml. of 0·1N-sodium hydroxide and made up to 100 ml. with 50% alcohol.

Potassium cyanide 10% (w/v) A.R.—an approximately saturated solution (50%) was extracted with dithizone, washed with chloroform and diluted to 10%. (Many chloroform washings were required to remove surplus dithizone.)

Nitric acid 1%—10 ml. of distilled nitric acid made up to 1 litre with glass-distilled water and saturated with chloroform.

Standard lead solution—a stock solution containing 0·1% Pb was prepared by dissolving 0·16 g. of pure dried anhydrous lead nitrate in water containing 3 ml. of redistilled nitric acid and making up to 100 ml. with glass-distilled water. A diluted lead standard solution containing 1 μg. per ml. was prepared daily by diluting 1 ml. of stock solution to 1000 ml. in two stages with 1 : 100 nitric acid.

Ammonia-potassium cyanide solution—20 ml. of 10% potassium cyanide and 75 ml. of ammonium hydroxide sp. gr. 0·9 diluted to 500 ml. with distilled water.

All reagents were stored in Pyrex glass bottles and Pyrex glassware was used throughout.

Nitric acid was found to be very readily contaminated with lead from soda-glassware, and particular care was needed in dealing with this reagent. High blank values were occasionally found and these were traced to contamination of the nitric acid.

b. *Extraction of lead*

1. To 50 ml. of wine boiled to remove alcohol or 10 g. of macerated grapes and duplicate blanks of glass-distilled water, add concentrated nitric acid (30 ml. for grapes or sweet wines or 10 ml. for dry wines) and evaporate slowly to 3–4 ml. total volume. Cool and add 3 ml. of concentrated sulphuric acid. Continue the digestion, with addition of nitric acid as required to avoid charring, until the solution is clear. Heat strongly until fuming for 5 minutes and cool. Add 25 ml. of glass-distilled water and evaporate with occasional shaking to fumes and then fume for 5 minutes. Cool and add 20 ml. of glass-distilled water. (The digestion should take about $2\frac{1}{2}$ hours and it is important not to overheat in the early stage.)

2. When cool add the following reagents with shaking: 10 ml. of ammonium citrate, 1 ml. of hydroxylamine hydrochloride, 2 drops of thymol blue indicator, ammonia solution until alkaline (yellow) and cool, 5 ml. of potassium cyanide and cool, and finally ammonia solution to pH 8·5–9·0 (greenish-blue).

3. Transfer to 125-ml. separating funnel (no Vaseline on stopper or tap) rinsing Kjeldahl flask with glass-distilled water and adding rinsings, and extract the lead by addition of several 2–5-ml. portions of the dithizone solution, transferring the extracts to a second separator containing 25 ml. of 1% nitric acid, avoiding passing any of the aqueous phase into the second separator as it will interfere with the subsequent estimation.

4. Shake the second separator thoroughly and discard the green dithizone. Shake with a few ml. of chloroform and discard the chloroform. The lead is now in 25 ml. of 1% nitric acid.

c. *Estimation of lead*

1. Prepare a range of standard solutions of lead containing 0 to 5 μg. in stoppered 25-ml. measuring cylinders and make each up to 5 ml. with the 1% nitric acid.

2. Pipette aliquots of the digested material (usually 5 ml. for wines and 10 ml. for grapes) into similar cylinders, together with blanks which have been similarly treated. Add 1 ml. of ammonia–potassium cyanide solution for each 5-ml. aliquot used and 5 ml. (accurately) of dithizone solution and shake thoroughly.

3. As soon as possible, and preferably in subdued light, draw off and discard the aqueous layer, pour dithizone into a spectrophotometer cuvette (1 cm. cell) and read the % transmission at 510 mμ using distilled water as a zero standard.

4. Prepare a curve from the standard solutions and calculate the lead content.

The blank value for a 5-ml. aliquot was usually 0·2–0·4 μg.

The method was modified for analysis of paraffin wax, a weighed quantity of which was dissolved in chloroform and extracted with water. The aqueous extract was digested, extracted and estimated as described above.

All the results listed are the means of duplicate or triplicate analyses. The investigations comprised about 600 separate analyses.

Other analyses

The following methods were used for the other analyses: ethanol by ebulliometer, specific gravity and Baumé by hydrometer, pH by glass electrode, titratable acid electrometrically using the Fisher Titrimeter, total sulphur dioxide by the Ripper method, and free sulphur dioxide by direct iodine titration on the acidified wine.

Preparation of grapes

The samples were divided into two equal portions, one of which was washed thoroughly with dilute HCl (1 + 9), tap water then distilled water, while the other sample remained untreated. The samples were then macerated in a high-speed macerator and 10-g. aliquots digested and analysed. In this way the lead content of the grape flesh was distinguished from lead contamination, in the form of lead arsenate spray residues and dust, present on the surface of the berries.

Finings and other materials examined comprised bentonite, tannin, gelatin, carbon, tartaric acid, citric acid, potassium metabisulphite and fortifying spirit; all were obtained from winery stores currently in use.

Results

Grapes

The lead content of the various grape samples is shown in Table I. The lead content of the grapes, without surface contamination, is very low, but the extent of the contamination can vary within wide limits, depending on the amount of lead arsenate spray residue or lead-containing dust on the grape surface. Lead arsenate sprays were used as far as was known only in the southern district of South Australia at the time when the samples were collected, and care was taken to include sprayed samples, one of which (Pedro variety from the southern district) showed a much higher lead content than the other samples. The range of samples covered four South Australian viticultural districts but should not be interpreted as being representative of the grapes of each district. In preparing the samples the grapes were washed individually as thoroughly as possible, but a commercial load could not be washed in such a manner.

Influence of fermentation

This was tested experimentally in the laboratory by adding known amounts of lead to grape juice contained in Pyrex flasks, and analysing the juice before and after complete fermentation. Samples of juice before and after fermentation were also obtained from wineries and the results are shown in Table II.

Table I

Lead content of grapes, p.p.m., before and after washing

Variety	Viticultural district	Lead arsenate sprayed	Before washing	After washing
Pedro	Southern	yes	0·98	< 0·01
,,	,,	yes	<0·01	<0·01
,,	,,	no	0·10	<0·01
,,	Barossa	no	0·07	0·05
Muscat	Southern	yes	0·09	0·09
,,	Murray River Irrigation	no	<0·01	<0·01
,,	,, ,,	no	0·18	<0·01
,,	Adelaide	no	0·05	0·05
Shiraz	Southern	yes	0·04	0·04
,,	,,	yes	0·10	0·02
,,	Barossa	no	0·10	0·10
Grenache	Southern	yes	0·06	0·05
,,	,,	yes	0·12	0·06
Mataro	,,	yes	<0·01	<0·01
,,	Barossa	no	0·13	0·10
Cabernet Sauvignon	Southern	yes	0·03	<0·01
Riesling	Adelaide	no	0·16	0·10
White Hermitage	Barossa	no	0·13	0·08
Hunter River Riesling (Semillon)	,,	no	0·15	0·10
Frontignac	,,	no	0·09	0·04
		Mean	0·13	0·05

Table II

Influence of fermentation on lead content of grape juice

Variety of juice	Where fermented	Pb before fermentation, p.p.m.	Pb after fermentation, p.p.m.	% decrease
Pedro (sprayed)	Laboratory	0·22	0·18	22
,, ,,	,,	0·32	0·19	41
,, ,,	,,	0·42	0·26	38
,, ,,	,,	0·72	0·50	31
,, ,,	,,	1·22	0·72	41
,, ,,	Winery	0·94	0·62	34
,, ,,	,,	0·76	0·68	11
,, ,,	,,	0·78	0·64	18
Grenache (not sprayed)	,,	0·22	0·14	36
,, ,, ,,	,,	0·11	0·06	45
Mean		0·57	0·40	30

Influence of finings and other materials

Six of these materials were analysed directly and three, which could not be satisfactorily analysed, were added to wine which was analysed before and after addition. Table III shows that the materials tested could be disregarded as sources of contamination.

The Möeslinger or blue fining is used in some countries to reduce copper and iron contamination in wine, and samples of wine before and after trial blue fining were analysed for lead and copper content. The results are shown in Table IV.

The fining had no influence on lead content although a marked reduction in copper was produced.

Influence of winery equipment

(a) *Rubber hoses.*—Wines were analysed before and after storage in winery hoses of various ages, and the results are shown in Table V. The important result from this table is that rubber hoses are a source of lead contamination. With the red rubber hoses the extent of contamination appears to depend on the age of the hose, which is doubtless related to deterioration of the inner surface with consequently greater contact area exposed. A sample of red rubber from a hose was found to contain 44 p.p.m. of lead.

Table III

Influence of winemaking materials on the lead content of wine

Material	Lead, p.p.m.	Normal max. addition per 100 gal.	Influence on lead content, p.p.m.
Tartaric acid	1·8	1 lb.	<0·002 increase (calculated)
Citric acid	0·8	1 lb.	<0·001 ,, ,,
Bentonite	—	1 lb.	no change (measured)
Potassium metabisulphite	4·2	8 oz.	0·002 increase (calculated)
Gelatin	0·8	4 oz.	<0·001 ,, ,,
Tannin	3·1	8 oz.	0·002 ,, ,,
Carbon	—	4 oz.	0·06 ,, (measured)
Corks	—	—	no change ,,
Fortifying spirit	0·04	10 gal.	,, ,, ,,
,, ,,	<0·01	,,	,, ,, ,,
,, ,,	<0·01	,,	,, ,, ,,
,, ,,	<0·01	,,	,, ,, ,,

Table IV

Influence of trial Möeslinger fining on lead and copper content of three wines

Wines	Lead, p.p.m.		Copper, p.p.m.	
	before fining	after fining	before fining	after fining
Sweet sherry	0·12	0·12	3·3	0·3
Dry sherry	0·21	0·19	2·4	1·5
,, ,,	0·34	0·33	2·7	1·1
Mean	0·22	0·21	2·8	1·0

Table V

Influence of storage in winery hose on the lead content of dry sherry

Age of hose, years	Type of rubber	Storage time, h.	Lead, p.p.m.		% increase
			before storage	after storage	
new, unused	red	½	0·18	0·18	0
7	,,	84	0·06	0·20	230
22	,,	84	0·06	0·44	630
6	,,	2½	0·32	0·37	16
6	,,	2½	0·32	0·46	44
20	white	2½	0·32	0·40	25
28	,,	2½	0·32	0·38	19

(b) *Pumps.*—Wine was stored in four winery pumps for 3½ hours and the lead content was determined before and after storage. The results are shown in Table VI, which indicates that pumps are a source of contamination. The wines after contact were all cloudy, indicating heavy metal contamination. The pumps were constructed mainly of casting brass, which normally contains up to 5% of lead, and lead–tin solder is normally used to unite various components and fill up any casting holes.

Although the contamination in wine stored in the pumps during the time of contact was very great, and it is likely that a similar quantity of lead would be picked up by wine passing through the pump in operation during this period, the increase in lead content in the total volume of wine pumped in 3½ hours (1200–2500 gal./h. or 55–114 hl./h.) would be very low (0·0001 p.p.m. calculated), providing the pumping was continuous ; that is, the extent of contamination would depend on the time of contact.

(c) *Bottle-filling machine.*—The influence of a rotary bottle filling machine on lead content was tested during an actual bottling run on claret. The machine was a hand-operated rotary siphon filler made of gunmetal. The lead content of the wine before entering the machine was 0·24 p.p.m. and after standing in the machine for ¾ hour over lunch—0·38 p.p.m., so that the wine bottled immediately after lunch was thus 0·14 p.p.m. higher in lead content. Gunmetal is a copper–tin alloy and commonly contains lead and zinc,[4] and contamination from this source could be expected.

(d) *Filter pad.*—A new D4 porosity cellulose asbestos pad was chosen for examination. Because a direct lead analysis of the pad was not practicable, an indirect test of lead contamination was carried out by placing the powdered pad in a sintered glass funnel and washing it repeatedly with dry sherry. The wine was analysed before and after the washing process, but no significant increase in lead content was observed.

(e) *Metal and plastic fittings.*—The influence of plastics, stainless steel and casting brass on the lead content of dry sherry was examined and the results are shown in Table VII.

Table VI

Influence of contact with winery pumps on lead content of dry sherry

(Time of contact, 3½ h. Pumps not operating)

Type of pump	Lead content, p.p.m. before contact	Lead content, p.p.m. after contact	% increase
Mechanical, positive action	0·32	2·8	780
Steam, positive action	0·32	3·8	1200
Centrifugal	0·32	4·8	1500
,,	0·32	4·2	1300

Table VII

Influence of plastic, stainless steel and casting brass fittings on lead content of dry sherry)

(Initial lead content, 0·29 p.p.m. 77 sq. cm. of each material exposed to 300 ml. of wine for 1 h.)

Material	Lead content of wine, p.p.m.	% increase
Polythene, 2·5-cm. tube	0·30	3
Polystyrene, 2·5-cm. rod	0·30	3
Cellulose acetate butyrate, 2·5-cm. tube	0·40	38
Stainless steel 18/8 Mo, 2·5-cm. tube	0·29	0
Brass hose nozzle, 2·5 cm. diameter	1·16	300

Influence of maturation

(a) *Changes during storage.*—Samples of 1956 wines recently fermented were taken before and after storage for short periods under winery conditions, and the lead content determined. The results are shown in Table VIII.

The various wines differ somewhat in their lead content before and after storage: the general effect is that the lead content is slightly lower after a short storage period.

(b) *Cask wood.*—Metal drillings of the wood from different types of cask were digested and analysed for lead. The influence of the various timbers on lead content of wine was further examined by soaking 5 g. of the fine drillings in 250 ml. of dry sherry for 8 weeks in Pyrex-glass reagent bottles. The wine was analysed before and after soaking and the results of this and the direct analyses are shown in Table IX.

Cask wood is low in lead content and is not a source of lead contamination.

(c) The influence of *bottle corks* was examined by leaving 10 g. of small cork pieces in contact with 250 ml. of dry sherry in a Pyrex reagent bottle for 6 weeks with occasional shaking. No change in lead content was detected in the wine before and after contact.

(d) *Paraffin wax.*—Paraffin wax is used widely in the industry as a coating for cement in contact with wine, in particular cement fermentation tanks and storage tanks, and also various other pieces of equipment, such as wooden slats used to head down fermentation tanks, and wooden casks made of Australian oak (*Eucalyptus obliqua*) used for shipping wine. The wax coating is applied hot before vintage and is renewed from time to time. The wax removed is usually melted down, cast into blocks for storage and subsequently reused.

Samples of paraffin wax were analysed directly and also after soaking in wine in a similar manner to that for cask wood described above. A sample of reused wax contained 4·1 p.p.m. whereas new wax was very low in lead content. Neither the old nor new wax however caused any increase in lead content when stored over wine for 5 weeks with occasional shaking.

(e) *Refrigeration.*—Refrigeration alters the composition of wine by deposition of potassium bitartrate, pigments and small amounts of other constituents. Two samples of tartrate deposit from different wineries were analysed with the following results:

Deposit from white wine 11 p.p.m.
,, ,, red ,, 17·5 p.p.m.

This lead content appears high but would correspond to a very slight overall decrease in the wine,

Table VIII

Influence of storage of newly fermented wines on lead content

Wine type	Lead content, p.p.m. before	after
Winery A : storage 2 months		
Grenache Sweet Fortified	0·12	0·06*
" " "	0·09	0·15*
" " "	0·09	0·09*
" " "	0·08	0·04*
Muscat " "	0·05	0·05*
" " "	0·01	0·01*
Winery B : storage 2 months		
Dry Red	0·14	0·02
Muscat Sweet Fortified	0·14	0·16
Winery C : storage 1 month		
Pedro Dry White	0·31	0·32
" " "	0·26	0·24
Sweet Red	0·04	0·03
" "	0·09	0·13
" "	0·07	0·05
Mean	0·11	0·10

* Racked and fined between analyses

Table IX

Influence of cask wood on lead content of wine

(5 g. of wood soaked in 250 ml. of dry sherry for 8 weeks. Initial lead content of wine, 0·29 p.p.m.)

Variety of wood	Lead content of wood*	Increase in lead content of wine
American oak	0·06	nil
German oak	0·06	"
French oak	0·07	"
Australian oak (*Eucalyptus obliqua*)	0·05	"
Jarrah	0·05	"

* Lower limit of analysis of wood, 0·05 p.p.m.

because only about 6–7 lb. of deposit are obtained from 1000 gal. of wine, which would correspond to a decrease in the two wines of 0·008 and 0·012 p.p.m. respectively. Thus refrigeration does not appear to influence the lead content.

(f) *Ion-exchange treatment.*—The treatment of wine with ion-exchange resins is a new development in the wine industry and claims have been made that the treatment is effective in removing metals from wine. The use of the resins has been investigated in this laboratory[5] with the finding that copper and iron were partially, but not satisfactorily, removed from wine. Samples of various wines from this investigation were examined for lead content before and after ion-exchange treatment by both cation exchange (4 experiments) using Zeo-Karb 225 in the sodium cycle, and anion exchange (4 experiments) using DeAcidite-E, -H, -G and -G highly crosslinked in the hydroxyl cycle. Apart from one isolated experiment in which the lead content decreased from 0·25 to 0·20 p.p.m. in a flor sherry treated by anion-exchange resin DeAcidite-E, no reduction in lead content was brought about by the treatments.

Influence of overseas shipment

Since the restriction in lead content of wine is at present only operating in Britain it was considered important to investigate the influence of sea transport of wine from Australia to Britain to ascertain if contamination could arise from contact with storage containers during the voyage.

Samples of a sweet red wine not exceeding 27° proof spirit were taken from hogsheads and stainless steel tanks immediately prior to shipment, and samples from the same containers were carefully drawn after arrival in Britain, and returned by ship to Australia and analysed. There was no significant difference in lead content in the wines before and after shipment.

Influence of wine type and composition

A representative range of wines from one manufacturer was obtained and analysed for normal constituents. A 250-ml. portion of each wine was left in contact for 30 minutes with frequent shaking with a small piece of casting brass (approximate composition : 80% copper, 5% zinc, 5% tin and up to 5% lead) with a surface area of 6·9 sq. cm. The wines before and after contact were analysed for lead content with the results shown in Table X. The composition of the muscat (Samples 15 to 21) was altered by addition of potassium metabisulphite and tartaric acid.

Table X

Influence of wine type and composition on lead uptake

(250 ml. of wine, 30 minutes' contact with casting brass (80% copper, 5% lead), surface area 6·9 sq. cm., gentle shaking)

Wine type	Composition						Lead content		
	Ethanol % v/v)	sp. gr. or ° Baumé	pH	Titratable acid, g/l. as tartaric acid	SO_2 content Total, p.p.m.	SO_2 content Free, p.p.m.	Before contact	After contact	% increase
1. Dry White	11·1	0·991	3·22	5·7	124	8	0·31	0·75	142
2. ,, ,,	11·7	0·999	3·13	6·6	159	36	0·23	0·80	248
3. ,, ,,	13·5	0·994	3·41	4·7	76	18	0·08	0·55	590
4. Sauternes	12·5	2·8	3·50	5·2	550	90	0·16	0·75	369
5. ,,	11·6	2·6	3·44	3·8	332	132	0·27	0·70	159
6. Dry Red	13·1	0·994	3·63	5·7	51	21	0·16	0·75	369
7. ,, ,, Sherry	13·5	0·993	3·56	6·7	54	13	0·25	0·70	220
8. pale, dry, delicate	20·2	0·987	3·35	4·7	13	6	0·94	1·26	29
9. ,, ,, ,,	20·5	0·992	3·32	5·0	72	9	0·41	1·10	168
10. ,, ,, ,,	16·9	0·985	3·46	4·3	73	11	0·28	0·90	221
11. fruity	19·7	0·3	3·50	5·3	14	5	0·39	1·15	195
12. ,,	18·5	0·995	3·71	3·6	11	2	0·22	0·78	254
13. sweet	17·6	4·3	3·50	4·2	74	9	0·23	1·19	418
14. Port	17·9	4·6	3·68	3·9	58	7	0·23	1·15	400
15. Muscat	18·6	4·8	3·79	3·6	69	11	0·16	0·95	494
16. ,, + SO_2	18·6	4·8	3·81	3·6	155	27	0·16	0·85	431
17. ,, ,, ,,	18·6	4·8	3·83	3·6	242	56	0·16	1·25	681
18. ,, acidified	18·6	5·0	3·31	5·6	67	10	0·16	1·10	587
19. ,, ,, + SO_2	18·6	5·0	3·29	5·7	139	24	0·16	0·93	481
20. ,, ,, ,, ,,	18·6	5·0	3·28	5·8	221	48	0·16	1·44	800
21. ,, ,,	18·6	5·2	2·94	9·1	67	9	0·16	1·62	913
						Mean	0·25	0·98	292

The results indicate that the amount of lead contamination is not closely related to the content of sulphur dioxide or titratable acid, nor to pH, but is higher in the sweet fortified wines, and the presence of added sulphur dioxide and tartaric acid in the muscat produced an increase in lead contamination. The reason for the increased uptake by these wines is difficult to explain. Lead may enter into combination with the sugar and alcohol but no reference to any reactions of this type could be found.

The uptake of copper was also determined and the values ranged from 0 to 1 p.p.m. This was a small uptake when it is considered that copper was present in 16 times the concentration of lead in the brass sample used. From these results it appears that wine is more readily contaminated with lead than with copper when a clean alloy of the two is exposed. It has been demonstrated repeatedly in wineries however that much greater copper contamination occurs from an uncleaned surface than from a cleaned one, and this is probably the reason for the relatively low copper contamination.

Discussion

Lead introduced with unsprayed grapes appears to constitute a small proportion of that present in the wine, but where lead sprays are used the lead content of the wine can be very high, and the spray residues appear to be the main source of contamination. The removal of lead during fermentation is surprisingly low when compared with the removal of copper and iron. A survey of the available literature[6] showed that about 80% of these two metals but only about 30% of the lead appears to be removed during fermentation. The reason for this lower percentage removal is not apparent although it may be related to the lower concentration in the wine. The fact that fermentation does not remove much of the lead in grape juice emphasizes the importance of a low initial lead content in the grapes. Lead sprays are not used widely in Australia. The low lead content of washed grapes agreed well with the findings of Gentilini[7] and

Bagchi[8] that grapes are not a source of lead contamination. Removal of surface lead contamination in practice would be difficult, and careful washing such as used in this work would not be possible.

The negligible lead content of the various finings and other materials added to wine was satisfying in view of the finding of Ferré & Jaulmes[9] that a sample of tartaric acid examined contained 48 mg. of lead per kg. (Assuming all this lead dissolved in the wine a normal addition of 1 lb. per 100 gallons would increase the lead content of the wine by 0·05 p.p.m.) The low lead content of fortifying spirit was also satisfying in view of the finding of de Almeida[10] that the spirit used to fortify Portuguese ports was a contributing factor to the lead content of these wines.

The contaminating effect of various pieces of winery equipment is of considerable importance. It is not possible to estimate accurately the relative effects of various pieces of equipment on the overall contamination of wine, because the rate of contamination depends on the time of contact. As a general rule brass is suspect, and unfortunately there is still a great deal of brass equipment in Australian wineries, whereas stainless steel, polyethylene plastic and glass appear to be satisfactory. There is a tendency in the industry to replace brass and copper fittings where possible with one or more of the other materials in order to eliminate copper and iron contamination, and this will likewise reduce lead contamination.

Rubber hoses are a source of contamination and this was also shown by de Almeida,[10] who considered them to be an important source. Lead compounds are used as vulcanizing accelerators and consequently it is expected that rubber hoses would be a source of contamination.

It appears that once lead is present in the wine it is not readily removed, although a small quantity may precipitate with the lees during maturation. This stresses the importance of preventing initial lead contamination, as the method of removing heavy metals by blue fining does not appear to be of use in removing lead. Probably the very small concentration of lead ferrocyanide formed is soluble in wine, as solubility tables indicate that lead ferrocyanide is slightly soluble in dilute acid, although it is insoluble in cold water.

This investigation has been concerned with wine up to the time of bottling and, although the influence of corks on lead content was measured, no examination of the influence of lead capsules was undertaken. There is ample evidence[9, 10] that the lead capsules (92% lead, 8% tin) are a very important source of lead contamination both during storage, when the wine slowly soaks through the cork and contacts the capsule, and during actual pouring of the wine where particles of lead and its salts may easily fall into the glass. Most of the wine now bottled in Australia carries aluminium foil or plastic capsules.

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Australian Wine Research Institute
c/o Waite Institute
Adelaide
S. Australia

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SYSTEMIC ACTION OF CAPTAN AGAINST *BOTRYTIS FABAE* (CHOCOLATE SPOT OF BROAD BEAN)

By EUNICE J. NAPIER, A. RHODES, DOROTHY I. TURNER, J. TOOTILL and A. DUNN

The control of *Botrytis fabae* (chocolate spot of broad bean) effected by foliage and root applications of captan were examined. Captan applied to the dorsal surfaces only of leaves protected the ventral surfaces of the same leaves and also leaves both above and below the foliage to which it had been applied. These systemic antifungal effects showed marked persistence. When watered on to the roots, captan gave indications of a similar systemic protection of the foliage against the same pathogen.

A nomogram is provided to facilitate calculation of fiducial limits from a population of different variance.

Introduction

A new class of organic fungicides, in which the fungicidal activity was attributed to the $>NS{\cdot}CCl_3$ group, was described by Kittleson[1] in 1952. One member of this group, *N*-trichloromethylthiotetrahydrophthalimide or captan (previously designated ' SR406 ' or ' Orthocide '), is now widely used as an agricultural fungicide.

McClellan[2] reported early and extensive field trials of SR406 in U.S.A.; he showed that this material gave good control of *Venturia inaequalis* (apple scab), but not of *Podosphaera leucotricha* (apple mildew). Good results were also obtained with Orthocide 406 by Horn,[3] who concluded that it was the best fungicide among those tested for the control of *Botrytis cinerea* (fruit rot) on strawberries.

In this country, Moore & Kirby[4] have reported that formulated captan caused no leaf damage on Cox's Orange Pippin apple at 0·1% and 0·2% of active material. When 0·1% captan was given at each time of application, it proved to be equal to a full schedule (2½ : 2 : 1%) of lime sulphur treatment for the control of *Venturia inaequalis* (apple scab), but the first year's results against *Podosphaera leucotricha* (apple mildew) were not promising. Similarly, Byrde & Marsh[5] found that 0·2% captan was superior to lime sulphur in the control of both apple and pear scab. More recent developments have amply confirmed these early results and shown that captan, despite its inactivity against mildews, remains one of the most promising of the new organic fungicides.

Captan was therefore adopted as a comparative standard in the evaluation of new antifungal antibiotics as systemic fungicides. As, however, captan itself was found to possess systemic antifungal properties, its action against *Botrytis fabae* (chocolate spot of broad bean) was examined in some detail. Results obtained in these experiments are presented here.

Experimental

The plant pathogen, *Botrytis fabae*, was used as test organism under greenhouse conditions, with *Vicia faba* (broad bean) as host plant.

Convenient groups of experimental plants were grown under glass in 4¼-in. whalehide pots containing an aggregate composed of equal volumes of vermiculite (' Exflor ' Grade C) and sand, with surface applications of nutrient solution (Mullard's M.15 Nutrient Solution for soilless cultivation) as required. Daylight was supplemented as necessary by the provision of artificial illumination from banks of 80-watt daylight fluorescent tubes spaced at 4-in. centres and suspended directly above the plants. Application of the treatments began when the plants were approximately 6 in. high and the first two leaves were fully expanded.

Captan was used as a suspension in distilled water, prepared from either 50% (w/w) commercial wettable powder or 90% technical captan. Manoxol O.T. or Agral L.N. at 0·05% (v/v) was used as wetting agent in all foliage application experiments unless otherwise stated. For root application the wetting agent was omitted.

In all but one of the experiments with foliage application, the captan suspension was applied on two successive days as fine spray from a B.E.N. model R.F.4 spray gun at a constant pressure

of 3 lb. to the dorsal surfaces of the two lowest leaves only of the treatment plant (approx. 1·2 ml. to each broad-bean plant). All other parts of the plant were shielded when spraying and the spray was applied in a routine manner from a standard distance of approximately 2 in. Control plants were similarly sprayed with water containing wetting agent alone. Subsequently the inoculum of pathogen was applied in the same way to the ventral surface of three or more of the lowest leaves on each plant.

Suitable inocula were obtained by growing *Botrytis fabae* on malt agar slopes at 25° and washing the spores from week-old cultures with distilled water. The spore concentration was estimated from the mean of four to ten counts; the suspension was then diluted to give approximately 40,000 spores per ml., and 1% glucose was added immediately before use.

After inoculation the plants were incubated for 18 hours at greenhouse temperature in a small humidity chamber, where the atmosphere was maintained as nearly as possible at saturation point. The plants were then returned to the bench for 1–3 days, when disease was estimated by counting the lesions within a circle of 2·2 cm. diameter made by the imprint of a metal cap pressed into the middle of each leaflet. The mean number of lesions per leaf (two leaflets) on treated plants was compared with the mean number on corresponding leaves of control plants. Thereby two estimates of fungicidal effect were obtained: the comparison on the two lowest leaves indicated the efficacy of the fungicide acting through the thickness of the leaf, and the others denoted the effect resulting from movement of active material out of the two lowest leaves into the stem and then into the third or higher leaves.

Persistence of the fungicide was estimated by varying the interval between the times of application of fungicide and of inoculation with pathogen.

In a single experiment on foliage application, captan was sprayed on two successive days on to the dorsal surfaces of leaves half-way up the plant, and the ventral surfaces of untreated leaves both above and below this region were subsequently inoculated with pathogen. The appropriate comparisons with untreated inoculated leaves provided estimates of movement of antifungal material both upwards and downwards from the site of application.

For root application, the captan suspension was watered into the aggregate of the pots at the rate of 100 ml. per plant on each of three successive days before inoculation of the foliage on the 4th day in the manner already described.

Results

The results obtained from foliage application experiments with captan in the form of 50% wettable powder when the concentration of active ingredient was 1, 2 or 8 mg./ml. are summarized in Table I. The time intervals between completion of spray application and inoculation with pathogen were restricted to 1–4 days in the experiments with the two lower concentrations, but when the active ingredient was raised to a concentration of 8 mg./ml., longer time intervals were allowed in order to test for persistence of the antifungal effect.

At that time captan was available only as a commercial 50% wettable powder, but later, 90% technical material became available and this was compared with the original material. The results are shown in Table II, where the effects of equivalent active ingredient are compared.

Results of tests for simultaneous upward and downward movement of fungicide, with foliage sprays prepared from 90% technical captan, are given in Table III and the results obtained from root watering with suspensions made from the same material in Table IV.

In each experiment the variability in the number of lesions recorded for the control plants differed from that with the treated plants, and there appeared to be no simple relation between the number of lesions and its standard deviation. It was therefore necessary to analyse the data without postulating any such relationship. This was done with the aid of Fisher Behrens Tables given in Fisher & Yates[6] and similar additional tables prepared by the same authors[7] and Fisher & Healy.[8] The results of the statistical analyses are presented with the relevant data.

Table I shows that captan from 50% wettable powder, when applied to the dorsal surfaces of the first and second leaves, resulted in a marked antifungal effect on the ventral surfaces of the same leaves and in leaves higher up the stem that had received no direct application of fungicide. This systemic antifungal action was persistent, being detected up to sixteen days after treatment.

Table I

Systemic antifungal effects of captan (50% wettable powder) foliage spray applied on two successive days at various intervals before inoculation of plants with pathogen

(Total application of spray to each plant approximately 1·2 ml.)

Expt. No.	Captan concentration, mg./ml.	Time interval between sprays and inoculation, days	Wetting agent	Mean number of lesions per leaf (with standard error)							
				Leaves sprayed on dorsal surface and inoculated on ventral surface				Leaves unsprayed but inoculated on ventral surface			
				1st leaf		2nd leaf		3rd leaf		4th leaf	
				Control	Treated	Control	Treated	Control	Treated	Control	Treated
B62	1	1	Agral L.N.	217 ± 18	68 ± 11*	175 ± 12	50 ± 7*	195 ± 16	119 ± 12*		
B46	2	1	Manoxol O.T.	214 ± 10	23 ± 11*	165 ± 14	34 ± 7*	191 ± 24	25 ± 5*		
B47	2	1	Agral L.N.	299 ± 25	59 ± 14*	256 ± 18	43 ± 7*	269 ± 27	89 ± 18*		
B52	2	1	Manoxol O.T.	120 ± 10	22 ± 3*	71 ± 7	18 ± 4*	70 ± 9	21 ± 6*		
B55	2	1	Manoxol O.T.	196 ± 18	12 ± 6*	132 ± 12	23 ± 4*	125 ± 11	41 ± 8*		
B56	2	1	Manoxol O.T.	69 ± 10	11 ± 6*	63 ± 3	20 ± 6*	77 ± 13	35 ± 5*		
B58	2	1	Manoxol O.T.	91 ± 6	11 ± 1*	92 ± 15	30 ± 17*	83 ± 14	37 ± 16*		
B60	2	1	Agral L.N.	419 ± 30	54 ± 14*	263 ± 15	79 ± 7*	256 ± 17	155 ± 12*		
B42	2	2	Manoxol O.T.	209 ± 24	70 ± 13*	145 ± 13	79 ± 14*	113 ± 7	108 ± 4	105 ± 9	84 ± 16
B59	2	2	Manoxol O.T.	151 ± 19	28 ± 3*	94 ± 5	27 ± 5*	109 ± 11	37 ± 4*		
B53	2	2	Manoxol O.T.	175 ± 15	60 ± 11*	141 ± 19	64 ± 18*	142 ± 28	66 ± 12*		
B48	2	2	Manoxol O.T.	154 ± 14	45 ± 12*	101 ± 4	50 ± 6*	75 ± 7	37 ± 4*		
B41	2	3	Agral L.N.	293 ± 27	62 ± 13*	181 ± 17	61 ± 7*	143 ± 16	114 ± 11	107 ± 9	83 ± 15
B45	2	4	Manoxol O.T.	152 ± 19	12 ± 3*	153 ± 22	19 ± 6*	128 ± 13	37 ± 5*	97 ± 12	49 ± 16*
B61	2	4	Agral L.N.	130 ± 17	49 ± 10*	114 ± 15	44 ± 7*	135 ± 9	108 ± 8*		
B67	2	4	No wetter	253 ± 19	88 ± 10*	207 ± 18	69 ± 10*	152 ± 18	77 ± 8*		
B76	2	4	Agral L.N.	180 ± 12	21 ± 6*	135 ± 12	37 ± 9*	112 ± 9	73 ± 9*		
B31A	8	7	Manoxol O.T.	82 ± 19	7 ± 2*	46 ± 8	16 ± 5*	44 ± 9	17 ± 5*	28 ± 7	12 ± 3*
B33A	8	7	Manoxol O.T.	197 ± 18	58 ± 16*	158 ± 16	61 ± 10*	164 ± 9	93 ± 13*	152 ± 16	89 ± 14*
B33B	8	10	Manoxol O.T.	91 ± 14	10 ± 2*	61 ± 10	17 ± 7*	46 ± 8	18 ± 3*	35 ± 8	29 ± 2
B33C	8	13	Manoxol O.T.	87 ± 9	8 ± 5*	72 ± 7	16 ± 4*	64 ± 5	49 ± 5*	59 ± 6	38 ± 2*
B33D	8	16	Manoxol O.T.	148 ± 14	10 ± 3*	113 ± 10	13 ± 2*	104 ± 9	63 ± 12*	116 ± 14	45 ± 2*
B31B	8	17	Manoxol O.T.	108	18 ± 5	74 ± 3	30 ± 4*	61 ± 5	56 ± 8	56 ± 8	47 ± 10

* Significant at 5% or lower level

Table II

Comparison of systemic fungicidal effects from two foliage applications of sprays containing 2 mg./ml. captan provided by different formulations

(Approximately 1·2 ml. of spray applied to each plant. Two days between sprays and inoculation)

Expt. No.	Treatments	Mean number of lesions per leaf (with its standard error)		
		Leaves sprayed on dorsal surfaces and inoculated on ventral surface		Leaves unsprayed but inoculated on ventral surface
		1st leaf	2nd leaf	3rd leaf
B84	Control (0·05% Agral L.N.)	66 ± 8	49 ± 3	44 ± 7
	50% captan wettable powder + 0·05% Agral L.N.	23 ± 4*	23 ± 1*	24 ± 4*
	90% technical captan + 0·05% Agral L.N.	33 ± 3*	33 ± 3*	31 ± 4
	90% technical captan	37 ± 4*	33 ± 2*	20 ± 3
B86	Control (0·05% Agral L.N.)	171 ± 19	138 ± 10	131 ± 12
	50% captan wettable powder + 0·05% Agral L.N.	80 ± 11*	62 ± 8*	80 ± 11*
	90% technical captan + 0·05% Agral L.N.	95 ± 18*	92 ± 12*	104 ± 15
	90% technical captan	90 ± 14*	90 ± 21	92 ± 16
B88	Control (0·05% Agral L.N.)	233 ± 20	211 ± 21	195 ± 17
	50% captan wettable powder + 0·05% Agral L.N.	56 ± 10*	64 ± 7*	110 ± 4*
	90% technical captan + 0·05% Agral L.N.	82 ± 8*	92 ± 15*	139 ± 16*
	90% technical captan	69 ± 12*	78 ± 13*	117 ± 8*

* Significant at 5% or lower level

Table III

Systemic antifungal effects in leaves above and below foliage sprayed on dorsal surface only with 2 mg./ml. captan (90% technical material)

(Total application of spray to each plant approximately 1·2 ml. Time interval between spray and inoculation 1 day)

Expt. No.	Treatments	Mean number of lesions per leaf (with its standard error)				
		1st leaf	2nd leaf	3rd leaf	4th leaf	5th leaf
B.91	Controls (0·05% Agral L.N.)	197 ± 4	190 ± 13	159 ± 20	122 ± 10	83 ± 9
	Captan + 0·05% Agral L.N. applied to dorsal surfaces of leaves 1 & 2 only	100 ± 10*	76 ± 13*	81 ± 9*	66 ± 8*	51 ± 7*
		Dorsal surfaces sprayed				
	Captan + 0·05% Agral L.N. applied to dorsal surfaces of leaves 3 & 4 only	118 ± 17*	108 ± 39	67 ± 12*	55 ± 10*	47 ± 10*
				Dorsal surfaces sprayed		

* Significant at 5% or lower level

From Table II it appears that captan formulated as the commercial wettable powder may exert a greater antifungal effect than the 90% technical material, but the difference is not significant. Whereas both materials gave consistent and statistically significant local systemic antifungal effects in the first and second leaves, only the wettable powder was consistently effective in reducing the lesion counts on the third leaf. The 90% technical material, although showing appreciable reductions in the mean lesion counts on the third leaf in all experiments, gave a statistically significant reduction only in experiment B88.

Table III shows that captan entered leaves of various ages with about equal facility and that active material then moved to leaves both above and below the foliage to which it had been applied.

Captan, at a concentration up to 2 mg./ml. watered into the roots of broad-bean plants, had no general toxic effect, but slight chlorosis of some leaflets of first leaves occurred in one experiment (B85) done at appreciably lower temperatures than usual. The fungicide, however, when applied to the roots, lowered the mean lesion counts on all leaves with one exception (Table IV). Thus the results as a whole support the conclusion of systemic antifungal action in the foliage, although individual experiments did not always show a statistically significant effect.

Table IV

Systemic antifungal effects on foliage resulting from root applications of captan (90% *technical material*) *suspensions in water*

(100 ml. applied on three days previous to inoculation of ventral surface of foliage with pathogen. Time interval between treatment and inoculation 1 day)

Expt. No.	Treatments	Mean number of lesions per leaf (with standard error)			
		1st leaf	2nd leaf	3rd leaf	4th leaf
B85	Control (untreated)	263 ± 13	205 ± 19	209 ± 13	191 ± 21
	0·5 mg./ml. captan	163 ± 5*	152 ± 17	150 ± 16*	137 ± 15
	2 mg./ml. captan	199 ± 10	195 ± 23	183 ± 14	139 ± 19
B87	Control (untreated)	256 ± 28	199 ± 26	137 ± 24	
	0·5 mg./ml. captan	146 ± 21*	124 ± 14*	107 ± 17	
	1 mg./ml. captan	114 ± 13*	96 ± 17*	82 ± 11	
	2 mg./ml. captan	96 ± 13*	86 ± 9*	81 ± 11	
B89	Control (untreated)	290 ± 33	233 ± 24	243 ± 26	
	0·5 mg./ml. captan	275 ± 31	244 ± 27	224 ± 5	
	1 mg./ml. captan	193 ± 32	199 ± 34	208 ± 26	
	2 mg./ml. captan	171 ± 22*	202 ± 20	198 ± 18	

* Significant at 5% or lower level

There remained the rather remote possibility that the systemic antifungal effects observed in these experiments may have been due to vaporization of captan. The supposition was tested in a further experiment conducted in a greenhouse under high-temperature conditions (minimum 70° F).

Spray containing 2 mg./ml. active ingredient provided by 90% technical captan was applied to the dorsal surfaces only of the two lowest leaves of the treatment plants, and the resulting antifungal effects were observed by inoculation of the ventral surfaces of the three lowest leaves and comparison against corresponding leaves of untreated plants, as in previous experiments.

In two other treatments, captan was placed in close proximity, but not applied, to the plants so as to facilitate any antifungal action that might result from vaporization. This was effected by dipping filter paper (5·5 cm. diameter) in the captan wash used for the foliage application already described and then suspending the paper in a horizontal position close to but not touching the stem and about 2 cm. above the surface of the aggregate in the pot. In one of these treatments, the papers were dipped again in the captan wash when the second foliage application was made to the sprayed plants; in the other, the filter paper was kept moist throughout the experiment by wicks dipping into reservoirs containing captan wash.

The results given in Table V confirm the systemic antifungal pattern previously observed with foliage applications of captan and exclude the possibility that this protection is due to vaporization of the fungicide.

Table V

Test to eliminate vaporization as possible explanation of observed systemic antifungal action of captan foliage sprays (*using* 90% *technical material*)

(Time interval between spray and inoculation 1 day)

Expt. No.	Treatments	Mean number of lesions per leaf with its standard error		
		1st leaf	2nd leaf	3rd leaf
B90	Control (untreated)	238 ± 16	132 ± 10	124 ± 12
	Foliage spray of 2 mg./ml. captan + 0·05% Agral L.N. applied to dorsal surfaces only of 1st and 2nd leaves on two successive days	56 ± 10*	64 ± 12*	71 ± 8*
	No foliage treatment but filter papers twice-dipped in 2 mg./ml. captan in close proximity	254 ± 18	141 ± 18	115 ± 9
	No foliage treatment but filter papers dipped and continuously moistened with 2 mg./ml. captan in close proximity	240 ± 10	154 ± 27	113 ± 16

* Significant at 5% or lower level

In these experiments captan levels were, in general, somewhat greater than those most commonly used in commercial protective washes. It was therefore of interest to observe the antifungal effects of a range of captan concentrations. Results from such experiments are summarized in Table VI, from which it may be seen that the systemic antifungal action of captan sprays gave a fairly smooth mean decrease in lesion count with rise in concentration, but that even the 0·05% level had a marked effect.

Table VI

Systemic antifungal effects resulting from captan foliage sprays of different concentrations (from 50% wettable powder) applied on two successive days before inoculation with pathogen

(Total application to each plant approximately 1·2 ml. of wetting agent, Manoxol O.T. Time interval between spray and inoculation 1 day)

Expt. No.	Captan mg./ml.	Mean number of lesions per leaf		
		Leaves sprayed on dorsal surface and inoculated on ventral surface		Leaves unsprayed but inoculated on ventral surface
		1st leaf	2nd leaf	3rd leaf
B55	0	196	132	125
	0·003	161	107	95
	0·125	70	86	82
	0·5	36	36	70
	2·0	12	23	41
	8·0	9	6	24
B56	0	69	63	77
	0·003	37	56	57
	0·125	30	47	67
	0·5	10	19	41
	2·0	11	20	35
	8·0	5	15	26
B58	0	91	92	83
	0·5	28	28	37
	1·0	24	48	29
	2·0	11	30	37

The application of the 0·05% treatment on two successive days approximates in effect to the application of a 0·1% captan spray commonly used for commercial purposes. It seemed therefore of interest to consider in greater detail the degrees of protection afforded by this treatment. For this purpose, the observed antifungal effects in various leaves were expressed by the ratio of the mean lesion count in treatment plants to the corresponding mean in control plants. The values of these ratios and their 95% fiducial limits (calculated as below) in the three experiments were for the first leaves 18·6% (12–27·1%), 14·2% (4·3–28·5%) and 31·3% (8·7–55·5%) and for the second leaves 27·3% (15·9–41·2%), 30·6% (14·9–47·5%) and 30·6% (8·5–63·0%). The corresponding values for the third leaves, unsprayed but inoculated, were 55·6% (25·0–91·5%), 52·7% (10·6–116·9%) and 44·9% (15·2–95·8%).

All the results except those for the third leaves in one experiment present significant evidence in favour of systemic antifungal action, but the ratios are much less precisely determined for the unsprayed third leaves than for the two leaves sprayed on the dorsal surfaces.

Discussion

It has been shown that captan foliage sprays on leaves of broad bean exert a general systemic antifungal effect against *Botrytis fabae* on the foliage and that these responses are elicited by captan concentrations commonly used in commercial washes. Should this phenomenon occur in the apple tree it might be expected that captan within the tissues may resist leaching by rain and may protect new growth after spraying. This would account for the greater persistence of captan than that of mercury when used as foliage sprays against *Venturia inaequalis* (apple scab) and would support expectations of benefit from systemic antifungal action.

Although treatment of foliage with captan consistently lowered the mean lesion counts on

all leaves, there was considerable variation between experiments in the degree of significance attained; this was particularly noticeable on the unsprayed but inoculated leaves. Since dye injection experiments on broad bean had shown good continuity between leaf traces of the first two leaves and those of the third and fourth leaves, it seems unlikely that differences in available paths of transport could account for the variations observed, although competition might occur between the third and fourth leaves. The much lower variation of the lesion counts on the sprayed leaves than on the unsprayed leaves does not indicate that these differences could be due to errors in technique of inoculation between experiments.

It seems likely that the greater variation of the results for unsprayed leaves may be due to uncontrolled factors affecting translocation of the antifungal material—for example, carbohydrate status of the plant, temperature and humidity.

Although captan was used as a systemic antifungal agent for root application in these experiments, it seems unlikely to be of general application as such, for it has been observed that it was very much more toxic when watered on to tomato roots, 0·05 mg./ml. resulting in necrosis in apices of leaflets.

Appendix

Calculation of fiducial limits

Finney[9] gives a method whereby the fiducial limits of the ratio of two means, each from a population of different variance, may be determined and shows how these limits depend on the Behrens Fisher distribution. This method is tedious if any number of such ratios are examined, as interpolative or iterative methods have to be employed. The value of d required depends on the limit itself, so that no explicit solution is obtainable.

A nomogram such as Fig. 1 allows the solution to be obtained directly. It consists of two scales at right angles evenly graduated for the coefficient of variation (as a percentage) of the control and the treatment means respectively. Quarter circular arcs, the radii of which are the reciprocal of various values of d measured in the same units as the coefficient of variation (C.V.) scales and with its centre at the inter-section of the scales are drawn downwards from the scales. Radii at 5° intervals from the intersection of the C.V. scales are drawn to cut the arcs and marked off in degrees as measured from the control C.V. scale. This diagram is used as described below.

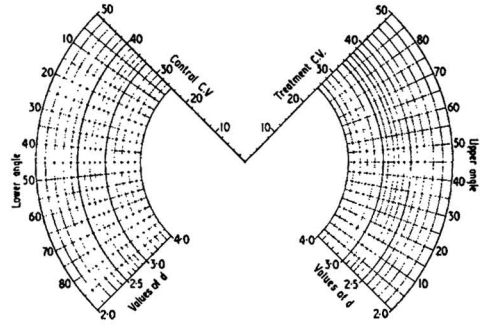

FIG. 1.—*Nomogram for fiducial limits of a ratio*

(1) For n_1 = degrees of freedom for treatment and n_2 = degrees of freedom of control, the tabulated values for d for the significance level required, given in the Tables against various angles, are read off.

(2) For each given tabulated angle a point is marked off on the radius, corresponding to the angle, and on the arc, corresponding to the value of d, if the value of d for the given angle does not fall exactly on one of the arcs, the point is marked by visual interpolation.

(3) This plotting is done on both sides of the diagram and the points are joined by eye to give a smooth curve.

(4) A ruler is placed to cut the control C.V. and treatment C.V. scales at the values observed.

(5) The ruler so placed will cut the smooth plotted curve on the left-hand side and the right-hand side, thus enabling the lower and higher angles to be read off.

(6) The fiducial limits of the ratio can thus be obtained from the formulae.

$$\text{Fiducial limit} = \frac{\text{Standard error of treatment mean}}{\text{Standard error of control mean}} \times \text{cotangent } \theta$$

where θ is the angle read off from the diagram, the lower angle giving the upper limit and the upper angle giving the lower limit.

In practice, a series of experiments is usually carried out with similar replication, involving the same values for n_1 and n_2 (degrees of freedom) for all the experiments. Thus one such plot will normally suffice for each experiment of the series. If the diagram is required for another series in which the replication is different, the plot may be rubbed out and the new plot substituted.

In practice, the accuracy of this method is limited by the accuracy of drawing involved, an error of about 1° being likely with scales four times greater than those of Fig. 1. For special circumstances greater accuracy may be required and this can readily be obtained as described below:

Let $\tan \psi = \dfrac{\text{C.V. of treatment}}{\text{C.V. of control}}$

Let d = value of d from the tables for the lower angle read on the diagram.

Then the accurate value for the lower angle is given by:

$$\sin (\psi - \theta) = \frac{d}{100} \times (\text{C.V. of control}) \times \psi$$

Similarly, where d is the value from the tables for the upper angle as found from the diagram, the accurate value for the upper angle is given by:

$$\sin (\theta - \psi) = \frac{d}{100} \times (\text{C.V. of control}) \times \psi$$

Glaxo Laboratories Ltd.
Sefton Park
Stoke Poges
Bucks.

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THE AVAILABILITY OF SOIL PHOSPHATE. IV.*—The Effect of Nitrogen on the Utilization of Phosphate by Italian Ryegrass

By S. H. YUEN and A. G. POLLARD

In pot and solution culture experiments with Italian ryegrass and oats and laboratory studies of soil, using Harlington medium loam and Buxted silty clay loam, repeated application of nitrogen as compared with a single addition was found significantly to promote the uptake of added fertilizer phosphate, but not that of difficultly-soluble native phosphate, by ryegrass.

In the very acid Buxted soil (pH 4·9), repeated use of physiologically basic nitrogen ($NaNO_3$ and KNO_3) more effectively increased the uptake of both native and added phosphates by ryegrass than did the physiologically acid nitrogen [$(NH_4)_2SO_4$ and NH_4Cl]. In these cases the uptake of phosphate was positively related to that of nitrogen. The effect of nitrogen fertilizers in the slightly acid soil (pH 6·4) chiefly depended on their influence on soil pH, the uptake of phosphate being favoured by those increasing soil acidity.

Comparison of various phosphate compounds containing ammonia showed that their efficiency in relation to the utilization of phosphate by ryegrass was closely associated with the ammonia content as well as with the uptake of nitrogen.

Repeated addition of physiologically acid nitrogen decreased the acid-extractable phosphate but increased the phosphate-fixing capacity in the slightly acid soil, while physiologically basic nitrogen behaved in the reverse manner. None of the nitrogen treatments appreciably affected either the acid-extractable phosphate or fixing capacity in the very acid soil, which had a low phosphate status and high fixing capacity.

Results of solution culture with oat seedlings showed increased absorption of phosphate at pH 5·5 6·2 with maximum at pH 5·8 6·0. The intake of phosphate decreased sharply beyond the pH range 4·0 6·2.

Introduction

Although phosphorus has certain specific functions in plant metabolism, it often appears that the intake of this element by plants is to some extent influenced by the supply of nitrogen. Nitrogen-phosphate relationships in the soil have already received much attention, but a survey of literature reveals little definite information probably because the influence of nitrogen fertilizers on the availability of phosphate differs with the form in which the nitrogen is applied as well as with the soil conditions.

Since nitrogen fertilizers may affect soil reaction[1, 2] and the availability of phosphate is known to vary with the soil pH, the intricate relation between nitrogen supply and phosphate intake is possibly linked with soil acidity. Repeated use of a particular nitrogen fertilizer therefore may modify the phosphate status of soil as well as the absorption of phosphate by plants. For instance, in the slightly acid Slough soil, as reported in previous communications,[3] evidence has been obtained that repeated application of ammonium sulphate reduced the concentrations of both native and added phosphates in soil solutions and in Morgan and Truog soil extracts, and increased the phosphate-fixing capacity of the soil.

The present investigation with Italian ryegrass, *Lolium multiflorum*, was planned to examine the following: (i) the effect of repeated application of nitrogen on the uptake of phosphate, (ii) the effect of different forms of nitrogen on the uptake of phosphate and (iii) the relative efficiency of some phosphate compounds containing varying amounts of ammonia. Since various forms of nitrogen affect the intake of phosphate differently according to the existing soil condition, attention was also given to (iv) the effect of nitrogen compounds on the pH, acid-extractable phosphate and phosphate-fixing capacity of soil and (v) the behaviour of the root membrane at various pH levels towards the diffusion of phosphate ions.

Experimental

Methods

Three Italian ryegrass experiments were carried out each involving two to four replications of a number of treatments. Ryegrass was sown in glass pots having a capacity of about 500 g.

* Part III: *J. Sci. Fd Agric.*, 1951, **2**, 260

of dry soil, which had previously received a basal supply of potassium chloride. Two types of soil were used: Harlington medium loam, Middlesex, and Buxted silty clay loam, Sussex; the former (pH 6·4) had a moderately high phosphate status and the latter (pH 4·9) an extremely low acid-extractable phosphate content.

The crops of ryegrass collected in from three to four cuts at about 2½-week intervals were dried in a steam-oven, weighed and then finely ground. Phosphate was determined in the samples after digestion with hydrogen peroxide and sulphuric acid by the modified absorptiometric Fiske-Subbarow method,[4] and the nitrogen in the same digest determined by distillation and titration of the ammonia in 1% boric acid.[5] Percentages of both phosphate and nitrogen in ryegrass varied considerably with the stage of growth. Although the percentages in samples taken at the same time are comparable with each other, they are not used in the interpretation of the present work. The total uptakes from all cuts, i.e., the sum of the overall response from treatments, were used to compare the fertilizer effects.

The pH of the soil was measured and available phosphates determined in the Morgan extracts (1 : 5 soil : extractant ratio, 30 minutes' shaking)[7] after decolorization with purified activated charcoal.[6] The percentage of added water-soluble phosphate which was not recovered by extracting the treated soil with Morgan's reagents was designated the phosphate-fixing capacity. Soil samples were wetted with water and then dried in a steam-oven before extracting their original available phosphate content.

(i) *Effect of the repeated application of nitrogen*

In the study of the repeated application of nitrogen, a 3 × 3 × 2 design was adopted. Phosphate was applied at three levels: 0P, 1P and 2P (0, 1 and 2 g. of superphosphate per 500-g. pot), and the nitrogen treatment in three forms: NH_4 (ammonium sulphate), NH_4 NO_3' (ammonium sulphate + sodium nitrate) and NO_3' (sodium nitrate) at 0·1 g. of N per pot. Superphosphate was added before the sowing of ryegrass (0·25 g. per pot). In Series I nitrogen was applied once before sowing and in Series II once before sowing and also once after each cut of grass. Results of total uptakes of phosphate and nitrogen are summarized in Table I.

The repeated application of nitrogen, irrespective of the form in which it was applied to the Buxted soil, did not increase the uptake of native phosphate by ryegrass, as the uptakes recorded in Series I and II were essentially the same. On the other hand, the repeated application of nitrogen stimulated the assimilation of added phosphate, the uptake from the single and double dressings of superphosphate being 21 and 22% respectively greater in the repeated than in that of a single application of nitrogen. Failure of the repeated application of nitrogen to increase the intake of native phosphate clearly suggests that phosphate is one of the limiting growth factors in this soil.

Table I

Effect of repeated application of nitrogen on the uptake of phosphate and nitrogen, Buxted silty clay loam

(Mean values of total P_2O_5 and N in mg. per pot for 4 cuts, 4 replicates)

Nitrogen treatment	Zero P_2O_5			N		
	0P	1P	2P	0P	1P	2P
I. One application of nitrogen						
NH_4	41	70	91	120	123	128
$NH_4 + NO_3'$	55	84	100	169	165	167
NO_3'	58	90	97	154	160	151
Mean	51	81	96	147	153	149
II. Repeated application of nitrogen						
NH_4	46	93·5	107	243	244	251
$NH_4 + NO_3'$	47	99	122	262	283	279
NO_3'	63·5	103·5	123	303	298	319
Mean	52	99	117	269	275	283
P_2O_5: S.E. for 2 × 9 tests ± 4·5; for 2 × 3 tests ± 2·6 C.V. 11·0%						
N: ± 7·3 ± 4·2 6·9%						

In both series, the nitrate consistently led to the highest uptake of phosphate, ammonia to the lowest, the ammonia–nitrate combination being intermediate. This order of efficiency in promoting the uptake of phosphate remained the same with all three levels of phosphate treatments. The uptake of nitrogen from nitrate was greater than that from ammonia. The increased uptake of phosphate in presence of nitrate was associated with the higher uptake of this form of nitrogen. Repeated addition of nitrogen caused a significant increase in the nitrogen uptake from 79 to 89%. The uptake of nitrogen was only slightly increased in the presence of superphosphate. This suggests that phosphate deficiency does not limit the absorption of nitrogen to any great extent.

(ii) *Effect of different forms of nitrogen*

In this investigation effects of five forms of nitrogen, i.e. ammonium sulphate, ammonium chloride, ammonium nitrate, sodium nitrate and potassium nitrate, were examined in association with two levels of superphosphate (oP and 1P) on both soils. Nitrogen was applied twice at the rate of 0·1 g. of N per pot once before sowing and again after the first cut. Results of total uptakes of both phosphate and nitrogen and percentages of phosphate are shown in Table II. It was evident that application of nitrogen to the Harlington soil significantly increased the phosphate uptake to extents which varied considerably with the form of the nitrogen. In the Harlington soil both ammonium sulphate and chloride led to greater increase in the phosphate uptake than did ammonium nitrate, which in turn was superior to sodium and potassium nitrate. This also applies to the pots to which superphosphate was added except that ammonia appeared to be more effective than nitrate in promoting the uptake of phosphate. In the Buxted soil there

Table II

Effect of different forms of nitrogen

(Mean values of total P_2O_5 and N in mg. per pot for 3 cuts, 2 replicates)

A. Uptake of phosphate and nitrogen

Nitrogen treatments	oP		1P			
	P_2O_5	N	P_2O_5	N	P_2O_5	N
Harlington medium loam						
Control	27	26	44	42	S.E. ± 5·2	± 9·6
Ammonium sulphate	77	219	92	210	C.V. 12·5%	7·7%
Ammonium chloride	76	202	91	220		
Ammonium nitrate	57	211	63·5	208		
Sodium nitrate	41	192	51	201		
Potassium nitrate	40	205	44	194		
Buxted silty clay loam						
Ammonium sulphate	21	114	54	199	S.E. ± 4·7	± 13·3
Ammonium chloride	21	122	41	150	C.V. ± 15·5%	± 10·2%
Ammonium nitrate	29	169	63·5	235		
Sodium nitrate	36·5	191	64	212		
Potassium nitrate	35	215	64	226		

B. Percentages of phosphate, % P_2O_5, mean of two replicates

Nitrogen treatments	1st cut		2nd cut		3rd cut	
	oP	1P	oP	1P	oP	1P
Harlington medium loam						
Control	1·28	1·26	2·04	2·31	2·44	3·04
Ammonium sulphate	1·16	1·24	1·48	1·55	1·24	1·39
Ammonium chloride	1·17	1·25	1·42	1·46	1·18	1·26
Ammonium nitrate	1·04	1·14	1·12	1·28	0·80	0·94
Sodium nitrate	0·95	1·04	0·96	1·16	0·80	0·81
Potassium nitrate	0·88	0·98	0·82	0·94	0·62	0·76
Buxted silty clay loam						
Control	1·16	1·24	—	—	—	—
Ammonium sulphate	0·67	1·19	0·75	1·18	0·48	0·76
Ammonium chloride	0·62	1·10	0·77	1·18	0·57	0·91
Ammonium nitrate	0·74	1·15	0·71	1·15	0·67	0·85
Sodium nitrate	0·74	1·18	0·80	1·22	0·85	1·28
Potassium nitrate	0·76	1·26	0·80	1·27	0·87	1·17

was a reversal of the order of nitrogen effect on phosphate uptake. Sodium and potassium nitrates induced higher uptake of phosphate than did ammonium sulphate and chloride. In the pots with added superphosphate, the uptake of phosphate was lowest where nitrogen was given as ammonium chloride; with all three nitrates almost equal quantities of phosphate were absorbed.

The uptake of phosphate by ryegrass was probably associated with the uptake of added nitrogen as seen in both control and superphosphate-treated Buxted soils. In the Harlington soil, the relation between absorptions of phosphate and nitrogen was not clearly illustrated as there was no significant difference between the total uptakes of nitrogen among nitrogen treatments.

In both soils, the application of nitrogen decreased the percentage of phosphate in ryegrass both in the oP and 1P tests. This is obviously due to the ' dilution ' effect. However, with the five nitrogen treatments, the more effective the nitrogen as shown by the uptake of phosphate, the higher were the percentages of P_2O_5 in the crop. The extremely high concentration of phosphate in the absence of nitrogen fertilizer particularly in the second and third cuts of grass for the Harlington soil was associated with the stunted growth of the grass as a result of extreme starvation of nitrogen.

(iii) *Relative efficiency of phosphate compounds containing varying amounts of ammonia contents*

In the third ryegrass experiment, the relative efficiency of some phosphate compounds containing ammonia was compared, the following substances being tested: diammonium phosphate, monoammonium phosphate, two ammoniated superphosphates: (A) 17·5% P_2O_5, 4·76% N and (B) 19·1% P_2O_5, 2·35% N, and superphosphate. Two levels of lime, oL and 1L (o and 1 g. per 300-g. pot), were included. The phosphates were applied at the rate of 0·3 g. of P_2O_5 per pot before sowing. Results of total uptakes of phosphate and nitrogen are shown in Table III.

Table III

Efficiency of phosphate compounds containing varying amounts of ammonia, in Buxted silty clay loam

(Mean values of total P_2O_5 and N in mg. per pot, for 3 cuts, 2 replicates)

Phosphate compounds	N, mg. in the fertilizer added	oL		1L	
		P_2O_5	N	P_2O_5	N
Diammonium phosphate	117	47·5	109	40	98
Monoammonium phosphate	59	42	73·5	46	73·5
Ammoniated superphosphate (A)	81	41·5	87	33	75
Ammoniated superphosphate (B)	37	33	57	37	71·5
Superphosphate	o	29	42	31	43

P_2O_5: S.E. ± 5·0 C.V. 18·9%
N: ± 7·8 15·3%

Data recorded in Table III show that the total uptake of both phosphate and nitrogen increases with the ammonium content in the phosphate compounds added. Addition of lime apparently increased the total uptake of both phosphate and nitrogen by ryegrass treated with superphosphate and ammoniated superphosphate of low ammonium content, but decreased the uptake by those receiving ammonium phosphate and ammoniated superphosphate of high ammonia content. The ammonia contained in the phosphate compound is therefore likely to show a beneficial effect on plant growth although the efficiency of a particular compound somewhat depends on the soil condition, such as lime status.

(iv) *Effect of different forms of nitrogen on the pH, available phosphate and phosphate-fixing capacity of soil*

The effect of nitrogen on the pH, available phosphate and phosphate-fixing capacity of the soil was examined in a similar manner to the second ryegrass experiment except that the soil was uncropped. Both Harlington and Buxted soils were treated four times at two-week intervals with various forms of nitrogen at the rate of 0·1 g. of N per pot. Soil samples were taken for test at the end of the experiment. Results are shown in Table IV.

Table IV

Effect of nitrogen on the pH, available phosphate and phosphate-fixing capacity of soil

Nitrogen treatments	pH		Available phosphate, p.p.m. P_2O_5 on dry soil		Phosphate-fixing capacity, %	
	oP	1P	oP	1P	oP	1P
			Harlington medium loam			
Control	6·4	6·0	44	95	76	68
Ammonium sulphate	5·8	5·6	38	89	78	78
Ammonium chloride	6·0	5·8	40	95	80	77
Ammonium nitrate	5·9	6·1	39	96	79	66
Sodium nitrate	6·6	6·4	46	98	70	66
Potassium nitrate	6·5	6·2	45	99	63	58
			Buxted silty clay loam			
Control	5·0	4·6	2	14	93	88
Ammonium sulphate	4·7	4·5	2	14	94	91
Ammonium chloride	4·6	4·5	2	13	94	92
Ammonium nitrate	4·7	4·6	2	14	94	89
Sodium nitrate	4·9	4·7	2	15	94	88
Potassium nitrate	4·8	4·6	2	15	93	86

In the Harlington soil, repeated addition of ammonium sulphate, chloride and nitrate reduced the pH by 0·4–0·6 unit and that of sodium and potassium nitrates increased it by 0·1–0·2 unit. In the superphosphate-treated soils the pH were generally lower than those of the corresponding controls. Ammonium sulphate and chloride decreased the pH by 0·2–0·4 unit but sodium and potassium nitrates increased it by 0·2–0·4 unit. In the Buxted soil, the addition of these nitrogen fertilizers reduced the soil pH, though only very slightly. In the pots with added superphosphate, none of the nitrogen treatments had a significant effect on soil pH.

After repeated application to the Harlington soil, ammonium sulphate, chloride and nitrate tended to reduce the available phosphate content, but sodium and potassium nitrates increased it. In the pots with added superphosphates, sodium and potassium nitrates caused somewhat higher recoveries of available phosphate but ammonium sulphate reduced it. No significant effect was shown by ammonium chloride and nitrate treatments. Since the Buxted soil was strongly acid and had a high phosphate-fixing capacity, none of the nitrogen fertilizers altered the native phosphate status, nor that of the superphosphate-treated soil.

Ammonium sulphate, chloride and nitrate increased the fixing capacity of the Harlington soil while sodium and potassium nitrates considerably reduced it. In the pots with added superphosphate the fixing capacity was generally lower than that in the corresponding controls (oP). In the Buxted soil, the nitrogen treatments did not affect the phosphate-fixing capacity to any great extent, although in presence of superphosphate, ammonium sulphate and chloride tended to increase slightly but potassium nitrate tended to reduce the fixing capacity.

(v) *Diffusion of phosphate ions through the root membrane as affected by acidity*

To investigate the relationship between the acidity of the medium and the uptake of phosphate, oat seedlings were grown in dilute phosphate solutions in concentrations analogous to those in the soil solutions. Seedlings which were grown together for 18 days in coarse sand were watered with a dilute nutrient solution* during the first 10 days after germination. The test seedlings were washed with water and their roots were subsequently immersed in water for 5 hours. The seedlings were then placed in four series of 50-ml. conical flasks (5 per flask) containing 50 ml. of 2·0 (total 100 μg.) or 5·0 p.p.m. P_2O_5 (total 250 μg.) at various pH, for 4 or 12 hours at 22°. After culturing, the volume of solution was noted and the pH and concentrations of phosphate were examined. Results of phosphate absorbed by oat seedlings by the time of sampling are shown in Fig. 1.

It was evident that oat seedlings removed from both 2 and 5 p.p.m. P_2O_5 solutions essentially the same amount of phosphate in 4 hours, irrespective of the pH. In 12 hours, oats absorbed a greater amount of phosphate from 5 p.p.m. than from 2 p.p.m. solutions. It was also clear that

* The concentrated solution contained 0·01M-KH_2PO_4, 0·05M-KNO_3 and 0·005M-$(NH_4)_2SO_4$, being diluted 50 times with water before use

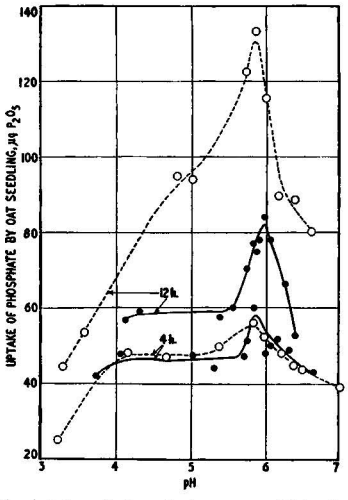

FIG. 1.—*Effect of pH on the diffusion of phosphate into oat roots*

---○--- initial 250 μg. P_2O_5
—●— initial 100 μg. P_2O_5

the intake of phosphate was rapid in the pH range 5·5–6·2, with maximum at pH 5·8–6·0, the rate of absorption sharply decreasing when the pH was above 6·2. On the strongly acidic side there was practically no difference in phosphate uptake between pH 4·5–5·5 for the 2 p.p.m. solution and between 4·5–5·0 for the 5 p.p.m. solution. In both cases the absorption of phosphate was greatly retarded below pH 4·0.

Discussion and conclusion

No definite conclusion can be drawn from the reports published during the past 25 years indicating that various nitrogenous fertilizers applied to the soil affect the utilization of phosphate by plants differently. Volk[7] observed that as compared with sodium nitrate, ammonium sulphate or urea, when used with rock and waste-pond phosphate, greatly increased the uptake of phosphate by sorghum and oats grown on unlimed and limed Cecil clay. Coleman[8] found that cotton and oats when treated with larger amounts of sodium nitrate gave a higher response to phosphate. Ensminger & Cope[9] reported that, on the unlimed plots receiving sodium nitrate, superphosphate gave higher increases in seed cotton yields than did those receiving ammonium sulphate. In comparing various nitrogenous fertilizers, Fudge[10] made the generalization that physiologically basic nitrogen fertilizers increased, but acid-forming nitrogen decreased, the availability of phosphate. Chapman,[11] however, considered the reverse to be true in calcareous soils.

The present ryegrass experiments indicate that the effect of various forms of nitrogen on the uptake of phosphate is closely associated with the existing soil conditions, such as pH, available phosphate content and phosphate-fixing capacity. In the slightly acid Harlington soil (pH 6·4), which had a moderately high available phosphate content and medium phosphate-fixing capacity, repeated use of physiologically acidic nitrogen (ammonium sulphate and chloride) more effectively increased the uptake of native and added phosphate than did the physiologically basic nitrogen (sodium and potassium nitrate). These fertilizers behaved in exactly the opposite manner in the very acid Buxted soil (pH 4·9), which had low available phosphate content and high phosphate-fixing capacity. The uptake of phosphate was not necessarily dependent on that of nitrogen. In the Buxted soil which was deficient in both phosphate and nitrogen, the response of phosphate was limited by the supply of nitrogen and utilization of these two elements appeared to be positively related. In the Harlington soil the uptake of nitrogen was almost entirely independent of their form, although the absorption of phosphate was considerably affected by the form of nitrogen used.

It has been reported that monoammonium phosphate was less efficient than other types of phosphate in promoting seed cotton yield.[9] Rhind & Tin[12] showed that the yield of rice significantly decreased after repeated use of ammonium phosphate. Ammoniated superphosphates, especially those containing more than 3% ammonia, have not always proved as effective as non-ammoniated superphosphate.[13] One of the ryegrass experiments shows that the ammonia contained in phosphate compounds is likely to give a favourable effect on the utilization of phosphate by plants, but its efficiency is influenced by liming. The decreased efficiency of phosphate compounds containing ammonia is probably not due to the direct effect of ammonia on the absorption of phosphate by plants, but to the fact that ammonia may interfere with the availability of phosphate by conversion of soluble phosphate to difficultly-soluble tricalcium phosphate in the case of ammoniated superphosphate,[14] or to the increased fixation of phosphate by soil as a result of residual acidity owing to nitrification of ammonia.[10]

Laboratory studies of soil show that repeated use of nitrogen tends to change not only the pH but also the phosphate status and phosphate-fixing capacity. The extent of the effect varies with the form of nitrogen as well as with the original pH, available phosphate and fixing capacity of soil. Addition of superphosphate consistently reduced the pH and phosphate-fixing capacity but increased acid-extractable phosphate content in the soils examined. Nitrogen treatments caused a more noticeable effect, on the Harlington soil, where sodium and potassium nitrate increased the pH and available phosphate but reduced the phosphate-fixing capacity, while ammonium sulphate and chloride reversed the reaction. Various nitrogen treatments which had no apparent effect on the pH, available phosphate and fixing capacity in the Buxted soil had a different effect on the phosphate uptake. It may be assumed that the effect of nitrogen on pH in the plant root–soil zone is so delicate that the change of pH and nature of phosphate may not necessarily be detectable in certain types of soil by the methods employed.

The present work leads to the belief that the pH of soil, which may be altered by repeated use of nitrogen, is an important factor affecting the utilization of phosphate. It is necessary to understand the effect of acidity on the diffusion of phosphate through root membranes. McGeorge[15] stated that the pH of plant sap usually lay between 5 and 6, at which $H_2PO_4^-$ was the dominant form, and plants preferred this form of phosphate for nutritional purposes. Apparently acidity is the sole factor controlling the nature of phosphate ion; thus $H_2PO_4^-$ ions are dominant below pH 5 and HPO_4^{2-} at pH 8–9. At pH 6·8 soluble phosphate is present as $H_2PO_4^-$ and HPO_4^{2-} in practically equal concentrations and proportions. Mattson *et al.*[16] demonstrated that the diffusion of phosphoric acid through a permeable membrane was a function of pH, the rate of diffusion through a cellophane membrane being $H_3PO_4 > H_2PO_4^- > HPO_4^{2-}$. A reduction of pH from neutral to slightly acid in soil would therefore facilitate the uptake of phosphate by plants, by shifting the proportion of phosphate ion from HPO_4^{2-} to $H_2PO_4^-$; an increase of pH from acid to neutral reversed the action. This change in the nature of phosphate ion is particularly critical in the range of pH 6–7.

The result of the solution culture experiment is in agreement with the work of McGeorge and of Mattson. Oat seedlings, when transferred to the culture solution, tend to absorb increased amounts of phosphate between pH 5·5–6·2. The absorption of phosphate declined sharply above pH 6·2 due to the alteration of the proportion of phosphate from $H_2PO_4^-$ to HPO_4^{2-}.[15] Although $H_2PO_4^-$ is dominant below pH 5, the intake of phosphate by oats in the strongly acidic solution is increasingly restricted possibly because of the effect of acidity in disturbing the normal physiological function of the root. In soil, it would seem that in the root zone where the soil solution is possibly saturated with carbon dioxide $H_2PO_4^-$ ions must be dominant and HPO_4^{2-}, if present in adjacent soil, should change to $H_2PO_4^-$ as they penetrate into the root–soil zone. Outside the root zone the state of phosphate ions will be entirely governed by the soil reaction. The optimum soil reaction for maximum absorption of phosphate by plants is apparently somewhere around pH 6.

The increased availability of phosphate to oat seedlings at pH 5·8–6·0 in cultural solution accords well with results of ryegrass and soil experiments. It explains why addition of physiologically basic nitrogen to the slightly acidic Harlington soil, although increasing the acid-extractable phosphate and reducing the fixing capacity, actually inefficiently promoted the intake of phosphate. From the relationship between nitrogen supply and phosphate uptake by

plants with reference to soil pH, it may be concluded that repeated use of physiologically acidic nitrogen on soils of pH above 6·2 favours the efficient utilization of phosphate, but the reverse is true with physiologically basic nitrogen. On soils below pH 5·5 these nitrogen fertilizers would behave in exactly the opposite way.

Agricultural Chemistry Dept.
Imperial College of Science and Technology
London, S.W.7

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TALL OIL: ISOLATION OF THE BRANCHED-CHAIN FATTY ACID (+)-14-METHYLHEXADECANOIC ACID AND OF THE STRAIGHT-CHAIN ACID PALMITIC ACID

By R. P. HANSEN and N. JUNE COOKE

Investigations on a sample of New Zealand tall oil produced in the manufacture of wood pulp from New Zealand-grown *Pinus radiata*, have shown it to contain trace quantities of the branched-chain fatty acid (+)-14-methylhexadecanoic acid. Palmitic acid has also been shown to be present. Branched-chain acids have not formerly been detected in tall oil, although their presence in wool grease,[1] milk fat,[2, 3] animal depot fats,[4, 5] shark-liver oil,[6, 7] the coccygeal glands of ducks and geese,[8] and the lipids of certain bacilli[9, 10] is well established.

Experimental

A sample (J/23, wt. 1757·0 g.) of crude acidified sodium soaps of tall oil, produced by the sulphate process, was fractionally distilled *in vacuo* in a 480 × 4·5 cm. Vigreux column. The fifth fraction distilled (denoted A6, wt. 33·19 g., sap. equiv. 297·5, I val. 59·0) was taken for this study. After removal of unsaponifiable matter, 29·46 g. of this fraction (sap. equiv. 269·9, I val. 37·8, m.p. 51·0–52·0°) were converted to methyl esters and crystallized from 40 vol. of acetone at −40° to give a soluble fraction A6L (wt. 14·56 g., m.p. −11·0 to −9·5°, I val. 68·6) and an insoluble fraction A6S (wt. 14·90 g., m.p. 28·5–29·5°). Fraction A6L, denoted A10, was crystallized from 40 vol. of light petroleum at −70° yielding a soluble fraction A10L (wt. 9·77 g., m.p. −45·0 to −23·0°) and an insoluble fraction A10S (wt. 4·45 g., m.p. 2·0–3·0°). A10L, when crystallized from 20 vol. of light petroleum at −70°, gave A10LL (wt. 9·07 g.) and A10LS (wt. 0·68 g., m.p. 1·5–2·0°). Fractions A10S and A10LS were combined, denoted A12

and fractionated in a semi-micro column. Three fractions and a residue resulted. The second and third of these fractions (A12L2, wt. 2·21 g., m.p. 3·5–4·0°, sap. equiv. 281·5, I val. 1·6: A12L3, wt. 1·33 g., m.p. 4·0–4·5°, sap. equiv. 282·8, I val. 8·7) were combined, denoted A13, and as acids, crystallized successively at −40° from 40 vol. of each of the following solvents: light petroleum, methanol and acetone. In each case the soluble fraction was removed and the insoluble fraction recrystallized to yield finally fraction A13S5S (wt. 2·20 g.) which had the chemical and physical properties shown in Table I compared with those of (+)-14-methylhexadecanoic acid.

Table I

Chemical and physical properties of fraction A13S5S compared with those of (+)-14-methylhexadecanoic acid

	Fraction A13S5S	(+)-14-methylhexadecanoic acid
Melting point	38·8–39·6°	36·8°,[1] 36·0–36·8°,[11] 36·0,[12] 39·5–40·1°,[13] 38·0°[14]
(Mixed m.p. with (+)-14-methylhexadecanoic acid, m.p. 36·6–37·2°, from ox fat)	37·2–38·0°	—
Sap. equiv.	269·9	270·4 (calc.)
I val.	0·0	0·0
Elemental analysis		
% C	75·5	75·5
% H	12·3	12·7
C-methyl value	9·86 (≡1·77 mole of acetic acid)	—
$[\alpha]_D$	+5·0° ($CHCl_3$, *c* 22·58, 17·2°)	+5·0° (acetone, *c* 20·04, 26°)[1]; +5·16° (acetone, 20°)[11]
X-ray long spacing	30·6 Å	29·9 Å[13]

Fraction A6S referred to above was denoted A9, crystallized from 40 vol. of acetone at −40° and then fractionated in column E[14] to give fractions A9S1 to A9S4 (and a residue) all four of which have the properties of methyl palmitate (Table II).

Table II

Fractionation of fraction A9

Fraction	Wt., g.	M.p.	Sap. equiv.
A9S1	1·67	29·0–30·0°	271·9
A9S2	2·86	29·0–30·5°	271·9
A9S3	5·05	30·2–30·5°	270·3
A9S4	2·45	29·2–29·8°	270·0

When combined and converted to acids these fractions constituted A16 (wt. 10·53 g.) with the following chemical and physical properties characteristic of palmitic acid: m.p. 62·7–63·0°; mixed m.p. with pure *n*-hexadecanoic acid (m.p. 63·0–63·3°), 63·0–63·3°; sap. equiv. 256·9; combustion analysis C 75·4%, H 12·9%, I val. 0·0; *X*-ray long spacing 35·0 Å.

Two fractions resulting from the original fractional distillation of this tall oil sample fraction 3 (denoted A8), and fraction 4 (denoted A7) when freed of unsaponifiable matter (A8, 27·48 g., sap. equiv. 254·9, I val. 15·1, m.p. 57·8–58·6°: A7, 20·11 g., sap. equiv. 257·1, I val. 7·2, m.p. 59·5–61·1°) were separately converted to methyl esters and after two crystallizations of each fraction from 40 vol. of acetone at −40° yielded fractions A8SS (21·10 g.) and A7SS (17·20 g.). These fractions were converted back to fatty acids which had the characteristics of palmitic acid. The values obtained for the methyl esters and the free acids are shown in Table III.

Melting points were determined in closed capillaries and are uncorrected. Combustion analyses were made by Dr. K. W. Zimmermann, Organic Microanalytical Laboratory, C.S.I.R.O., Melbourne, Australia. The *C*-methyl value was determined by Dr. A. D. Campbell, University of Otago, New Zealand. *X*-ray measurements were made with a Philips Geiger *X*-ray spectrometer using manganese-filtered Kα radiation. In the case of A13S5S, the material for *X*-ray analysis was prepared by evaporation from benzene, while with fractions A7SS, A8SS and A16, samples were prepared from the melt.

Table III

Characteristics of the methyl esters and acids rom fractions A7SS and A8SS

	Fraction A7SS	Fraction A8SS	Palmitic acid
Methyl ester			
M.p.	30·2–30·7°	30·0–30·4°	29·2–29·8°
(Mixed m.p. with methyl palmitate)	29·2–30·2°	29·2–30·0°	—
Free acid			
M.p.	62·7–63·7°	62·4–63·2°	63·0–63·3° (62·9°[15]; 62·4°[1])
(Mixed m.p. with pure palmitic acid)	63·0–63·2°	62·7–63·1°	
Sap. equiv.	256·1	255·6	256·4
I val.	0·0	0·0	0·0
Elemental analysis			
% C	75·3	75·1	75·0
% H	12·7	12·7	12·6
X-ray long spacing	35·4 Å	35·8 Å	35·6 Å[16]; 36·0 Å[15]

Discussion

The chemical and physical properties of fraction A13S5S identify it as the C_{17} *anteiso*-branched-chain saturated fatty acid (+)-14-methylhexadecanoic acid, while fractions A16, A8SS and A7SS are identified as palmitic acid.

Although the presence of palmitic acid has been reported in American tall oil by Anderson & Wheeler,[17] in Finnish tall oil by Hasselstrom,[18] and in Swedish tall oil by de Keghel,[19] Sandquist[20] and Dittmer[21] both stated that they could detect no palmitic acid in hydrogenated fatty acid fractions of Swedish tall oil. Burch *et al.*,[22] working on Canadian tall oil, were unable to obtain evidence for the presence of palmitic acid. They thought that the main saturated fatty acid constituent was stearic acid. It is noteworthy that Hasselstrom[23] subsequently stated that palmitic acid could be formed from oleic acid by ' alkali cooking '. Other saturated fatty acids whose presence in tall oil have been reported are formic, acetic, lauric, myristic and lignoceric acids. (See Nicholls.[24])

The fatty acid composition of New Zealand tall oil has not previously been investigated, nor has that of the lipid extract of locally grown *Pinus radiata*. Recent work has been carried out on the Finnish hardwoods, aspen and birch: by paper chromatography have been detected a number of odd and even *normal* saturated acids from formic to octanoic inclusive, together with the remaining even-numbered members of the series up to hexacosanoic acid.[25]

Although prior to this work branched-chain acids had not been isolated from a vegetable fat, the recent work of James & Martin,[26] using gas–liquid chromatography in association with the gas-density meter, suggests that traces of C_8, C_9, C_{11}, C_{12} and C_{13} branched-chain acids are present in olive oil.

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Fats Research Laboratory
Dept. of Scientific and Industrial Research
Wellington, N.Z.

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THE USE OF CHLORTETRACYCLINE (AUREOMYCIN*) TO RETARD THE SPOILAGE OF POULTRY CARCASSES

By D. H. SHRIMPTON

A preliminary study is made of the use of chlortetracycline under conditions approaching those used in the United States: the results are compared with those obtained when some of the features of present British practice were retained. The American results were confirmed but, as was expected, the application of chlortetracycline produced no worthwhile extension of the short storage life of eviscerated birds subsequently kept at ordinary temperatures. It was, in fact, evident that no advantages would be gained by the use of chlortetracycline in this country unless, as in America, refrigerated transport and distribution were also employed.

Introduction

In November 1955, the Food and Drug Administration of the United States, exercising its responsibility for the establishment of tolerances for pesticides in foodstuffs, permitted the presence of chlortetracycline in uncooked poultry. The Order[1] stated that ' a tolerance of 7 p.p.m. is established for residues of chlortetracycline in or on uncooked poultry and this tolerance level should not be exceeded in any part of the poultry '. The issue of the Order followed the demonstration by the American Cyanamid Co. after extensive field work[2, 3] that the storage life of eviscerated poultry could be lengthened by the use of chlortetracycline, and that 7 parts or less of the antibiotic per million parts of poultry flesh were completely destroyed by cooking. The extension of the storage life of eviscerated poultry held at 3° was 14 days,[3] the period at 3° without chlortetracycline being not more than 7 days.

In the standard process, termed ' Acronize ', the chlortetracycline in the form of Acronize PD is added to give an effective concentration of 10 p.p.m. of antibiotic in the tanks of slush ice which are used in American plants to cool rapidly the freshly-killed, eviscerated carcasses. After immersion for two hours, the carcasses are removed, left to drain and packed without any further washing. Acronize PD also contains[4] citric acid, salt and a small proportion of a wetting agent. The Acronize process is controlled by patent rights and administered by the American Cyanamid Co. under a system of franchise, whereby high standards of sanitation and refrigeration are ensured in the licensed plants; it is known that the effectiveness of the process is impaired when the bacterial contamination of the flesh is high.

* The trade-mark of the American Cyanamid Company for the antibiotic chlortetracycline is Aureomycin

In the United Kingdom, evisceration in poultry plants is often practised if the carcass is to be frozen, but dressed poultry going into direct consumption is usually distributed in the uneviscerated form and either eviscerated on retail sale, or after sale by the buyer. The reason for this is that refrigeration at all stages between plant and consumer is seldom applied to unfrozen carcasses in this country, and that under such conditions uneviscerated birds keep better than eviscerated ones. In the United States and Canada, where refrigeration is usually applied at all stages, there has been a steady increase over a number of years in the proportion of birds that are eviscerated in the plant before going into direct consumption. Another difference from American practice is that in this country, even when the freshly killed birds are artificially cooled in the plant, this is usually accomplished by cooling in cold air or by the use of contact-cooling racks or, occasionally, by immersion in water ;[5] whereas it is standard American practice to cool carcasses in slush ice.

This paper describes a preliminary study of the use of chlortetracycline under conditions approaching those used in the United States, and a comparison of these results with those obtained when some of the features of present British practice were retained. The length of storage life in the experiments described with eviscerated poultry was taken as the time required for the appearance of off-odours, mould or slime.

As a matter of interest, a smaller number of uneviscerated birds were submitted to similar treatments. The first sign of spoilage of uneviscerated carcasses stored without refrigeration is a green discoloration around the vent, which has been attributed by Pennington & Sherwood[6] to the action of hydrogen sulphide, produced by the intestinal microflora, on the haemoglobin in the skin.

Experimental

Origin of birds

The birds were one-year-old White Leghorns which had been hatched at the Houghton Poultry Research Station and reared there under known controlled conditions. Of the 140 birds used, 72 had been housed in individual cages in a laying battery and 48 in a deep litter house. Only water was supplied for the 24 hours prior to killing. At this time the average live weight was 4 lb. 9 oz. None of the birds received any treatment with drugs, such as sulphonamides, during the three months preceding slaughter. They were delivered alive to a nearby poultry packing station on the morning of the experiment and, as far as possible, each experimental group was similar, composed of birds from both the laying battery and the deep litter house.

Preparation of chlortetracycline 'dip'

An aqueous solution containing one part of powdered chlortetracycline per thousand was prepared immediately before use. Enough solution was stirred into the iced water (temperature about 6°) in the cooling tank to give a concentration of 10 p.p.m. of chlortetracycline calculated on the total volume of the contents of the tank.

Procedure at the poultry packing station

Eviscerated birds.—Ninety-two birds were weighed and then stunned, killed, bled and plucked. The birds were 'wet plucked' mechanically after they had been semi-scalded by dipping in water at 53·4° for one minute. Sixty-eight of these carcasses were hot-eviscerated by hand on stainless steel tables in a separate room. Lungs and kidneys were removed with a stainless steel claw and the body cavities were washed out with a spray of cold water. The 68 carcasses were then arranged in three groups and treated as follows:

Treatment (1) (ice–water–chlortetracycline). Twenty-two carcasses were immersed in 33 gal. of iced water containing chlortetracycline. Slush ice was then poured in until the surface was covered. After two hours (water temperature, 5°), the carcasses were transferred to aluminium trays to drain.

Treatment (2) (ice–water–no chlortetracycline). The twenty-four carcasses of this group were treated as in (1) but the chlorotetracycline was omitted.

Treatment (3) (water-chlortetracycline). Twenty-two carcasses were treated as in (1) without the addition of ice. The temperature of the water before the carcasses were immersed was 11·8° and after two hours was 12·6°.

Treatment (4) (contact cooling-no chlortetracycline). After plucking, the remaining 24 carcasses were placed, without evisceration, on aluminium-faced wooden racks, back down, with the vents pressed against a cold pipe.[5] Two hours later, when the fat had hardened, the carcasses were eviscerated, but not washed. This treatment is the one normally used in the packing station before carcasses are frozen.

Uneviscerated birds.—Forty-eight birds were weighed, stunned, killed, bled and plucked, and divided into four equal groups which, without evisceration, were treated in the same manner as the eviscerated carcasses; as fewer uneviscerated birds were used, the volumes of cooling water used were correspondingly reduced. The four treatments are denoted by 1A, 2A, 3A and 4A.

Storage.—Immediately after cooling, all the carcasses were brought to the laboratory by road (half an hour) on aluminium trays. Twelve carcasses from each of treatments 1–4 and six from each of treatments 1A–4A were placed on greaseproof paper on aluminium shelves in a constant-temperature room at 15°, the remainder being similarly stored in a constant temperature room at 1°.

All the carcasses were packed in cardboard boxes lined with greaseproof paper; up to six carcasses were placed in each box in two layers separated by greaseproof paper.

Examination of stored birds.—Every box was opened daily and each carcass inspected in the storage room, and examined for ' off ' odours, development of ' slime ' and the growth of moulds. In addition, the uneviscerated carcasses were examined for the development of ' greening '.

Results

Appearance of the carcasses at the packing station after cooling

All the carcasses which had been wet-cooled, with or without ice, had a particularly pleasant appearance. There was no staining of the carcasses by chlortetracycline.

Changes during storage

Eviscerated birds.—The periods of storage required for (*a*) a mild ' off ' odour, (*b*) a strong ' off ' odour, to become evident are recorded in Tables I and II. Because of the subjective nature of observations of this type, the two stages of deterioration have always been recorded. The behaviour of carcasses having the same history was so consistent and the differences between groups were so great, that no additional information could be obtained by statistical analysis.

The storage life of carcasses at 15° was short, irrespective of the treatment. Dry cooling (Treatment 4) gave a storage life of 3 days, whilst when chlortetracycline was present in the slush ice (Treatment 1), the carcasses kept for 4 days. After this time, moulds and yeasts grew throughout the body cavity until, after 7 days, there was an extensive growth protruding from the abdominal cavity. The storage life of the carcasses kept at 1° was never less than 12 days. With wet-cooling in ice-slush, the use of chlortetracycline increased the storage life from 12 to 21–24 days. As with carcasses stored at 15°, Treatment 4 was superior to Treatment 2.

Where carcasses had received Treatment 1, the storage life was terminated by the extensive growth of moulds within the body cavity during storage at 15° and along the back and shoulders at 1°. Of the carcasses which had received no treatment with chlortetracycline, only 3 (Treatment 4; storage at 1°) supported a growth of moulds and that only on the shoulders. In general, the storage life of these groups was finally terminated by the appearance of ' off ' odour and slime.

Uneviscerated carcasses.—The periods of storage at 15° before the appearance of (*a*) a mild ' off ' odour or (*b*) a strong ' off ' odour are recorded in Table III. As with eviscerated carcasses, the behaviour of carcasses having the same history was so consistent that no additional information can be obtained by statistical analysis. Quite unlike the eviscerated carcasses, there were no differences in the storage lives of carcasses which had received different cooling treatments. In all cases at 15° and irrespective of whether or not chlortetracycline was used, the period of storage to a mild ' off ' odour ranged from 5 to 8 days and at 1° from 18 to 21 days.

Table I

Storage of eviscerated poultry at 15°

Treatment No.	Method of cooling	No. of days before the development of ' off ' odours			
		Slight ' off ' odour	No. of carcasses	Strong ' off ' odour	No. of carcasses
1	Ice slush and chlortetracycline	3 4 *4·0* 5	4 4 4	7 8 *7·6*	5 7
2	Ice slush, no chlortetracycline	2 *2·0*	12	3 *3·0*	12
3	Water and chlortetracycline	2 *2·0*	12	5 *5·0*	12
4	Dry cooling on racks, no chlortetracycline	3 *3·0*	12	5 *5·0*	12

Table II

Storage of eviscerated poultry at 1°

Treatment No.	Method of cooling	No. of days before the development of ' off ' odours			
		Slight ' off ' odour	No. of carcasses	Strong ' off ' odour	No. of carcasses
1	Ice slush and chlortetracycline	21 24 *22·5*	5 5	24 26 *24·8*	6 4
2	Ice slush, no chlortetracycline	12 *12·0*	12	13 *13·0*	12
3	Water and chlortetracycline	17 18 *17·7*	3 7	21 24 *22·2*	6 4
4	Dry cooling on racks, no chlortetracycline	13 14 *13·7*	4 8	14 17 *16·0*	4 8

Table III

Storage of non-eviscerated poultry at 15°

Treatment No.	Method of cooling	No. of days before the development of ' off ' odours			
		Slight ' off ' odour	No. of carcasses	Strong ' off ' odour	No. of carcasses
1A	Ice slush and chlortetracycline	5 6 *5·8* 8	3 2 1	8 10 *9·7*	1 5
2A	Ice slush, no chlortetracycline	5 6 *5·7* 7	3 2 1	8 10 *8·3*	5 1
3A	Water and chlortetracycline	5 6 *5·7* 8	4 1 1	8 9 *8·5* 10	4 1 1
4A	Dry cooling racks, no chlortetracycline	5 6 *5·7* 8	4 1 1	8 10 *9·0*	3 3

On carcasses stored at 15°, the earliest sign of spoilage was a green discoloration on the muscle of the vent and on the skin adjacent to it, but not on the abdomen. The storage period before the vent appeared green, however, was variable and details are therefore given for each carcass in Table IV. Moulds finally grew, as they did on eviscerated carcasses, only on those which had been cooled in slush ice containing chlortetracycline (Treatment 1A).

Table IV

' Greening ' on non-eviscerated poultry stored at 15°

Treatment No.	1a	2a	3a	4a
Method of cooling	Ice slush and chlortetracycline	Ice slush, no chlortetracycline	Water and chlortetracycline	Dry cooling on racks, no chlortetracycline
No. of days before ' greening ' observed on vent	2 3 5 8	3 4 5	3 4 5	2 3
No. of carcasses	1 1 3 1	3 2 1	2 1 3	1 5
Period during which vent ' greened '	2–8 days	3–5 days	3–5 days	2–3 days

Discussion

The general problems arising from the use of antibiotics for preserving meat and fish have been recently reviewed[8] and need not be discussed here.

American results on poultry have been largely confirmed by the present work, but from the point of view of British practice the most significant result is the complete failure of chlortetracycline to extend the storage life of eviscerated poultry carcasses in the absence of refrigeration. When the carcasses were kept in refrigerated storage at 1°, however, chlortetracycline was an efficient preservative, the extension of storage up to three weeks being similar to American results.[3]

Although chlortetracycline did not postpone spoilage when the carcasses were stored at 15°, the type of spoilage was unusual. Ayres *et al.*[9] have reported that the predominant flora on eviscerated poultry, stored at 10°, at the time of sliming, is a bacterial one consisting of motile Gram-negative rods which appear as glistening circular colonies ranging from grey through white to cream or buff in colour. This characteristic spoilage pattern, which was present in those carcasses which had been dry-cooled as well as in those which had been wet-cooled, was entirely changed by the presence of chlortetracycline in the slush ice. The extensive growth, not only of yeasts (which have been reported[10] on chlortetracycline-treated carcasses stored at 2·2°) but also of moulds, was a striking demonstration of the rapid growth of a micro-flora resistant to chlortetracycline and, under traditional methods, suppressed by the growth of the typical bacterial spoilage flora. The presence of such an abundant growth of yeasts and moulds in the later stages of storage raises doubts as to whether there had also been a growth of some strain of bacteria which are known to be resistant to chlortetracycline and which, at 15°, could multiply, for example, the Salmonellas. This might be a hazard if attempts were made to use chlortetracycline in this country without subsequent refrigeration of the treated carcasses.

The spoilage of uneviscerated carcasses from off odours and from greening was not checked by the presence of chlortetracycline in the cooling medium.

Acknowledgments

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Low Temperature Research Station
University of Cambridge

and

Dept. of Scientific and Industrial Research
Downing St.
Cambridge

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ARSENIC IN CIGARETTE TOBACCO

By J. H. WEBER

Since 1939 routine determinations of arsenic have been made on a number of brands of cigarettes sold in the U.K. The results show that there was an irregular but fairly considerable increase up to 1953. This was, however, followed by a sharp decline, and present levels are well below the 1939 figures.

Introduction

Growers of agricultural and horticultural crops have made extensive use of arsenical dusts and sprays over the last sixty years as a protection against insect attack. It has therefore been found desirable to carry out analytical checks in this country on any contamination of tobacco which might arise from such treatment. Reports of isolated analyses of tobacco products for arsenic have appeared from time to time.[1–7]

Satterlee[8] has collated some of these results in an attempt to show the long-term trend between 1932 and 1951 but no systematic investigation made over a long period on adequate samples appears to have been reported hitherto.

Sampling

To obtain representative samples of tobacco is not easy since practical difficulties render it almost impossible to spread insecticides uniformly over growing leaves. Tobacco from different sources receives varying treatment in the field and complete mixing is difficult to achieve in the blending of different growths. Sampling, therefore, needs a good deal of care if the analytical results are to represent true average residues.

The results reported here are the annual averages of seven leading brands of cigarettes on sale in the U.K. Samples were purchased from retailers either weekly or fortnightly, usually at the rate of ten cigarettes of each brand per week. At the end of six months each brand was bulked and the tobacco separated, milled and very carefully mixed before a portion was withdrawn for analysis.

Experimental

The Gutzeit technique for determination of arsenic has an established use in the examination of drugs and foodstuffs, and has been found very suitable for the regular testing of tobacco. A modification of the B.P. method is applied using an unconstricted tube of 1 cm. bore to carry a mercuric bromide paper disc held in position by a telescoping black polythene holder. Aliquots of the usual acid digest from 2·5-g. samples are tested directly in the apparatus and stains matched. Good blanks are obtained and recoveries of known additions are usually very near to 100%.

Results

The arsenic contents of bulked samples of tobacco used for cigarette manufacture for the years 1939–56 varied from 7 to 51 p.p.m. of arsenic as As_2O_3. Fig. 1 shows the considerable variation in different years.

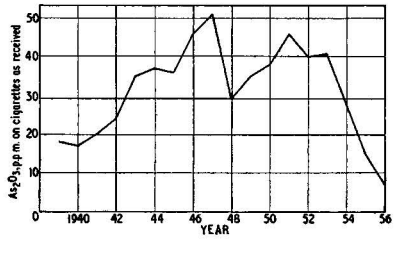

FIG. 1.—*Arsenic content of bulked tobacco samples for years 1939–1956*

Discussion

The curve in Fig. 1 shows an erratic but appreciable rise in insecticide residues from 1939 to 1953. This gave rise to considerable concern and since such residues were present almost solely on American and Canadian leaf, vigorous representations were made to the authorities concerned in an endeavour to reverse the trend. These, coupled with the availability of alternative insecticides, have brought about the pronounced decrease in arsenical residues on tobacco.

With the cessation of arsenical spraying there remains the possibility of uptake by roots from soils on which sprayed crops have been grown for a number of years.

If the trend of the above curve is maintained it will indicate that the effect is at most very small, in fact recent determinations show some samples to be free of arsenic. A few samples of unsprayed leaf tobacco have also been examined and although these were grown on tobacco lands which have almost certainly been sprayed with arsenic for many years the element could not be detected. Further support comes from the work of Jones & Hatch[9] on a number of other crops.

Acknowledgment

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Research Department
The Imperial Tobacco Co. (of Great Britain and Ireland), Ltd.
Bristol, 3

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CHEMICAL CHANGES OCCURRING IN COD MUSCLE DURING CHILL STORAGE AND THEIR POSSIBLE USE AS OBJECTIVE INDICES OF QUALITY*

By J. M. SHEWAN and N. R. JONES

Studies have been made on the extractives and volatile substances (amino-acids, amines and other nitrogenous compounds, sugars, etc.) of freshly caught cod muscle and of some of the changes that occur during autolysis (storage at 0° under sterile conditions) alone or combined with bacterial spoilage as in normal trade practice (storage in ice). The merits of some of these changes as objective indices of quality, particularly in relation to taste panel assessment, are discussed.

Introduction

We wish to give a short résumé of some aspects of work on the chemical changes occurring in the muscle extractives of white fish as the result of autolysis and/or bacterial spoilage during

* Read at meeting of London Section of Food Group, March, 1956

storage at 0° or in ice, and to examine how far any such changes might be used as objective indices of quality. We shall confine ourselves to cod, because it is commercially the most important single species of white fish and most of our work has been done with it.

Composition of newly caught cod

On the average, newly caught cod contains about 81% water, 15% protein,[1–5] 1·5% extractives,[5] 1·5% ash[4, 6] and 0·2% fat.[1–4, 6] The values for fat, protein and water are known to undergo slight seasonal variations,[2–4] but present evidence suggests that the total amount of extractives remains fairly constant, although individual constituents are subject to considerable fluctuations.

In cod muscle the extractives comprise a heterogeneous mixture of nitrogenous bases (lower amines and trimethylamine oxide, etc.), free amino-acids (taurine and glycine, etc.), simple peptides (anserine and glutathione),[7] purines and purine derivatives, and carbohydrates such as glycogen and glucose. Although relatively small in amount, these extractives are important in that many play an essential rôle in muscle metabolism (e.g. creatine) and several almost certainly form the main flavouring constituents in fresh fish, as do their breakdown products in the spoiling muscle. In fish generally, these extractives are present in greater amounts than in mammalian muscle, the latter containing about 900 mg./100 g.,[9–10] while in teleosts (cod) the value is about 1400 mg., in selachians (skate) 3000 mg. and crustacea (lobster) 3500 mg./100 g. respectively.[11]

The relative amounts of the various extractives in cod muscle are given in Fig. 1, which shows that the most important constituents are creatine (400 mg.-%), trimethylamine oxide (350 mg.-%), taurine (300 mg.-%) and anserine (150 mg.-%). The other free amino-acids account for a further 70 to 75 mg.-%, while the purines and purine derivatives probably contribute at least a further 200 mg.-%.

Calculations have shown that most of the extractive-nitrogen in cod muscle can now be accounted for (Fig. 2), as indeed can that of mammalian (rat), and selachian (skate) muscle, but about 20 to 25% of the lobster (crustacean) muscle extractives are still not identified.

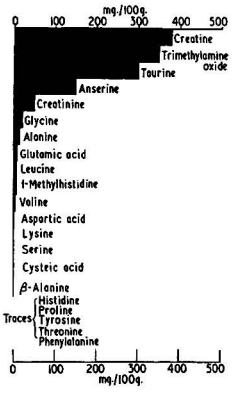

FIG. 1 (left).—*Nitrogenous extractives of cod muscle*

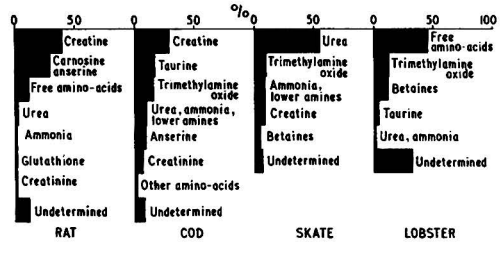

FIG. 2 (above).—*Comparison of nitrogenous extractives in rat, cod, skate and lobster*

Some of these nitrogenous constituents undergo variations due to season or other factors. Thus both lysine and taurine[12] vary markedly throughout the year, the former falling from a peak of 12 to 13 mg.-% in January to nil in April–May, and the latter rising from 140 mg.-% in January to peaks of 450 mg. in June–July.[12] Trimethylamine oxide also suffers seasonal variations with peaks in June–July and minimum amounts in September–October.[13] The reasons for these variations are not yet understood, but in the case of lysine, they are probably linked with the spawning cycle (North Sea cod spawn in January to February), and the mobilization of the muscle lysine for the formation of milt and roe.

In addition to the nitrogenous constituents, freshly killed muscle contains small amounts of glucose (9–35 mg.-%),[14] the quantity probably depending on the state of exhaustion of the fish when caught. Glycogen is also present, MacLeod & Simpson[15] finding 10 to 60 mg.-%.

Changes in the extractives due to autolysis and bacterial spoilage

In this work extensive use has been made of two-dimensional paper chromatography utilizing the chloroform-separated, 80% ethanol-soluble extractives after concentration by freeze-drying. For the autolysis studies, blocks of muscle were dissected out from freshly killed codling under aseptic conditions and incubated in dishes at 0°. Only muscle shown to be bacteriologically sterile by subculture into fluid and on to solid media, was extracted for subsequent chromatographic estimations. In no experiment has use been made of toluene, irradiation or other such means for suppressing or eliminating bacterial activity.

Autolytic changes

Serial chromatograms have shown that little change occurred in most of the free amino-acids during storage at 0°.[16] Individual acids varied somewhat in the extent of the change but the overall patterns were similar even in batches examined at and after the spawning period. Fig. 3*b* shows that, in sterile cod muscle, glycine remains unchanged while alanine and lysine both fall in amount, and glutamic acid increases by over 300%. The significance of these changes is at present unknown but are of considerable biochemical interest and further work on the subject is contemplated.

Of the major constituents (Fig. 4*b*) it is known that taurine is unaffected by autolysis,[16–18] and indirect evidence suggests that little or no change occurs in the amounts of either creatine + creatinine or trimethylamine oxide. Anserine on the other hand disappears under the action of the muscle anserinase,[18–20] giving rise to its constituent amino-acids, 1-methylhistidine and β-alanine. The nucleotides, nucleosides and other purine derivatives seem to undergo considerable changes during autolysis; ATP (adenosine triphosphate), for instance, is usually assumed to have disappeared when full rigor had been reached, i.e. before bacterial action has got properly under way.[21]

Of the carbohydrates, glucose appears to be present only in traces after about 5 days at 0°,[14] but the fate of the glycogen in sterile cod muscle has not been determined, but probably it has also disappeared by the 5th day. Ribose, present only in traces in the newly killed muscle, increases to about 4–5 mg.-% after 12 days and up to 6 to 7 mg.-% after 21 days.[14] Such increases are the result of the activity of the muscle ribosidases.

Bacterial spoilage

The changes that occur when cod muscle is allowed to spoil during storage in ice are different from those produced by autolysis alone. Here there are at least two factors to be considered, (1) leaching losses due to the ice melt water and (2) the transformation by both the bacterial and autolytic enzymes.

Fig. 4*a* shows that all the major nitrogenous constituents suffer losses,[12, 18, 22] (cf. Fig. 4*b*). It appears that the main losses in taurine are due to leaching and not to bacterial attack. Creatine also undergoes leaching, but is almost certainly attacked by bacteria as well, to give so far unknown degradation products although ammonia is almost certainly present. Further, trimethylamine oxide is readily attacked by several groups of marine bacteria giving rise to trimethylamine,[23–26] but again leaching losses also occur.

Anserine is broken down by the muscle anserinase, and possibly by bacterial action, although the amounts of 1-methylhistidine and β-alanine finally produced are less than expected —again probably as a result of leaching and/or utilization by certain bacteria.

In spoiling cod muscle the behaviour of the remaining free amino-acids (Fig. 3*a*) is quite distinct from that in the sterile autolysing muscle (Fig. 3*b*). Several show a slight fall, probably due to leaching, over the first few days (e.g. glycine, alanine and glutamic acid); but afterwards they all increase until about the 10th day after which they again fall or remain stationary. Lysine, on the other hand, increases steadily all the time, particularly over the first 10 to 12 days.

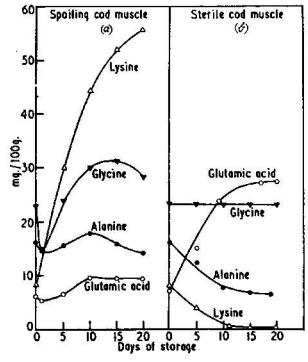

FIG. 3.—*Changes in free amino-acids in* (a) *spoiling,* (b) *autolysing cod muscle*

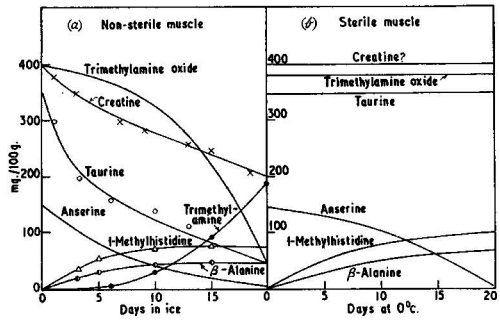

FIG. 4.—*Changes in nitrogenous extractives in* (a) *spoiling,* (b) *autolysing cod muscle*

The precise reasons for these changes are not known, but are obviously linked with bacteria activity. It is probable that the steady increase in lysine is caused by the splitting off of the terminal lysine of the fish protein by the proteolytic bacteria. Thus a comparison of the chromatograms of the free amino-acids of sterile muscle and muscle inoculated with a pure culture of a proteolytic pseudomonad present normally on fish, shows the large production of lysine, leucine, valine, aspartic acid etc. and disappearance of alanine. Similar results have recently been reported by Ranke using ling and coalfish undergoing normal bacterial spoilage at 2–4°.[23, 27, 28]

As regards the carbohydrates, glucose disappears by the 10th to 12th day and ribose rises steadily until the 10th day when it begins to decrease, probably as a result of leaching and bacterial action.[14]

The 10th–12th day seems to be a critical period in the dynamics of the changes occurring during storage in ice, and it may be significant that also at this point spoilage (as judged by the sensory data) becomes rapid and putrefaction rapidly ensues. In addition recent work has shown that a significant alteration in the character of the bacterial flora occurs at the same point.

To complete this general picture of the fate of the extractives during ice storage, a study has been made recently by one of us (N. R. J.) concerning the nucleotides, nucleosides and related compounds in cod muscle stored in ice, but there are no data available yet for sterile muscle. It has already been well established that in mammalian muscle, phosphorylated adenosine compounds undergo various changes post-mortem and that these are intimately related to the onset

and resolution of rigor mortis. These adenosine compounds are present in the muscle of a number of fish species, but apart from a study of dephosphorylation in fresh-water fish by Partmann and a demonstration of extensive ribosidase activity in some Canadian species by Tarr,[29, 30] little work has been done so far on this group of compounds. At Torry it was quickly established that the level of ribosidase activity in codling was much lower than that in the species studied by Tarr; but it was found that the enzymic removal of the phosphate and amino-nitrogen from adenine nucleotides takes place rapidly during the early period of storage, following the scheme given in Fig. 5. A number of these intermediates have now been separated and isolated by the extensive use of ion-exchange chromatography.

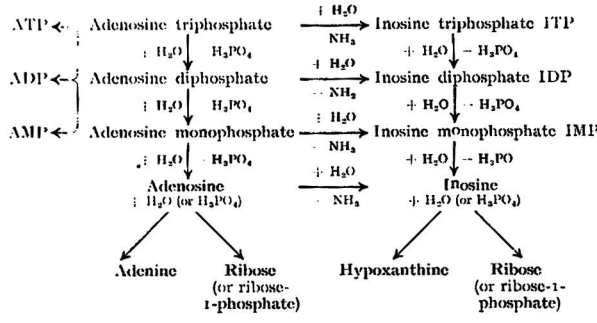

FIG. 5. - *Enzymic degradation of adenosine triphosphate* (ATP)

Phosphorylated compounds in this series (ATP, ADP, AMP, ITP, IDP and IMP in Fig. 5) are all precipitable by barium acetate at pH 8·2 in 80% ethanol. By carrying out ribose determinations on trichloroacetic acid extracts of muscle before and after barium precipitation under these conditions, one can estimate the extent to which dephosphorylation has occurred. The results given in Fig. 6 show that by the 5th day of storage in ice, almost all the ribose is present in the non-precipitable form. Of the ribose not precipitable by barium, 90 to 95% is in the form of inosine, which has been isolated in quantity from 10-day-stored fish. From this work, it will be seen that application of paper and ion-exchange chromatography has given a much clearer picture of the relative contribution of autolysis and bacterial activity to the spoilage of iced fish.

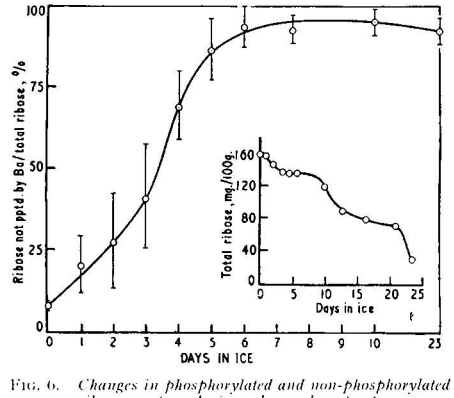

FIG. 6. *Changes in phosphorylated and non-phosphorylated ribose compounds in cod muscle extracts*

Objective indices of quality

In this connexion we are concerned only with the complex of factors such as appearance and odour of the raw fish and odour and flavour of the cooked fish, factors which affect the sensory judgments of the buyer or consumer, and which may be summed up by the term 'eating quality'. For this purpose it is absolutely fundamental that the sensory data be obtained by as reliable and accurate a method as possible. At the Torry Station, the best technique evolved consists of using a trained taste panel to assess the main organoleptic characters (appearance, odour and texture of the raw fish and odour, flavour and texture of the cooked fish), according to a points scale that has been developed over a period of years.[31] From a thorough statistical investigation, it is claimed that this technique possesses a fair degree of objectivity and is independent of personnel or time of assessment.[32]

By this method it has been found that, in the passage from absolute freshness to putridity during storage in ice, cod may be divided into at least four categories on the basis of general appearance and texture of the raw fish and into 9 or 10 categories on the basis of odour of the raw fish or odour or flavour of the cooked fish.[31] At Torry the taste panel usually decline to eat the steamed fish with a raw odour score of 4 or below, and hence the point of edibility could be defined by the description of the raw odour score at 4, i.e. 'some lower fatty acid (e.g. acetic or butyric acids) or grassy, slightly sweet fruity odours'.

In the work at Torry on iced codling, four broad quality groups can be discerned as the fish spoil from absolute freshness to putridity.[33]

These, with their corresponding raw odour scores, are as follows:

Group	Raw odour scores
Group I	10–7½
,, II	7–6
,, III	5½–4½
,, IV	< 4½

These groups correspond to stowage in sufficient ice for approximately 0–6, 7–10, 11–15 and more than 15 days, respectively. For the sake of argument these Groups might be looked upon as corresponding to trade categories for freshing (Group I), smoking (Group II), salting (Group III) and fish meal manufacture (Group IV), although the standards set may be much higher than those in operation commercially.

In theory, it should be possible to use each or all of the chemical changes for defining a quality index, but as has already been pointed out, before any particular chemical entity can be used for this purpose it should satisfy the following conditions:

(1) It should be absent or present in constant amount in the freshly caught fish.
(2) It should accumulate or disappear quickly and at a steady rate during spoilage, and
(3) It should be capable of being simply and speedily estimated.

An examination of all the chemical data suggests that possibly only the following entities satisfy these criteria:

(*a*) (Ratio of ribose not precipitable by barium to total ribose) × 100; (*b*) trimethylamine and total volatile bases, and (*c*) lysine, although the determination of the latter is somewhat lengthy. The correlation of chemical changes and sensory data is shown in Fig. 7.

Thus Group I, i.e. fish 0–6 days in ice and with raw odour scores of from 10 to 7½, should then have (*a*) not more than 80% of total ribose in non-barium precipitable form, (*b*) less than 1·2 mg.-% trimethylamine N, (*c*) lysine not more than 30 mg.-%.

Similarly the values for Group II, III and IV could be given.

Sufficient data has not yet been accumulated regarding the % of non-barium precipitable ribose/total ribose or the lysine values to examine statistically the correlation between them and the sensory data; but the ribose relationship at present looks promising, particularly for defining objectively qualities within Group I. Thus results obtained suggest that good quality frozen fish that is to be kept in cold store for any length of time, can only be produced if the original material has been stored in ice for not more than 3 days after catching. Such a quality could be defined as one whose % of non-barium precipitable ribose/total ribose was of the order of 50%

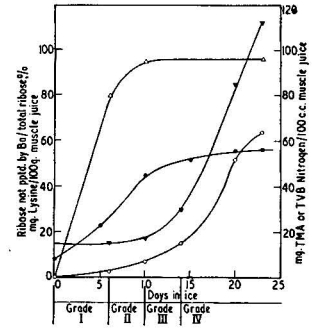

FIG. 7.—*Correlation between quality grading and some of the chemical changes occurring in cod muscle during storage in ice*

O—O—O trimethylamine (TMA)
▼—▼—▼ total volatile bases (TVB)
●—●—● lysine
Δ—Δ—Δ ribose not pptd. by Ba/total ribose

Raw odour scores:
Grade I 10 to 7½ Grade III 5½ to 4½
Grade II 7 to 6 Grade IV 4½

or less. It is just in this range, however, that sensory assessments are found to be difficult, e.g. to distinguish organoleptically a fish stored 3 days in ice from one stored 4 or even 5 days.

An extensive critical examination has been made[34, 35] on the relationship between sensory score and trimethylamine (TMA) and total volatile base (TVB) values for fish stored on ice for more than 5 or 6 days, taking into account size, sex and seasonal factors. From data collected over 3 years for North Sea cod, the following relationship has been evolved by statistical calculation.

$$\text{Flavour} + 0{\cdot}74 \log 10(1 + \text{TVB}) = 15{\cdot}9$$

which fits all the flavour and TVB batch means to within ± 1 unit on the flavour scale, most of the discrepancies, in fact, being less than $+ 0{\cdot}5$ unit.[35]

Similarly with raw odour and TMA an equation such as

$$+ 0{\cdot}47 \log 10(1 + \text{TMA}) = 10{\cdot}1$$

fits all raw odour and TMA batch means to within $\pm 1{\cdot}1$ unit.[35]

It is considered that the spread of values is due to the inherent differing rates of spoilage of the different catches. The reasons for the differing spoilage rates are still unknown but are almost certainly linked in part at least with differences in the types and numbers of bacteria present in each catch.

The above correlations might be sufficiently accurate for many trade purposes and at least have the advantage of being ' objective '. If a more accurate objective index is required for fish stowed in ice for more than 6 days, a more detailed study of the products of bacterial spoilage, particularly those giving rise to sensory (odour and/or flavour) reactions, will have to be undertaken. A study of the volatile matter would, it is considered, lead to most useful results in this connexion.

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Torry Research Station
Dept. of Scientific and Industrial Research
Aberdeen

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AN ACCELERATED STORAGE TEST FOR DEHYDRATED VEGETABLES

By E. G. B. GOODING and R. B. DUCKWORTH*

When dehydrated vegetables are stored at high temperatures (e.g. in the tropics) they eventually become unacceptable, in most cases because of the development of brown pigments. Testing for 'tropical' storage life is usually carried out at 37°, but preliminary tests on a number of different dehydrated vegetables suggested that the deterioration of culinary qualities (colour, flavour and texture) occurring in a certain number of days at 55° was approximately the same as that occurring in the same number of months at 37°. Further experiments on dehydrated potato have confirmed that there is a close correlation between the extent of browning developed under these two sets of conditions.

Introduction

Dehydrated vegetables are free from enzymic and bacterial spoilage but deteriorate slowly during storage as a result of various chemical reactions. Certain of these, for example those leading to alterations in texture and some flavour changes, are still obscure; others, such as oxidation of lipids, carotenoids or ascorbic acid are better understood and are prevented in practice by packing the foodstuff in an inert atmosphere. A third type of deterioration is the development of brown pigments resulting from reactions between reducing sugars and amino-acids. These reactions have a high temperature coefficient and the development of these pigments, with associated off-flavours, is usually the first cause of unacceptability in dehydrated vegetables stored in the tropics.

Although some control of the browning reactions can be obtained by appropriate manufacturing techniques, accurate predictions of storage life at high temperatures are virtually impossible. The possibility of carrying out accelerated storage tests by holding dehydrated vegetables at temperatures considerably higher than the usual 'tropical' test temperature (37°), appears to

* Now at the Department of Food Science, Royal College of Science and Technology, 1, Horselethill Rd., Glasgow, W.2

have been first suggested by Tomkins[1] who considered that the brown discoloration appearing at 37° and that which appeared, much more rapidly, at 55° or 70°, was similar in nature. More recently Legault *et al.*[2] showed that the curve relating browning and moisture content in dehydrated potato was similar in form at 120° F (49° C) and 100° F (38° C) but that the rate of browning was accelerated about ten-fold at the higher temperature. It did seem possible, therefore, that storage for a short time at some such elevated temperature might give an indication of the way in which dehydrated vegetables would behave during storage at the lower temperature of 37°.

Between 1950 and 1952 a series of storage experiments was carried out at Aberdeen on a number of dehydrated vegetables, including culinary tests in which marks were awarded by a taste panel for colour, flavour and texture to vegetables stored for various periods at 70°, 55° and 37°. On the basis of the results of these experiments, further tests were carried out on dehydrated potatoes produced during the 1953–54, 1954–55 and 1955–56 seasons, comparing the rate of development of brown discoloration during storage at 55° and 37°.

Experimental

The 1950 series of experiments

Material.—The dehydrated vegetables were either tender samples which had been sent by the commercial dehydration factories to the Ministry of Food, or samples of current production obtained directly from the factories. All tests were started within a few days of the arrival of the samples at the laboratory.

Sampling and storage.—For each test the contents of two 4-gallon cans (approximately 9 kg. in the case of root vegetables and 3·5 kg. in the case of leafy vegetables) were thoroughly mixed and repacked into A1 cans (type 211 × 400). Each A1 can contained approximately 70 g. of root vegetable or 28 g. of leafy vegetable. All cans except those containing potato were gas-packed with nitrogen.

Cans were stored at 70°, 55°, 37° and −5° and were withdrawn for examination at intervals of 12 hours, 24 hours and 14 days respectively for the first three treatments; control material from the −5° store was withdrawn every time a sample was taken from one of the other treatments. Each sample was examined for moisture content, sulphur dioxide content, and culinary properties.

Analytical and taste panel methods.—Moisture content was determined by heating the powdered material, ground to pass a 40-mesh sieve, for 5 hours at 70° and 1 mm. Hg pressure; the sulphur dioxide content was determined by a modified Monier-Williams method.

Culinary qualities were assessed by taste panels at which the samples were submitted to six or more tasters. Colour, flavour and texture were scored separately. There were eight specifically stated points under each heading, the eight points being arranged, so far as possible, for decreasing scores to indicate decrease in quality.

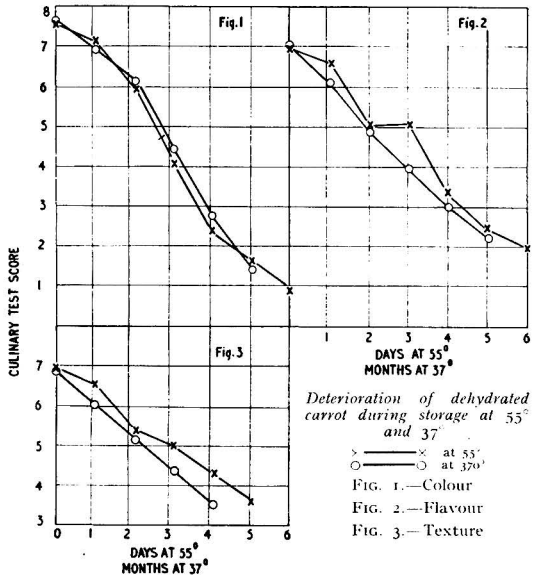
In preliminary experiments with the taste panel, identical samples were submitted on several occasions and the consistency of scoring by the members of the panel was statistically examined. Some members proved to be highly inconsistent and were replaced. By the time the actual storage tests started, the panel consisted of reasonably well-trained and consistent individuals.

Results

The deterioration of all the vegetables was extremely rapid when the temperature of storage was 70°, and severe browning took place within 12–24 hours; it was therefore possible to obtain only one or two points for material held at 70°, and plots could not be made illustrating the course of deterioration at this temperature.

For most vegetables the curves relating the scores obtained for culinary properties with the period of storage at 55° or 37° were similar in form, but deterioration in most cases at 55° appeared to be approximately 30 times more rapid than at 37°. This was more clearly shown when the data were plotted between the same ordinates, with the horizontal scale reading in days for the material stored at 55° and in months (28 days) for that stored at 37°. The behaviour of

dehydrated carrot was typical and is shown in Figs. 1–3. This relationship, however, did not hold for sulphur dioxide, the disappearance of which was proportionally more rapid at 55



Deterioration of dehydrated carrot during storage at 55° and 37°

× —— × at 55°
○ —— ○ at 37°

FIG. 1.—Colour
FIG. 2.—Flavour
FIG. 3.—Texture

The 1953–56 experiments

The results of the 1950 experiments so strongly suggested that storage behaviour of dehydrated vegetables at 55° might be useful as an approximate prediction of storage behaviour at 37°, that further experiments, on a more systematic basis in which attention was confined to the development of the brown discoloration, were started on dehydrated potato.[3]

Material.—During the 1953–54, 1954–55 and 1955–56 seasons, King Edward potatoes, grown at Tarland, Aberdeenshire, were used for an investigation into the connexion between post-harvest storage conditions and the development of brown discoloration during high-temperature storage of the dehydrated product.[4] Material showing a wide range of behaviour was obtained, and samples of this material were used in the present tests.

Assessment of browning.—The method hitherto employed in this laboratory for estimating browning had been to extract 5-g. duplicates of the ground sample with 100 ml. of 66% aqueous alcohol, either by blending for 5 minutes in a homogenizer or by leaving overnight, with occasional shaking, followed by centrifuging and filtering. The extinction value of the extract was then determined in a Unicam spectrophotometer at a wavelength of 400 mμ with 66% alcohol used as a blank. For examining large numbers of samples a more rapid method was required, and one employing direct visual comparison of the test sample with a range of colour standards was devised.

Visual assessment of browning in dehydrated potato strips is difficult because the strips do not brown uniformly along their lengths, and individual strips within a batch vary considerably in the extent of browning undergone during a given treatment. Powdered material is also unsuitable since light dispersion from fine particles largely obscures the true colour. To overcome these difficulties, colour standards and test samples were prepared by ' kibbling ' the potato strips by coarse grinding in a mill, the fragments held by a 10-mesh sieve being retained, though

pieces more than ¼ in. in length were discarded. This procedure gave samples of reasonably uniform appearance but in which the overall colour of the material was accurately presented.

It was felt that the moisture content of material prepared in this way might not be representative of the whole sample, i.e. that material of the lowest moisture content in the original strips might be differentially ground to a finer particle size and therefore lost to the final sample. This point was checked by carrying out moisture content determinations on the different fractions obtained during coarse grinding of a particular sample (during the course of the 1954–55 tests). The results showed a range from 8·19–8·63% compared with a value of 8·37% for a similar sample ground directly to the particle size required for moisture determination (passing a 40-mesh sieve).

Kibbled material prepared in the manner described above was packed in nitrogen in 1-lb. Kilner jars, and held at 55° for different periods to induce a range of brown discoloration. An arbitrary selection of eight degrees of browning was made, standard '8' being entirely free of browning (viz., material which had not been held at 55°) and standard '1' very severely browned; when not in use, the standards were kept in the dark at −5°.

Sampling and storage.—Samples of each type of material under test were kibbled and packed in Kilner jars in the same way as the standards, and held at 55° and 37°. The material from the 55° store was examined at daily intervals, and that from the 37° store at monthly (28-day) intervals. In each case the samples were compared with the standards, by three independent observers, and scored to the nearest half-mark.

Comparison of visual assessment of browning with other methods of evaluation

Duplicates of some of the samples examined in this way were also subjected to culinary tests and to estimations of browning by measurement of the optical density of the 66% alcohol extract.

In Fig. 4 the scores obtained by visual assessment are plotted against the marks awarded to the samples by the taste panel. It is clear that the relationship is a close one (the correlation coefficient $r = +0{\cdot}94$).

The scores obtained by visual comparisons of a large number of samples against the standards are plotted against the extinction values obtained for the same samples in Fig. 5. Although there is some scatter, it is again evident that the relationship between these two methods of assessment of browning is close.

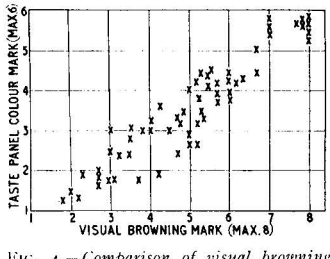


FIG. 4.—*Comparison of visual browning marks with taste panel colour marks (dehydrated potato)*

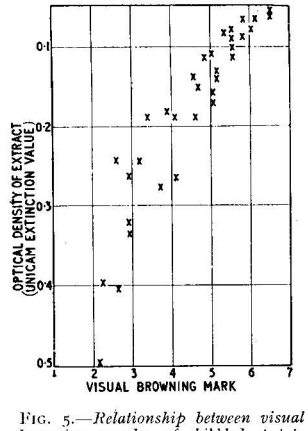


FIG. 5.—*Relationship between visual browning marks of kibbled potato strips and optical density of 66% ethanol extract*

Comparison of colour developed after storage at 55° and 37°

(1953–54 *series*)

From the 1950 experiments, as has already been stated, it appeared that the browning developed during each day when the vegetable was held at 55° would be approximately equal to that developed when the vegetable was held for the same number of months at 37°. In the present experiments, therefore, the browning marks for material stored for 2, 3, 4, 5 and 6 days at 55° were plotted on the horizontal axis (x) against marks given to a similar sample after the corresponding period, viz., 2, 3, 4, 5 and 6 months respectively, at 37° (y) (Fig. 6). There is a certain amount of scatter, but it is quite clear that the relationship is on the whole close. Calculation of the regression line gave $y = 1{\cdot}028x - 0{\cdot}041$, and correlation coefficient $r = +0{\cdot}91$.

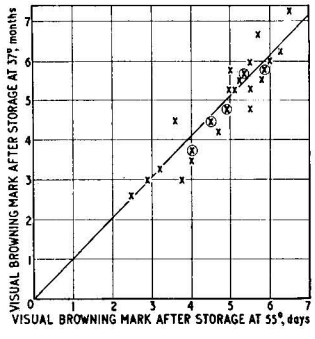


FIG. 6. *Regression of visual browning mark after months at 37° upon visual browning mark after the same number of days at 55° (1953–54). Dehydrated potato strips*

⊗ samples with low moisture content: 4·99%

(1954–55 *series*)

A new set of standards was prepared for the 1954–55 experiments, a slightly smaller size of portion being adopted in order to obtain an even more uniform sample: the new size of particle was that passing a 6-mesh sieve and retained by a 16-mesh sieve. Visual marking of material stored at 55° and 37° was carried out in the same manner as for the 1953–54 experiments, except that the number of standards was reduced to seven, designated 6–0. In Fig. 7 a comparison of the average day-to-day scores for duplicate samples stored at 55° is made with average scores for the corresponding months at 37°, and once again it is seen that the correspondence is satisfactorily close, although the scatter is somewhat wider than previously. The regression equation is $y = 0{\cdot}989x - 0{\cdot}013$ and the correlation coefficient $r = +0{\cdot}81$.

(1955–56 *series*)

The same technique was used in this series as for the 1954–55 experiments. An intervening test had, however, indicated that variations in visual browning mark between replicate samples during storage at 55° were related to small differences in temperature in the different parts of the oven occupied by the samples. Storage at 55° in this latest test was therefore carried out in a different oven in which more accurate control of temperature was possible. The results, shown in Fig. 8, again indicate a close relationship between the rates of browning at the two temperatures. The regression equation is $y = 1{\cdot}21x - 0{\cdot}88$ and correlation coefficient $+0{\cdot}933$.

Possible factors affecting relationship between colour developed at 55° and 37°

Although these experiments appear to have established a fairly close relationship between the rates of browning at 55° and 37°, it seemed possible that factors which influence browning at the latter temperature might not necessarily exert a proportional influence at the former. Important factors are the moisture, sulphur dioxide and reducing sugar contents of the vegetable, and the conditions of scalding before dehydration.

Moisture content.—In the majority of samples, the moisture content lay between 6·9 and 7·2%. One sample, however, had an exceptionally low moisture content, 4·99%. The points

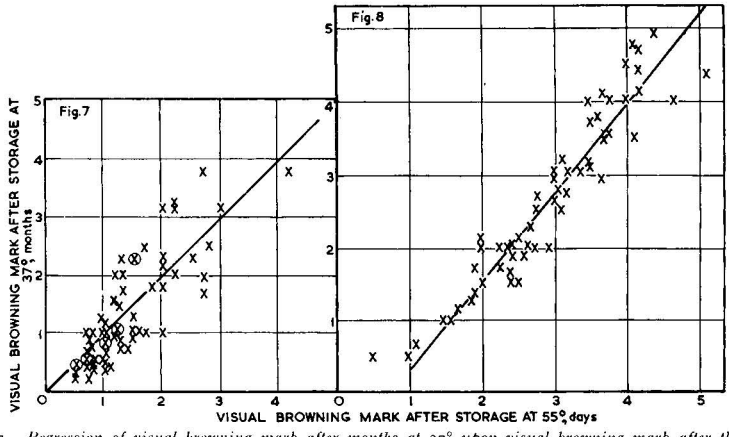


FIG. 7. *Regression of visual browning mark after months at 37° upon visual browning mark after the same number of days at 55° (1954–55). Dehydrated potato strips*

⊗ samples with low sulphur dioxide content: 195 p.p.m.

FIG. 8. *Regression of visual browning mark after months at 37° upon visual browning mark after the same number of days at 55° (1955–56). Dehydrated potato strips*

for this sample have been ringed in Fig. 6; these lie close to the regression line and there is nothing to indicate that the low moisture content has altered the general relationship.

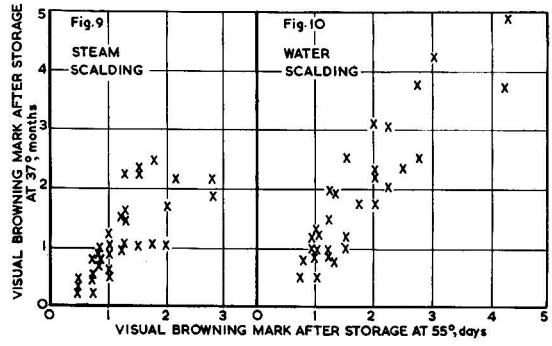
Sulphur dioxide content.—With one exception the sulphur dioxide content of the samples lay between 300 and 450 p.p.m. The exception had only 195 p.p.m., and the figures for this sample are ringed in Fig. 7. Again there is no evidence of any divergence from the general trend.

Reducing sugar content.—The reducing sugar content of the raw potatoes dehydrated during 1953–54 and 1954–55 covered the range 1·85–4·25%, but fell into two groups corresponding to the two seasons' productions; in 1953–54 all samples had relatively low reducing sugar contents, from 1·85 to 2·48%, but in 1954–55 the range was from 2·67 to 4·25%. It has already been shown (Figs. 6 and 7) that there was little difference in the relationship between browning at 55° and at 37° for the samples from these two seasons, and it seems clear, therefore, that the reducing sugar content does not affect the results.

Scalding treatment.—Water scalding alone was used in the 1953–54 experiments. Both water and steam scalding were used in 1954–55, and, in 1955–56, all the material was steam-scalded. The scores for each treatment were examined separately for the 1954–55 series, and the results (Figs. 9 and 10) show no appreciable difference in behaviour.

Conclusions and discussion

Results of these experiments indicate that there is a close relationship between the rate of browning that takes place in dehydrated potato stored at 55° and at 37°, and that for all practical purposes the rate in the former case is approximately 28 times greater than in the latter. This relationship appears to be independent of other factors which influence the rate of discoloration, and it therefore appears possible to predict with fair accuracy, from the results of a short-term test at 55°, the storage behaviour of any particular batch of dehydrated potatoes subjected to 'tropical storage conditions' at 37°. Such a test should be valuable not only in the laboratory, where investigation of the effects of different factors on the rate of browning would be greatly accelerated, but also as a means for ascertaining the suitability of consignments of dehydrated potato for storage and use in tropical regions.



FIGS. 9 *and* 10.—*Relationship between browning after storage at* 37° *and* 55°. *Dehydrated potato strips.* FIG. 9.—Steam scalding. FIG. 10.—Water scalding

It must be emphasized that careful control of temperature at 55° is extremely important: owing to the high temperature coefficient of the browning reactions small variations of temperature from the 55° level can cause considerable differences in the rate of discoloration.

The early experiments (1950–52) indicated that the type of relationship demonstrated in potato between browning at 55° and 37° probably holds good for other vegetables also, but no precise comparisons have yet been made.

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Ministry of Agriculture, Fisheries and Food
Research Establishment
The Experimental Factory
Greyhope Road, Aberdeen

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

AUGUST, 1957

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

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

AUGUST, 1957

I.—AGRICULTURE AND HORTICULTURE

General : Soils and Fertilizers

Missouri Agricultural Experimental Station, Annual Report, 1956. J. H. Longwell (*Missouri agric. Exp. Sta.*, 1956, Bull. 676, 124 pp.).—The annual Report of the Station for the year ending 30 June, 1956. A. H. CORNFIELD.

Chemical soil maps of Roumania. D. Davidescu (*Agrochimica*, 1956, **1**, 72-80).—Maps presented show the distribution of total N and P, HCl-sol. K, pH, org. matter and degree of saturation with bases in relation to production in Roumanian soils. A. G. POLLARD.

Physico-chemical characteristics of soils in the middle Ebro valley. P. Mela (*Agrochimica*, 1956, **1**, 81—90).—These soils, from arid areas, are characterized by high $CaCO_3$, Mg and K contents and deficiencies of org. matter and P. The agronomic significance of SiO_2/sesquioxide, C/N and base ratios is discussed. A. G. POLLARD.

Micropedology of some red soils from Cyprus. D. A. Osmond and I. Stephen (*J. Soil Sci.*, 1957, **8**, 19—26).—The micropedology and mineralogy of terra rossas and rotlehms associated with calcareous sediments and igneous rock material respectively are described. A. H. CORNFIELD.

Genesis of three soils derived from Wisconsin till in New Jersey. R. D. Krebs and J. C. F. Tedrow (*Soil Sci.*, 1957, **83**, 207—218).—Mechanical analyses, mineralogy and chemical analyses are reported for three profiles in the area. The formation of the soils is discussed in the light of these results. T. G. MORRIS.

Anisotropic hydraulic conductivity of soil. E. C. Childs (*J. Soil Sci.*, 1957, **8**, 42—47).—Mathematical. A. H. CORNFIELD.

Permeability measurements in the field as an assessment of anisotropy and structure development. E. C. Childs, N. Collis-George and J. W. Holmes (*J. Soil Sci.*, 1957, **8**, 27—41).—Very wide ranges in permeability occurred at 13 sites representing varying soil texture and structures. Anisotropy occurred in about half the soils. The use of permeability as a measure of structure in the field is discussed and variability in field structure is also considered. A. H. CORNFIELD.

Loss of nutrients in vineyard soil profiles by seepage. E. Knickmann (*Mitt. Wein -u. Obstbau, Wien*, 1957, **7A**, 15—21).—Long-term measurements show no excess of soil moisture during rainy summers, but over-saturation with consequent loss of nutrients by seepage into the subsoil may occur during rainy winters. (11 references.) P. S. ARUP.

Hydraulic tests of erosion-control structures ; sliced-inlet type entrance. R. P. Beasley and L. D. Meyer (*Missouri agric. Exp. Sta.*, 1956, Res. Bull. 599, 8 pp.).—Scale-model tube structures with sliced-inlet type of entrance for control of overfall erosion were tested for hydraulic characteristics and results compared with other types of entrances. A. H. CORNFIELD.

Soils and methods used in irrigation experiments at Geneva, N.Y. M. T. Vittum and N. H. Peck (*Cornell agric. Exp. Sta.*, 1956, Bull. 775, 56 pp.).—Soil moisture release curves (pressure membrane technique) indicated that water tension was ~2 atm. at 50% depletion. With sunflowers in pot tests 50% of the available water was held at <0·5 atm. and 75% at <1 atm. tension. In surface soils the wilting % (sunflower technique) was less than the % of water held at 15 atm. tension whilst the field capacity was greater than the moisture equivalent. Tests with the Bouyoucos resistance blocks for determining available water are described. A. H. CORNFIELD.

Moisture relationships and irrigation of vegetables. V. N. Lambeth (*Missouri agric. Exp. Sta.*, 1956, Bull. 605, 55 pp.).—An extensive paper dealing with the evaluation of irrigation treatments based on preceding rainfall, evaluation of soil moisture reservoir when irrigating deep-rooted crops, optimum range of soil moisture for Irish potatoes, sweet potatoes, and tomatoes, and response of deep-rooted crops to depth of wetting. A. H. CORNFIELD.

Effect of initial water content on stability of soil aggregates in water. C. R. Panaookke and J. P. Quirk (*Soil Sci.*, 1957, **83**, 185—195).—The stability of 5—10 mm. aggregates from a loam soil in both virgin and cultivated states was examined by wetting to various water contents by equilibration at pF values ranging from 1 to 6·2. After equilibrium had been reached the macro- (>0·25 mm.) and micro-aggregates were examined. The stability of the aggregates under a vac. was also studied. In the virgin soil the % of water-stable aggregates >5 mm. was unchanged at pF values of 0—3, and then decreased at higher pF. In the cultivated soil the % increased with pF values of 0—2, and then fell sharply at pF 3 and higher. Three clays were most stable when wetted initially to pF 1—3, and then broke down easily with increasing initial pF. The cohesion of natural aggregates 1 sq. cm. in cross section was greater in the virgin loam than in the cultivated. The most stable soil was the one in which there was the most swelling on wetting. The stability of the micro-aggregates of the virgin loam exceeded that in the cultivated plots irrespective of initial pF level. The micro-aggregates of the cultivated loam were most stable at an initial pF level of 2 to 3. T. G. MORRIS.

Thermal investigation of soil clays. R. C. Mackensie (*Agrochimica*, 1956, **1**, 1—22).—A review of methods and the interpretation of thermal analyses. Thermal, optical, chemical, X-ray, electron and i.r. methods afford complementary information. A. G. POLLARD.

Clay minerals and organic matter in Mediterranean soils. I. Sicily. A. Malquori and S. Cecconi (*Agrochimica*, 1956, **1**, 35—43).—In the black earth soils examined montmorillonite and, less frequently, illite are the dominant clay minerals. Humic matter is adsorbed at the surface of the clay particles through the agency of Ca^{2+} and is mainly responsible for the soil colour. A. G. POLLARD.

Basaltic soils of Northern Ireland. I. Cation-exchange properties. S. McConaghy and D. M. McAleese. **II. Contributions from the sand, silt and clay separates to cation-exchange properties.** D. M. McAleese and S. McConaghy (*J. Soil Sci.*, 1957, **8**, 127—134, 135—140).—I. Studies on nine basalt-derived horizons representing poorly-drained, freely-drained and mottled lower-horizon soils are reported. In all three types clay and org. matter contents decreased considerably with depth, but the cation-exchange capacity (CEC) did not follow changes in clay and org. matter content. The CEC in the C or G_{2-3} horizon was sometimes approx. equal to that in the surface horizon. In freely-drained profiles the CEC was usually lower in the B than in the A(S) or C horizon, whilst in poorly-drained profiles the CEC was usually lowest in the B, G_1 or A_2-G horizons, due mainly to fall in org. matter. Mg constituted up to 40% of the exchangeable cations present in the C (or the G_2) horizons in these soils, and was particularly high in poorly-drained soils.

II. The CEC (after destruction of org. matter) of the separates of basaltic soils was for clay (<2μ) 45—62 mequiv., silt (2—20μ.) 14—59 mequiv., fine sand (20–200μ.) 16—31, and coarse sand (>200μ.) 11—21 mequiv. per 100 g. The CEC of the clay fraction was not related to depth, whilst that of the other fractions tended to increase with depth. The CEC of the fractions was not related to drainage conditions. Part of the CEC of the coarser fractions may have been due to contamination by clay. A. H. CORNFIELD.

Penetration of phosphates through the soil profile. E. Alinari and C. A. Cecconi (*Agrochimica*, 1956, **1**, 56—71).—The downward movement of PO_4''' following surface application to soil of superphosphate is examined in columns of soil. In some cases 51—65% of the amount of P added was found at a depth of 6 cm. A. G. POLLARD.

Reaction between phosphate and phosphate-fixing soils. J. P. Leaver and E. W. Russell (*J. Soil Sci.*, 1957, **8**, 113—126).—A study of the effect of 12 blocking agents for reducing PO_4^{3-} sorption by soils showed that at pH 3·5 with a tropical red earth (latosol) aluminon had the greatest effect followed by alizarin S, whilst other reagents were relatively ineffective. At pH 5·5 there was a greater range of effectiveness, with cupferron, alizarin S and aluminon being the most effective, and $Fe(CN)^{4-}$ and H_2SeO_3 having little action. At pH 5·5 $Fe(CN)_6^{4-}$ reduced sorption of PO_4^{3-} by a loam high in free Fe oxides, but had little effect on PO_4^{3-} sorption by a loam high in Al oxides or by halloysite. $Fe(CN)_6^{4-}$ was a more effective blocking agent than was oxine for reducing PO_4^{3-} sorption by hydrated Fe and Al oxides. Fulvic acid (extracted from soil) was an effective blocking agent at pH 3·5 and 5·5 for soils and for Fe and Al oxides. Effective blocking agents were also those which caused soils to deflocculate in 0·1N-NaOAc. A. H. CORNFIELD.

Phosphate status of Irish soils with particular reference to farming systems. P. M. McDonnell and T. Walsh (*J. Soil Sci.*, 1957, **8**, 97—112).—There was appreciable accumulation of P in the surface 3 in. of permanent pasture, irrespective of whether P was added in org. or inorg. form. In a highly productive permanent pasture satisfactory mineralization of org. P occurred. Adsorbed P constituted a relatively small portion of the inorg. P present. Morgan's reagent was a good indicator of " continual-release " P. Differences in types of P present in soils in a hydrologic sequence were related particularly to degree of drainage. The main difference was found in the org. P level and was associated with the fine-clay fraction. Results are discussed in relation to farming systems and the P cycle. A. H. CORNFIELD.

Isotopically exchangeable phosphorus in soils. II. Factors influencing the estimation of " labile " phosphorus. O. Talibudeen (*J. Soil Sci.*, 1957, **8**, 86—96).—Methods of determining isotopically exchangeable P in soils are discussed. The influence of soil : solution ratio, PO_4^{3-} added with $^{32}PO_4$, equilibrium pH, and the removal of increasing amounts of PO_4^{3-} from the soil were studied. Soil P is subdivided into four fractions on the basis of their abilities to exchange PO_4^{3-} with the PO_4^{3-} in the soil solution. The relation between the labile pool of soil PO_4^{3-} and this scheme is discussed. A. H. CORNFIELD.

Potassium fixation in East Pakistan soils under different conditions. A. Q. M. B. Karim and M. A. Malek (*Soil Sci.*, 1957, **83**, 229—238).—Soil samples were treated in flasks with various K salts and left for different times, after which water-sol. and exchangeable K were determined. In all soils fixation of K increased with time. In 60 days all soils had fixed >50% of the K added. Of the forms of K tested, K_2CO_3 showed the highest fixation ; that from K_2SO_4 exceeded that from KCl or KNO_3. Soils with high levels of exchangeable Ca fixed most K from K_2CO_3 and K_2SO_4. Fixation increased with the amount of K or lime supplied. Superphosphate increased and $(NH_4)_2SO_4$ decreased fixation. Fixation decreased with falling acidity and increased with moisture content, being max. at the max. water-holding capacity. Removal of the org. matter resulted in greater fixation. T. G. MORRIS.

Potassium-supplying power of several Western Oregon soils. A. Pope and H. B. Cheney (*Proc. Soil Sci. Soc. Amer.*, 1957, **21**, 75—79).—Soils (20) representing seven soil series varied widely in their power of supplying K to 10 successive cuttings of ladino clover in pot tests. The total K uptake was very highly correlated with total K extracted by boiling the soils with N-HNO_3 and also with K extracted from non-exchangeable forms by boiling with N-HNO_3. The total K uptake was also highly correlated with the exchangeable K content of the soil before cropping. There was poor correlation between the K uptake from non-exchangeable forms in soil during cropping and the exchangeable K content of soil before cropping. A. H. CORNFIELD.

Potassium release characteristics of several soils from Ohio and New York. W. L. Garman (*Proc. Soil Sci. Soc. Amer.*, 1957, **21**, 52—58).—Potassium taken up from surface and subsoils by five successive crops was most highly correlated with K released by continuous leaching of soil with 0·01N-HCl, although very high correlations were also obtained with exchangeable K, Morgan-extractable K, and hot N-HNO_3-sol. K. The Great Soil types gave characteristic release curves of K when leached with 0·01N-HCl. K release from both surface and subsoils decreased in the order brown forest, grey-brown podsolic, brown podsolic, and podsolic. A. H. CORNFIELD.

Soil magnesium and the growth and chemical composition of plants. E. R. Graham, S. Powell and M. Carter (*Missouri agric. Exp. Sta.*, 1956, Res. Bull. 607, 20 pp.).—A low. concn. of Mg (0·5% Mg saturation of the exchange complex) as Mg ethylenediaminetetra-acetate (**I**) was as effective for increasing growth of soya-beans and uptake of Mg in sand-clay cultures as were high concn. of $MgCO_3$ (5—10% Mg saturation). A high concn. of **I** (5% Mg saturation) did not reduce the growth of soya-beans in all of the 15 soils studied. A. H. CORNFIELD.

Soil population studies. I. Effects of cultivation and treatment with insecticides. J. G. Sheals (*Bull. ent. Res.*, 1956, **47**, 803—822).—Cultivation of grassland markedly lowered the population of micro-arthropods in the soil and little change occurred during subsequent fallowing. Resowing with grass was followed by recolonization with Collembola. Application of BHC lowered the populations of most species of arthropods. DDT destroyed many mites but with subsequent increase of Collembola. Temporary effects of the two insecticides applied simultaneously and residual effects after 17 months are recorded. A. G. POLLARD.

Non-symbiotic nitrogen-fixing bacteria in soil. III. Total nitrogen changes in a field soil. C. A. Parker (*J. Soil Sci.*, 1957, **8**, 48—59).—The total N content of a silty clay loam was increased over three years by 60—70 lb. per acre per annum by growing rye-grass or medic (legume) on it. Soil N content on fallow soil was increased over three years by adding sucrose (3000 lb.), but not by adding straw (3000 lb. per acre). The increased N in the sugar-treated and grass soils was probably due to fixation by non-symbiotic bacteria and fungal endophytes. A. H. CORNFIELD.

Nitrate accumulation in Uganda soil. E. A. Calder (*J. Soil Sci.*, 1957, **8**, 60—72).—Nitrate accumulation during incubation of a non-laterized red earth was similar over the moisture range 15—35% (slightly above permanent wilting to waterlogging). No NO_3' accumulated under waterlogged conditions. The most favourable conditions for NO_3' accumulation were a fluctuating moisture content about an optimum average of 22—23%. Increase in NO_3' occurred without a decrease in total soil N, indicating that the NO_3' may be derived from NH_3 excreted by N-fixing organisms. A. H. CORNFIELD.

Effect of crop residues and nitrogen additions on decomposition of an Ohio muck soil. G. Stotzy and J. L. Mortensen (*Soil Sci.*, 1957, **83**, 165—174).—The rate of evolution of CO_2 from soil treated with rye, lucerne or wheat straw was determined over a period of 113 days. At all levels of application of the org. materials (>5 tons/acre), there was a net gain of C in the soil. The net gain of C showed a logarithmic increase with amount of residue added. The amounts of C retained from rye or lucerne were similar and less than that retained from wheat straw. After 20 days' incubation the rate of evolution of CO_2 was constant in all cases but at levels which increased with the amount of residue added. Addition of N as NH_4NO_3 had no effect on the evolution of CO_2. No significant losses of N as NH_3 occurred and the addition of N had no significant effect on the mineralization of P. T. G. MORRIS.

Afforestation and soil reaction. J. D. Ovington and H. A. I. Madgwick (*J. Soil Sci.*, 1957, **8**, 141—149).—The pH of the soil, litter and tree leaves at ten localities having a no. of different tree species 17—50 years after planting is reported. When grown under identical conditions the pH of leaves and litter varied considerably between species. Soil pH was modified by afforestation, largely depending on the nature of the established species. Tree species are graded according to their effect on soil pH. A. H. CORNFIELD.

Effect of Douglas-fir sawdust mulches and incorporations on soil microbial activities and plant growth. W. B. Bollen and K. C. Lu (*Proc. Soil Sci. Soc. Amer.*, 1957, **21**, 35—41).—Douglas-fir sawdust was low in both C and N, decomposed rather slowly in soil in spite of its high C/N ratio, showed no direct toxic effect on plants, and had little effect on soil pH. The apparent decomposition % based on CO_2 produced during decomposition in soil for 50 days, of material not receiving extra N, was for glucose 60, straw 48, alder 40, pine 33, red cedar 33, Douglas-fir 30, bark 26, and lignin 6%. Addition of N hastened decomposition but depressed CO_2 evolution. To avoid inducing N deficiency in the soil, 5—10 lb. of N per ton of applied sawdust was required in the first year, and smaller amounts in the second and third year after application. In some cases Douglas-fir sawdust when added to soil did not retard plant growth even when no extra N was added, and actually increased growth in some cases. A. H. CORNFIELD.

Geochemical field methods for the determination of tungsten and molybdenum in soils. A. A. North (*Analyst*, 1956, **81**, 660—668).—The soil sample (0·25 g.) is fused with Na_2CO_3, NaCl and KNO_3 and a solution of the melt is heated with $SnCl_2$ (in conc. HCl) to reduce interfering metals. It is then heated with *iso*amyl acetate containing toluene-3 : 4-dithiol until most of the ester is hydrolysed. The cooled liquid is extracted with colourless kerosene and the blue-green colour of the kerosene–ester layer is matched against standards. For Mo, after similar fusion and leaching, the aliquot is treated with hydroxylamine hydrochloride and extracted with the dithiol solution. The extract is shaken with conc. HCl and the yellow-green colour of the ester layer is matched against standards. The method is applicable to a range of 4—400 p.p.m. of W and 1—100 p.p.m. of Mo and can be modified for larger amounts. A. O. JONES.

Method for the microestimation of the nitrates in plant tissue and soil extracts. R. P. Murphy (*J. Sci. Food Agric.*, 1957, **8**, 231—234).—Nitrate in plant or soil extracts is reduced to NH_3 by Devarda's alloy and the NH_3 is then determined by the Conway microdiffusion technique. Results compared favourably with those obtained by the phenoldisulphonic acid method. The time required for the reduction was ~60 min. ; for extracts containing, e.g., 10 μg./l. of NO_3'-N, the amount of alloy used was 0·2 g. E. M. J.

Soil management in non-irrigated orchards and vineyards. R. L. Wishart (*J. Agric. S. Aust.*, 1956, **59**, 304–313, 315, 362—367).—Cover crops, manuring, cultivation, subsoiling and mulching are discussed. A. H. CORNFIELD.

Use of fertilizers in New Zealand. T. W. Walker (*Agric. Rev., Lond.*, 1957, **3**, 10—17).—A review dealing especially with the particular requirements of New Zealand pasture.
A. G. POLLARD.

Ammonium phosphates via wet process acid. G. Burnet (*J. agric. Food Chem.*, 1957, **5**, 258—263).—The process of making mono- or di-ammonium phosphate by treatment of phosphate rock with H_2SO_4 and neutralizing excess of acid with NH_3 is discussed. Details of five processes are given and compared, both technically and economically. E. M. J.

Rates of solution of calcium phosphates in phosphoric acid solutions. E. O. Huffman, W. E. Cate, M. E. Deming and K. L. Elmore (*J. agric. Food Chem.*, 1957, **5**, 266—275).—The rates of solution of eight synthetic Ca phosphates and three natural fluorapatites in dil. solutions of H_3PO_4 varied inversely with the pH of the solvent acid. For the different Ca orthophosphates values were in the same order as their solubilities, in the pH range 1—4. The effects of temp., stirring and salt compositions of the orthophosphates indicate that at a given pH, the rate is controlled by diffusion, while that for vitreous Ca metaphosphate is controlled by chemical reaction, probably hydrolytic degradation of polymeric phosphate chains. (41 references.) E. M. J.

Use of glasses or frits for enriching soils with trace elements. Yu. A. Zhdanov, K. P. Azarov and V. E. Gorbatenko (*Dokl. Akad. Nauk SSSR*, 1956, **108**, 1129—1131).—Addition to soils of powdered glasses or frits containing Cu, Mn, Mo or Zn gives considerable and lasting increase in yields of flax straw and linseed grown in soils deficient in these elements. Boron glasses are not effective, probably because their high solubility leads to excessive release of B to the soil. R. TRUSCOE.

Plant Physiology, Nutrition and Biochemistry

Daily course of "productivity of transpiration." W. Koch (*Planta*, 1957, **48**, 418—452).—Data obtained on a field of barley using the URAS and Thermoflux recorders is recorded. The productivity is defined as the assimilation/transpiration quotient calculated from the CO_2 and H_2O contents of the surrounding atm. Under sunshine or cloudy (intermittently or overcast) skies, the quotient shows, during the first third of the daily assimilation period, a marked max.; towards noon, it decreases to 50—33% of the morning max., and remains more or less constant until the evening decline. During rainy weather, the morning max. is not observed, but the average daily productivity exceeds that observed for the other types of weather. Analogous results are obtained in respiration-chamber experiments with various leaves. Fig and fir leaves, damaged by SO_2, show distinctly reduced productivity. Various possible methods for obtaining increased productivity are considered. (24 references.) P. S. ARUP.

Citric acid balance in seed germination. K. Täufel and U. Behnke (*Ernährungsforschung*, 1956, **1**, 277—283).—Data for the citric acid content of germinating seeds can be vitiated by infection with citric acid-forming moulds. Directions for avoiding infection are worked out. The citric acid content of poppyseed, sunflower seed and linseed decrease during the first four days of germination. Slowly germinating linseed contains during the third to the fifth day ~13% less citric acid than the fast-germinating seed.
P. S. ARUP.

Effect of various wave-lengths of light on selected ornamental plants. C. C. Fischer, F. B. Widmoyer and D. P. Watson (*Mich. agric. Exp. Sta. Quart. Bull.*, 1956, **39**, 259—262).—Effects of supplementary illumination of various plants with light of different colours included increased linear growth, shoot development and flowering response. A. G. POLLARD.

Effects of daily variations in temperature on growth, flowering and fertility. R. Knapp (*Ber. dtsch. bot. Ges.*, 1956, **69**, 399—411).—Responses by plants (5 spp.) are in most cases more favourable under moderate than under wide variations in temp., or at constant temp. Exceptional cases occur in which root-growth is promoted by wide variations, or in which the variations have no effect on most of the responses. (21 references.) P. S. ARUP.

Carotenoid synthesis and nitrogen metabolism. K. Paech (*Planta*, 1957, **48**, 587—591).—Wheat seedlings germinated in water, and in aq. 0·05, 0·1 or 0·2% urea show during the 6th to the 8th day, increasing synthesis of amino-acids, amides and proteins, but decreasing carotenoid synthesis with increasing concn. (up to 0·1%) of urea. When protein synthesis is stimulated by the presence of urea, the available energy of the plant is applied to protein- rather than to carotenoid-synthesis. This relationship can be observed only during the period mentioned, when amino-acid and protein synthesis is at its max.; during the 8th to the 10th day, the pattern is complicated by proteolysis. (10 references.) P. S. ARUP.

Comparative investigations of protein economy during elongation of petals and other organs. H. Matthaei (*Planta*, 1957, **48**, 468—452).—Comprehensive observations on the protein economy of the growing petals of 7 mono- and dicotyledons and the sporogoniophores of *Pellia epiphylla* show that cell elongation can be accompanied by increases, decreases or increases followed by decreases in protein content. In *Hydrocleis* petals, protein contents remain constant over prolonged periods. Chicory petals show characteristic changes during certain hours of the morning. Protein economy can be altered by environmental changes (notably in temp.) and is not causally connected with cell elongation. Discrepancies in the literature are explained by these observations. (86 references.) P. S. ARUP.

Leaf chlorosis in red clover. S. B. Hrushovetz, J. B. Lebeau and H. B. Stelfox (*Plant Dis. Reptr*, 1957, **41**, 120—122).—Moderate symptoms of chlorosis occurred in red clover when the soil was treated with NaCl or $BaCl_2$, but severe symptoms occurred when KOH, NaOH, Na_2CO_3 or $NaHCO_3$ was added. A pH greater than 8·48 was more important than the amount of Na present in inducing chlorosis. A. H. CORNFIELD.

Influence of low temperature on water- and nitrogen-uptake of plants, and its significance in relation to the "xeromorphy problem." H. Greb (*Planta*, 1957, **48**, 523—563).—The effects on xeromorphic symptoms of temp. and N-supply are investigated by growing beans and oats with divided root systems, one half in nutrient solutions at 0—2°, and the other at 15—20°, and also by growing the entire plants under comparable conditions in solutions within the two temp. ranges. Inhibition of growth of the divided roots in the cold solutions and of water-uptake by these roots is slightly counteracted by considerable increases in the N-supply. Doubling the normal N-supply of the plants grown with undivided roots in temperate solutions tends to check growth (the normal supply giving optimum growth), but for corresponding plants grown in cold solutions the doubled supply gives increased but subnormal growth with xeromorphic symptoms. The xerophytic characteristics of the stunted half-root systems or of the whole plants grown in cold solutions cannot be attributed to lack of water or of N, but are due to the inhibitory effect of low temp. on N-uptake. Xeromorphy should be regarded as a special case of a wider concept for which the term "Peinomorphie" is proposed. (86 references.)
P. S. ARUP.

Translocation and distribution of radioactive phosphorus in wheat. J. C. Frazier, J. F. Schaff, R. E. Hein and R. H. McFarland (*Bot. Gaz.*, 1956, **118**, 122—127).—The ^{32}P from nutrient solutions follows the transpiration stream to the upper part of the plant, and enters developing, but not mature kernels. During the late stages of development, more ^{32}P is deposited below the crease and in the embryo than in the endosperm. During the early stages, the concn. of ^{32}P reaches high levels in the endosperm and (especially) the bran-layer; subsequently the level in the endosperm becomes approx. uniform during ripening. In the bran-layer the level remains intermediate between those in the endosperm and the embryo. (24 references.) P. S. ARUP.

Relationships between copper, iron and molybdenum in the growth and nutrition of lettuce. I. Experimental design and statistical methods for characterizing the response surface. R. J. Hader, M. E. Harward, D. D. Mason and D. P. Moore. **II. Response surfaces of growth and accumulations of copper and iron.** D. P. Moore, M. E. Harward, D. D. Mason, R. J. Hader, W. L. Lott and W. A. Jackson. (*Proc. Soil Sci. Soc. Amer.*, 1957, **21**, 59—64).—I. The basis of construction of rotatable and composite designs are presented and illustrated by a practical example. These designs allow the calculation of response surfaces with much fewer treatments than where factorial designs are used.

II. The effects of five levels each of Cu, Fe and Mo, source of N (NO_3' alone or $NO_3' + NH_4^+$), and source of Fe (Fe^{3+} or Fe^{2+}) on yields and Cu and Fe content of the leaves of lettuce grown in culture solutions for six weeks are presented.
A. H. CORNFIELD.

Influence of zinc on the growth and nutrient uptake of *Zea mays* and *Vicia faba*. K. Scharrer and J. Jung (*Agrochimica*, 1956, **1**, 23—32).—In water-cultured maize 0·05 mg. of Zn per l. of nutrient solution was required for normal growth. The plants tolerated 5·0 mg. per l. without ill effects on growth. *Vicia faba* in a Zn-free nutrient showed no evidence of Zn deficiency; additions of Zn >0·05 mg. per l. produced toxic effects. In solutions of the same Zn concn. *V. faba* absorbed much more Zn than did maize.
A. G. POLLARD.

Plant utilization of zinc from various types of zinc compounds and fertilizer materials. L. C. Brown, F. Viets, jun., and C. L. Crawford

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(*Soil Sci.*, 1957, **83**, 219—227).—On a Zn-deficient loam, treated with Zn (2 p.p.m.) as ZnEDTA, the dry wt. of bean plants were not affected but the Zn contents of the plants increased in the order control, $ZnSO_4$, ZnEDTA. Stripping acid residue (a smelter by-product containing Zn with Mn, S and N) was comparable with $ZnSO_4$ as a source of Zn, and $Zn_3(PO_4)_2$, $ZnCO_3$ and ZnO were as readily utilized as $ZnSO_4$. The Zn in blast furnace slag (17% of Zn) and three Zn frits was not readily available; Zn granules were similar to $ZnSO_4$. T. G. MORRIS.

Protein changes during curing of burley tobacco and pectin methylesterase of cured leaf. B. M. Pogell, J. M. Moseley, C. J. Likes and D. F. Koenig (*J. agric. Food Chem.*, 1957, **5**, 301—304.)—Changes in protein fractions during curing are discussed. Evidence is presented that the rapidly sedimenting high-mol. wt. protein component of leaf cytoplasm is rapidly broken down during curing, whereas the remainder of the proteins which includes many enzymes is relatively more stable. The enzyme activity of pectin methylesterase in the protein fractions was followed by colorimetric measurement of the methanol liberated. (15 references.) E. M. J.

Rapid method for quantitative determination of peroxidase activity in plant press-juices. R. Kaul (*Planta*, 1957, **48**, 622—626).—The suitably diluted juice is mixed (in the order given) in a centrifuge tube, with aq. $Na_4P_2O_7$ and aq. $MnSO_4$ (which act as buffer and catalyst), benzidine + guaiacol solution (in 10% AcOH), aq. H_2O_2, and $CHCl_3$. (The vol. used and the concn. of the reagents are specified.) The mixture is shaken, after which the coloured $CHCl_3$ layer is quant. separated by centrifuging and measured spectrophometrically at 510 mμ. A calibration graph is given for this purpose. P. S. ARUP.

Wet ashing in agricultural chemical analysis. K. Scharrer and H. Munk (*Agrochimica*, 1956, **1**, 44—55).—Wet and dry ashing methods are discussed. Resistant org. matter is best dealt with by H_2SO_4–$HClO_4$ digestion. Sources of error and counter-measures are considered. A. G. POLLARD.

Synthetic plant hormones. IV. Aryloxymethylphosphinic acids. M. H. Maguire and G. Shaw (*J. chem. Soc.*, 1957, 311–314).—Several aryloxymethylphosphinic acids are prepared by the reduction of the corresponding phosphonic dichlorides with $LiAlH_4$ and aerial oxidation of the products. Aryloxymethyl chlorides are synthesized from aryloxymethyl phosphonic acids and PCl_5. 2 : 4-Dichlorophenoxymethyl chloride undergoes an Arbuzov reaction with triethyl phosphate. Preliminary biological tests show that 2-chlorophenoxymethylphosphinic acid has weak auxin activity but the 2 : 4-dichloro-deriv. is inert. In contrast the 4-chloro- and 2 : 4 : 5-trichloro-deriv. are anti-auxins. The products are described. I. JONES.

Gibberellins for crop production. S. H. Wittwer and M. J. Bukovac (*Mich. agric Exp. Sta. Quart. Bull.*, 1957, **39**, No. 3, 28 pp.).—A useful summary of recent work and of possible developments in promoting growth, early flowering, seed setting, fruiting etc. of various crop plants. A. G. POLLARD.

Gibberellins. S. H. Wittwer (*Farm Chem.*, 1947, **120**, No. 5, 55—57).—A concise summary of the present knowledge. A. G. POLLARD.

Effect of gibberellic acid on growth and development of plants of various genera and species. P. C. Marth, W. V. Audia and John W. Mitchell (*Bot. Gaz.*, 1956, **118**, 106—111).—A detailed account is given of the effects of the acid, applied as a lanolin paste, spray, dip or soak to 49 economic plants. Responses vary widely with different spp., the main effects being stem and petiole elongation accompanied by decreased root-development. Cases of accelerated flower-growth are recorded, but no increases in the no. or size of fruits were observed. P. S. ARUP.

Effect of fusaric acid on water-permeability of tomato plant pith cells. E. Gäumann and W. Loeffler (*Phytopath. Z.*, 1957, **28**, 319—328).—After immersion during 30 min. in aq. fusaric acid (**I**) of varying concn., and subsequent immersion in 0·8M-aq. sucrose, the pith cells of stem sections increase rapidly in permeability with increasing concn. of **I** over the range 5×10^{-8}—10^{-7}M. With further increasing concn. of **I**, the permeability decreases rapidly, showing subnormal values for 10^{-6}—10^{-3}M. Owing to the establishment of concn. gradients of **I** in infected plants, and to the ambivalent effects of **I**, the transpiration of the plants is affected by a complexity of factors. (11 references.) P. S. ARUP.

Influence of fusaric acid on the water permeability of plant protoplasts. E. Bachmann (*Phytopath. Z.*, 1956, **27**, 255—288).—The irreversible action of fusaric acid (**I**) in lowering the permeability of plant cells is examined in detail. The effect of pyridine homologues in this respect increased with the no. of C atoms in the aliphatic side-chain with a max. at C_4 (*n*-butylpyridine) in the case of *Rhœo* but which is extended up to C_6 in *Spirogyra*. The mechanism of action of **I** is examined in relation to non-osmotic water intake, to enzymic metabolism and to the tomato wilt disease. A. G. POLLARD.

[A] Changes in redox potentials during light-induced oxidative inactivation of indol-3-ylacetic acid. [B] Effect of heavy metals on light-induced oxidation of indol-3-ylacetic acid. G. Rummeni (*Ber. dtsch. bot Ges.*, 1956, **69**, 325—336; 1957, **70**, 31—38).—[A] The photochemical action occurring on exposure of solutions containing the acid (IAA) and various colouring matters to light in an atm. of N_2 results in rapidly increasing negative potentials and oxidation of the IAA with the formation of inactive products. Acidic dyes (e.g., eosin) are most effective in oxidizing IAA at pH 3·0, whilst basic dyes (e.g., phenosafranin) show max. activity at pH 6·0. The extent of oxidation depends partly on the reactivity of a basically dissociated group of the IAA, and partly on the state of dissociation of the dye. At pH 7, the oxidation process is, with all the (7) dyes, considerably inhibited, probably owing to associative effects undergone by the reactive IAA group. (17 references.)

[B] The presence of $FeCl_3$ promotes the atm. oxidation of IAA through the medium of non-auto-oxidizable dyes (e.g., methylene blue or Safranine-T). The effect is small in presence of some other dyes (e.g., Rhodamine-B or Nile-blue), the increase being less than that obtained with $FeCl_3$ (at 10^{-5}M) in the absence of dye. In conjunction with riboflavin, $FeCl_3$ has no effect. A catalytic effect on the light-induced atm. oxidation of IAA can be obtained with $FeCl_3$ in the absence of dye; this effect increases rapidly with the concn. of $FeCl_3$, and is probably due to the formation of an auto-oxidizable Fe-complex with IAA. (13 references.) P. S. ARUP.

Variability of [response of] carrots to auxin, casein hydrolysate and coconut milk. S. M. Caplin (*Amer. J. Bot.*, 1956, **43**, 749—754).—Approx. 20% of the explants from a large no. of carrot roots show, when grown on White's medium, no growth-response to indol-3-ylacetic acid (IAA) or to casein hydrolysate and very varying responses to coconut milk. Growth response by normally responsive explants to coconut milk are 3—4 times greater than those obtained with IAA. Addition of casein hydrolysate increases growth under all treatments, including the controls. A similarity between the growth-distribution profiles due to IAA and casein hydrolysate is observed. (22 references.) P. S. ARUP.

Enhancement of phosphatase activity of latex of *Euphorbia verrucosa* L. parasitized by *Uromyces scutellatus* (Schr.) Lev. Interactions with growth-substance metabolism. G. Turian (*Phytopath. Z.*, 1957, **28**, 275—280).—The latex of the parasitized plant contains ~100% more free inorg. P than does normal latex, shows considerably greater phosphatase activity, and has a higher content of heteroauxin. Addition to normal latex of Pilet's heteroauxin ABIA (in concn. of 10^{-3} mol.) increases its phosphatase activity. An interaction between heteroauxin and phosphatase activities, and the responsibility of the increased activity for floral sterilization are suggested. (13 references.) P. S. ARUP.

Photochemical degradation of 2 : 4-dichlorophenoxyacetic acid and structurally related compounds in the presence and absence of riboflavin. G. R. Bell (*Bot. Gaz.*, 1956, **118**, 133—136).—The products formed by u.v. irradiation of 2 : 4-D include phenols and other products, the nature of which is investigated by chromatographic and optical methods. An investigation of the effect of photoactivated riboflavin on 10 related aromatic ethers indicates that decomposition with formation of the corresponding phenol depends on the structure of the side-chain and on the presence of the carboxyl group in the 1-position relative to the etheric O atom. P. S. ARUP.

Histological responses of plant leaves to hydrogen fluoride and sulphur dioxide. R. A. Solberg and Donald F. Adams (*Amer. J. Bot.*, 1956, **43**, 755—760).—Histological changes produced in fruit-tree foliage by periodic fumigation with SO_2 (0·5 p.p.m.) are indistinguishable from those produced by HF (5 parts per 10^9). The first symptoms, which are detectable microscopically before macroscopically visible injury occurs, are collapse of the spongy mesophyll and lower epidermis. The injured tissue can be selectively stained with Safranine. The means of entry into the plant, and ways of distribution of toxic air-contaminants are discussed. (20 references.) P. S. ARUP.

Chromatography of lipin-soluble leaf pigments. A. Hager (*Planta*, 1957, **48**, 592—621).—The pigments are extracted by a mixture of acetone and light petroleum (4 : 1); they can then be quant. transferred to the light petroleum phase by the addition of aq. 10% NaCl and cautious agitation in a separating funnel. With the use of a suitable developer (light petroleum 60, C_6H_6 35, $CHCl_3$ 1·25, acetone 0·55 and Pr^iOH 0·06 pt.), the mixture can be separated on a starch column into six bands comprising two xanthines, two

chlorophylls, lutein and carotene. An improved paper-chromatographic technique (described) is operated under reduced pressure with the use of an ascending solvent similar to the above. By this method, the pigments (extracted by means of $CHCl_3$) comprising individually the carotenes and their deriv., xanthophylls, and the chlorophylls and their deriv. can be separated and determined spectrophometrically. (50 references.) P. S. ARUP.

Crops and Cropping

Tracer studies in Saskatchewan on the utilization of phosphates by grain crops. J. Mitchell (*J. Soil Sci.*, 1957, **8**, 73—85).—A review. A. H. CORNFIELD.

Effect of boron on cereals growing in water-deficient soil at their critical period of development. F. D. Skazin and V. G. Rozhkova (*Dok. Akad. Nauk SSSR*, 1956, **108**, 962–964).—Resistance of barley plants to drought at the critical stage of tetrad formation is greatly enhanced by spraying the leaves with 0·1% aq. H_3BO_3, the yield of grain being five times greater than that from control plants. The effect is ascribed to reduction of loss of water by transpiration, and to more active carbohydrate metabolism, leading to more active transport of sugars to the ear rudiment. R. TRUSCOE.

Zeleny sedimentation test applied to German wheats. W. R. Schaefer (*Cereal Sci.*, 1957, **2**, 16—19).—The application of the Zeleny sedimentation test in Germany is described and discussed. The procedure is associated with various analytical requirements of which the possibility of uniform and comparable production of flour is particularly important. If the procedural details of the test are followed, including the use of a suitable shaking apparatus, the method gives useful practical results. J. S. C.

Changes in carbohydrate metabolism of barley under conditions of high soil humidity. R. I. Lerman (*Dokl. Akad. Nauk SSSR*, 1956, **108**, 1191—1193).—The content of all carbohydrate fractions of barley leaf grown in waterlogged soil is less than that of control plants, except at the stage of ear formation. The same effect is found for grain, the yield of which is lowered; the lowest yields are obtained when waterlogging occurs at the end of the vegetative period or during that of ripening of the grain. R. TRUSCOE.

Effect of raised soil temperature at different stages of ontogenesis on growth and development of oats. R. S. Limar' (*Dokl. Akad. Nauk SSSR*, 1956, **108**, 1194—1196).—Raising soil temp. by 7° during the spring growth period increases the number of tillers and the yield of grain and straw, but has the opposite effect at later stages of development. R. TRUSCOE.

Effect of fertilizers on metabolism of sprouting potato tubers. A. F. Kalinkevich and V. A. Aleksandrovskaya (*Dokl. Akad. Nauk SSSR*, 1956, **108**, 683—685).—Addition of ^{32}P-labelled superphosphate to a podsol in which seed potatoes are planted is followed by appearance of activity in both the deep and the superficial parts of the tubers. Addition of P + N fertilizers stimulates respiration and amylolysis, and leads to higher wt. of the root and leaf systems of the resulting plants, as compared with P or P + N + K manuring. The effect of K is chiefly on the leaf system, for which reason the best results are obtained when K fertilizer is placed in the furrows between rows. R. TRUSCOE.

Chemical changes during growth of sugar beet. E. Kottelanne (*C. R. Acad. Sci., Paris*, 1957, **244**, 491—494).—During the development of the sugar beet, the max. concn. of P occurs in the leaves during the first vegetative phase. During the second (tuberization) phase, P migrates towards the stem. Mg attains a max. concn. in the dry matter during the first phase. Quant., K is the mineral most intensely absorbed by the plant. The Na content increases regularly in the leaves as the plant develops. The joint influence of P, N and Mg governs the growth of the young central leaf. The K/Na ratio is stable during stem growth. During tuberization, the P/K ratio in the stem remains constant. The roles of P, Mg, K, Na, N, S, and other elements in the metabolism of the plant are described and discussed. J. S. C.

Leaf and crude protein percentages among strains of some forage grasses. W. R. Kneebone and V. G. Heller (*Oklahoma agric. Exp. Sta.*, 1956, Tech. Bull. 65, 23 pp.).—There were significant differences in % of protein among selections and among lines of all species except sideoats grama. There were significant differences in % of protein in both leaf and stem of smooth bromegrass and sand bluestem. In sand bluestem the % of protein was significantly correlated with % of Ca but not with height, diameter or yield. Protein, Ca and P contents of the leaves were correlated with those of the stems. A. H. CORNFIELD.

Effect of sodium and potassium on maize and crimson clover grown on Norfolk sandy loam at two residual K levels. R. L. Wehunt, M. Stelly and W. O. Collins (*Soil Sci.*, 1957, **83**, 175—183).—To plots having initially low and high exchangeable contents due to previous K manuring, were added five levels of Na and K in different ratios, together with P and N. Clover and maize were grown. The Na treatments did not increase the exchangeable Na in the soils. On the low-K soil only the K treatments increased the exchangeable K. The control and all Na-treated high-K plots showed decreases in exchangeable K. The pH value decreased annually. Yields of maize grain and stover were not affected by any treatments on the high-K plots, but were doubled by all K treatments on the low-K plots. Na treatments increased yields of clover forage on the high-K plots but did not affect those on the low-K plots. K treatments on both soils increased yields significantly especially on the low-K soil. The Na content of maize leaves was unaffected in either soil by the treatments. The K content of the leaves was slightly increased by Na treatment. Use of K markedly increased the K content of the maize. The Na and K content of the clover was related to the initial soil K level and to the treatments. T. G. MORRIS.

Response of lucerne to applications of a soluble borate and a slightly soluble borosilicate glass. E. R. Holden and A. J. Engel (*J. agric. Food Chem.*, 1957, **5**, 275—279).—Supplemental B as coarsely ground borosilicate glass, applied to a light soil, was compared with equivalent treatments with borax of lucerne grown under greenhouse conditions. The concn. of B in the plant was approx. proportional to application and reached a min. value during late summer. Significant increases in yield were produced by borax 5 to 80 lb. and by glass from 12 to 360 lb. per acre. The use of a more sol. glass is suggested. E. M. J.

Effect of time and rate of fertilizer application on the yield, composition and longevity of lucerne. W. W. Nelson and J. M. MacGregor (*Proc. Soil Sci. Soc. Amer.*, 1957, **21**, 42—46).—Highly significant yield increases of lucerne (cut three times per year) over three years were obtained only where K was present in the fertilizer. Highest yields were obtained with 1000 lb. of a 0–20–20 (N–P_2O_5–K_2O) mixture applied in spring prior to sowing followed by 200 lb. of a 5–20–20 mixture in spring during the second and third years. Withholding N did not reduce yields. Autumn and spring applications were equally effective. The % of N, P and K in the plant was usually highest in the second and lowest in the first cutting. There were no differences due to fertilizer treatment in degree of winter-hardiness or in stand after four years. A. H. CORNFIELD.

Effect of rainfall and fertilization on the yield and chemical composition of lucerne over a ten-year period in North Central Oklahoma. H. J. Harper (*Proc. Soil Sci. Soc. Amer.*, 1957, **21**, 47—51).—Climatic factors had a greater influence than had soil treatments on the nutrient content of 26 cuttings of lucerne over 10 years. In most years the protein content of the first cutting was not less than that of the second and third cuttings. The Ca content of the hay was inversely related to soil moisture content. There was a low % of P in the hay when rainfall was low for several weeks before cutting. On unlimed soil utilization of fertilizer P was similar from both rock phosphate and superphosphate, but on limed soil rock phosphate was slightly inferior. The N and P content of stems varied to a greater extent than did the N and P content of the leaves. A. H. CORNFIELD.

Fertilizer trials on lucerne, cotton and sorghum in New Mexico. R. W. Leamer (*New Mexico agric. Exp. Sta.*, 1956, Bull. 408, 12 pp.).—Trials on loams and silty clay loams showed that optimum yields of lucerne, cut yearly for hay, were obtained with application of 60—80 lb. of P_2O_5 per acre per annum. Good yields of cotton were obtained when sufficient P was added to give good yields of lucerne in the rotation. The greatest responses to N and P occurred on new land or on fields which had not been in lucerne for a number of years. Sorghum gave good responses to N, but not to P. None of the species responded to K. A. H. CORNFIELD.

Resting period of fruit trees. Ya. S. Nesterov (*Dokl. Akad. Nauk SSSR*, 1956, **108**, 738—741).—Opening of leaf and flower buds of fruit trees (apple, plum, peach) exposed to winter weather until March and then brought indoors preceded that of similar plants kept indoors during the winter, by 2—3 months. Grafts from wintering trees on indoor ones produced few leaves and flowers, and often failed to take, whereas grafts from indoor plants on those which had been exposed to frost took well, and grew vigorously. The effects are ascribed to enhancement of permeability of the tissues of plants exposed to frost. R. TRUSCOE.

Increasing the fruit crop of greenhouse-grown tomatoes. S. H. Wittwer and F. G. Teubner (*Mich. agric. Exp. Sta. Quart. Bull.*,

1956, **19,** No. 2, 12 pp.).—Increased flower and fruit production by chilling the seedlings (50—55°F. for 3 weeks) by spraying older plants with *N*-*m*-tolylphthalamic acid combined with adequate periodic fertilizer application is described. A. G. POLLARD.

Bitter flavour in carrots. II. Field and storage experiment. J. D. Atkin (*Cornell agric. Exp. Sta.*, 1956, Bull. 774, 30 pp.).—A bitter flavour developed in carrots during refrigerated storage but not during common storage. There were little differences in ability to develop bitterness between strains and varieties. Extreme differences were found between individual roots, indicating that genetic or micro-environmental factors are responsible. Carrots grown intensively on muck and sandy soils were more bitter than those grown on general farms or upland soils. A. H. CORNFIELD.

Equilibrium desorption of water vapour on tobacco. E. E. Locklair, L. G. Veasey and M. Samfield (*J. agric. Food Chem.*, 1957, **5,** 294—298).—To evaluate dryer operations, data on the equilibrium moisture contents of various types of tobaccos over a temp. range of 80 to 140° and a R.H. range of 10 to 80% were obtained. The isosteric (constant moisture content) net heats of desorption varied from ~9000 to 16000 B.Th.U. per lb.-mol. in the region of 1% moisture to ~700 to 1800 B.Th.U. per lb.-mol., in the 20% moisture region, depending on the type of tobacco. Apparatus and procedure are described. E. M. J.

Development of oil in linseed *Linum usitatissimum* on soils of different productivity. B. G. Prasad and B. Biswas (*J. Proc. Oil Technol. Ass., India,* 1954, (1955), **10,** 37—42).—Trial cultivations of linseed showed that (1) the moisture content of seed, initially high, decreases with seed age; (2) as seed ages, the oil content increases to a max. and then remains almost constant; (3) the yield of seed per acre is increased by manuring, $(NH_4)_2SO_4$ being preferable to compost; (4) the oil yield % is not affected by manuring; (5) the I value of the oil increases with seed ageing. (12 references.) J. S. C.

Nitrogen, phosphorus and potassium content of castor bean hulls. W. Parkey, J. E. Webster, and D. L. van Horn (*Oklahoma agric. Exp. Sta.*, 1956, Tech. Bull. 61, 11 pp.).—Castor bean hulls averaged N 1·64, P 0·82, K 3·81 and ash 10·38%. Differences due to variety were relatively low compared with those due to location of growth or season. A. H. CORNFIELD.

Incomplete determination of a measure of quality in a series of experiments. D. J. Finney and Ø. Nissen (*J. Agric. Sci.*, 1956, **48,** 124—128).—A statistical procedure suitable for dealing, e.g., with cropping trials. A. G. POLLARD.

Pest Control

Technique for testing liquid or solid substances for fungicidal activity. K.-H. Kuhfuss (*Phytopath. Z.*, 1957, **28,** 281—284).—Cotton wool fibres of standard dimensions, treated with the fungicide under standard conditions are stretched diametrically across the surface of nutrient agar (in Petri dishes) which has been inoculated with spore suspensions of the test-fungi. After incubation at 26° during three days, the width of the resulting inhibition zones is measured. P. S. ARUP.

Determining the distribution of organic insecticides in powder formulations. L. B. Norton and G. D. Butler, jun. (*J. agric. Food Chem.*, 1957, **5,** 279—282).—Since most toxicants are very sol. in certain org. solvents, exposure of a formulation to the vapour of a suitable solvent causes deliquesence of the toxicant, but not of the other constituents. Microscopical examination of the resulting droplets could give an index to the location and quantity of toxicant originally present. E. M. J.

Compatibility of organic fungicides and antibiotics. E. T. Palm and R. A. Young (*Plant Dis. Reptr*, 1957, **41,** 151—155).—The fungicidal activity of dichlone and captan and the bactericidal activity of streptomycin sulphate and nitrate and Terramycin were not reduced significantly after aq. mixtures of the materials had aged for one week. Maneb showed an immediate loss in toxicity when mixed with Terramycin, but only a gradual loss when mixed with streptomycin. Maneb was less effective than Terramycin, but more effective than streptomycin as a bactericide. A. H. CORNFIELD.

Phytopathological importance of cuticular excretions. A. Kovács and É. Szeöke (*Phytopath. Z.*, 1956, **27,** 335—349).—The effects of leaf excretions from various plants on the spores of several pathogenic fungi are recorded; germination of *Botrytis cinerea* was more readily affected than that of *Puccinia tritici*. Excretions from different plant species varied in electrical conductivity, pH and content of readily-oxidizable org. matter, but these factors did not afford an explanation of the action on spores. The excretions contained both inhibitory and stimulatory substances. A. G. POLLARD.

Rôle of phenols in resistance of tomato plants to infections. R. Menon and L. Schachinger (*Ber. dtsch. bot. Ges.*, 1957, **70,** 11—20).—The content of polyphenoloxidase in tomato leaves and shoots is increased after infection with *Fusarium lycopersici*, and is greater in a resistant than in a non-resistant variety. Infection increases the total phenol content of resistant, but not that of non-resistant varieties. The extent of formation of total phenols is in rough proportion to the resistance of the variety, and to amount of the enzyme that is formed. (26 references.) P. S. ARUP.

Effect of copper on potato plants and its fungicidal action within the plant system. W. Zeck (*Phytopath. Z.*, 1956, **27,** 353—404).—The effects of Cu on the rate and duration of growth and on yields of potatoes are apparent only in the year of application and are influenced by the nature of the soil. The intake of Cu by the plants increases with the amount supplied and is most rapid in young plants. Within the plant Cu is combined with nitrogenous substances, 1 mg. of N being associated with 0·5 μg. of Cu. The intake of Cu is increased by application as a complex ion, the safe limit being much greater than for Cu-ions. No fungicidal action of Cu assimilated via leaves or roots is apparent. Detached leaves in which the Cu content has been increased 40-fold above normal show no increased resistance to fungal infection. A. G. POLLARD.

Action of quinoneoxime-benzoylhydrazone on seedling diseases of some crop plants. P. E. Frohberger (*Phytopath. Z.*, 1956, **27,** 427—455).—The protective action of the hydrazone [the active agent (10%) in Cerenox (Bayer) seed dressing] against, e.g., *Pythium* is not due to direct action on the fungus tissue or spore but depends on its uptake by the germinating seed. A. G. POLLARD.

Effects of fungicides on the honey bee. W. Parsons and E. C. Martin (*Mich. agric. Exp. Sta. Quart. Bull.*, 1956, **39,** 248—258).—Comparative tests with 14 proprietary fungicides commonly used in orchard practice are recorded. Death rates of bees reached 50% in some cases. A. G. POLLARD.

Analysis of commercial insecticide dusts containing two chlorinated hydrocarbons. D. P. Johnson (*J. Ass. off. agric. Chem., Wash.*, 1956, **39,** 1017).—If the material is free from ether-sol. and Cl-bearing materials other than those being determined, a reasonably accurate estimate of the amount of each constituent may be made by determining the total Cl. content and the ether-sol. extract. A. A. ELDRIDGE.

Separation and identification of chlorinated organic pesticides by paper chromatography. VIII. Technical DDT, *pp'*-DDT, DDA, DDD, DDE, 4 : 4'-dichlorobenzophenone and 2 : 4'-dichlorobenzophenone. Lloyd C. Mitchell (*J. Ass. off. agric. Chem., Wash.*, 1956, **39,** 980—985).—Four sets of spotted papers are prepared; three sets are sprayed with soya-bean oil in ether (1 diluted to 200) and developed respectively with aq. acetone, aq. 2-methoxyethanol, and aq. pyridine. The fourth set is sprayed with dimethylcyanamide in ether (20 diluted to 100) and developed with 2 : 2 : 4-trimethylpentane. When dry, the papers are sprayed with a reagent prepared by dissolving $AgNO_3$ (1·7 g.) in water, adding 2-phenoxyethanol (10 ml.) and ethanol (50 ml.) and diluting to 200 ml. with water. After air-drying, the papers are exposed to strong u.v. light, Nearly all the pesticides tested are identifiable by the order of separation and distance. The chromogenic reagent reveals as little as 1—3 mmg. A. A. ELDRIDGE.

Separation and identification of chlorinated organic pesticides by paper chromatography. IX. Aldrin, DDE, dieldrin, DDT, lindane, methoxychlor, Perthane and Rhothane (DDD). Lloyd C. Mitchell (*J. Ass. off. agric. Chem., Wash.*, 1956, **39,** 985—990).—Two sets of duplicate spotted papers are prepared. One set is sprayed with dimethylcyanamide in ether (20 ml. diluted to 100 ml.) and developed with 2 : 2 : 4-trimethylpentane or colourless mineral oil, $d^{15.5}$ 0·775—0·825. The other set is sprayed with soya-bean oil in ether (1 ml. diluted to 100 ml. or 200 ml.) and developed with aq. methanol (15 ml. of water diluted to 100 ml. with methanol). When dry, the papers are sprayed with the chromogenic agent prepared by dissolving $AgNO_3$ (1·7 g.) in water, adding 2-phenoxyethanol (10 ml.) and ethanol (50 ml.) and diluting to 200 ml. with water. After evaporation of the water the papers are exposed to strong u.v. light. A. A. ELDRIDGE.

Determination of benzene hexachloride in presence of pentachlorocyclohexene. F. R. Bradbury and H. Standen (*Chem. & Ind.*, 1957, 140).—The colorimetric method for determining benzene hexachloride (BHC) (cf. J.S.F.A. Abstr., 1954, ii, 116) is specific and not subject to interference by pentachlorocyclohexene (PCCH), benzene or chlorobenzenes. When ^{14}C-labelled γ-BHC is used in

experiments on fly metabolism, the addition of 100 g. of unlabelled γ-PCCH to CCl_4 extracts, followed by nitration and isolation of the tetrachloroadipic acid formed, can be made the basis for an isotopic dilution method of γ-PCCH determination reasonably free from interference by other compounds. J. S. C.

Detection of phytoalexines. K. O. Muller (*Phytopath. Z.*, 1956, **27**, 237—254).—Phytoalexines are defined as antibiotics produced by the interaction of metabolic systems of host and parasite and which inhibit the growth of micro-organisms pathogenic to plants. In experiments described the inner epidermis of pods of *Phaseolus vulgaris* serves as host and *Sclerotinia fructiola* and *Phytophthora infestans* as test parasites. The presence of an antibiotic factor is shown by bio-assay in the diffusate from the host tissue eight hours after invasion by the fungus. The antibiotic is not specific in its action and is also toxic to plant cells. The active material probably contains two components, is dialysable and resistant to heat (100°) and cold (< 0°) and retains its activity over wide ranges of pH. A. G. Pollard.

Determination of lindane residues on pickles. G. W. Gehrke and J. L. Bevirt (*Missouri agric. Exp. Sta.*, 1956, Res. Bull., 606, 24 pp.).—A critical study was made of the Schechter–Hornstein method (*Analyt. Chem.*, 1952, **24**, 544) for determining trace amounts of γ-$C_6H_6Cl_6$. The value of the original and a modified method for determining the lindane content of pickles is reported. A. H. Cornfield.

Effect of ions of certain metals on development of stem rust in wheat. F. R. Forsyth (*Nature, Lond.*, 1957, **179**, 217—218).—It has been found that wheat seedlings, normally resistant to rust, become susceptible after an uptake of Zn, Mn or Co ions by the roots, but that resistance is enhanced by Fe^{3+} ions. J. S. C.

Streak mosaic of wheat in Nebraska and its control. R. Staples and W. B. Allington (*Nebraska agric. Exp. Sta.*, 1956, Res. Bull. 178, 40 pp.).—The biology of *Aceria tulipæ*, K., the vector of streak mosaic, and epidemiological studies over two years are described. No satisfactory method of control of the disease through chemical control of the vector are yet known. Cultural methods of control are discussed. A. H. Cornfield.

Rice seed treatment tests. J. G. Atkins, E. M. Cralley and S. J. P. Chilton (*Plant Dis. Reptr*, 1957, **41**, 105—108).—Agrox, Ceresan M, Ceresan M-2X, Panogen 15 and MEMA treatments of rice seed gave good stand increases, whilst Arasan SFX, Delsan AD and Phygon-XL increased stands consistently. A. H. Cornfield.

Seed disinfectants for crops. W. E. Brentzel (*N. Dakota agric. Exp. Sta.*, 1956, Bull. 402, 31 pp.).—Seed disinfectants for cereals, flax, maize legumes, peas, beans and grasses are described. A. H. Cornfield.

Control of potato seed-piece decay. W. M. Epps (*Plant Dis. Reptr*, 1957, **41**, 148—150).—Dipping seed pieces in Phytomycin or Agrimycin (100 p.p.m.), streptomycin (50—100 p.p.m.) (instant to 5 min. dip) or treatment with streptomycin (0·05—0·20%) dusts increased the stands and yields of irrigated potatoes. Captan was not quite as effective, and caused reduced yields when seed decay organisms were not present. A. H. Cornfield.

Injury to potatoes caused by application of *iso*propyl phenylcarbamate (IPPC) for preventing sprouting. G. R. Edwards (*J. Agric. S. Aust.*, 1956, **59**, 274—275).—Surface blemishes on tubers which had been treated with IPPC at normal rates and stored for some months are reported. The extent of trouble was not related to the IPPC content of peelings at the end of storage. The trouble occurred only when tubers were treated when wet or where lenticel enlargement (water spot) was present. A. H. Cornfield.

Effects of 8-azaguanine on sugar beet infected with beet yellows virus. G. E. Russell and A. R. Trim (*Nature, Lond.*, 1957, **179**, 151).—Tests with 8-azaguanine (5-amino-7-hydroxy-1-*v*-triazolo-[4′ : 5′-2 : 3]-pyrimidine) applied to sugar beet infected by aphids with beet yellows virus showed a delay in appearance of symptoms of about the same magnitude as those observed with tobacco mosaic virus. J. S. C.

Inoculation studies related to breeding for resistance to bacterial wilt, *Xanthomonas lespedezæ*, or annual lespedeza. M. S. Offutt and J. D. Baldridge (*Missouri agric. Exp. Sta.*, 1956, Res. Bull. 603, 47 pp.).—Studies are reported of host–parasite relationships and of inoculation and evaluation methods as related to breeding for wilt resistance of annual lespedeza. A. H. Cornfield.

Insects and diseases of fruit nursery stock and their control. F. L. Gambrell and R. M. Gilmer (*Cornell agric. Exp. Sta.*, 1956, Bull. 776, 50 pp.).—Descriptions of and control measures for the principal insects and diseases of fruit nursery stocks in New York State are described. A. H. Cornfield.

Control of the grape cane girdler, *Ampelogypter ater*, LeC. W. D. Whitcomb (*Mass. agric. Exp. Sta.*, 1956, Bull. 484, 22 pp.).—The pest was controlled by spraying the canes with DDT (1 lb.), PbHAsO (2 lb.), γ-$C_6H_6Cl_6$ (0·125—0·400 lb.), or methoxychlor (1·5 lb. per 100 gal.), commencing when beetle activity started in late May—early June, and repeating every 4—5 days when growth increased 5—6 in. A. H. Cornfield.

Control of collar rot and shell bark of lemons. Anon. (*Agric. Gaz. N.S.W.*, 1956, **67**, 522—525).—Collar rot, due to *Phytophthora citrophthora*, is prevented by spraying the trunk and surrounding soil with Bordeaux mixture. Where the disease occurs the affected portion is cut out and the wood painted with Bordeaux mixture. Shell bark, probably caused by a virus, is avoided by maintaining a high level of tree vigour through correct cultural treatment, fertilizer applications and irrigation. A. H. Cornfield.

Control of root-knot nematodes of tomatoes. B. Lear and I. J. Thomason (*Plant Dis. Reptr.*, 1956, **40**, 981—986).—Application of Nemagon (1 : 2-dibromo-3-chloropropane) between the rows at 0·3 gal. per acre gave excellent control of nematodes and increased yields of tomatoes. Good control was also obtained with D-D (8 gal.) and ethylene dibromide (2·4—6·0 gal. per acre). A. H. Cornfield.

Control of downy mildew, *Phytophthora phaseoli*, of lima beans. D. F. Crossan, P. J. Lloyd, R. A. Hyre and J. W. Heuberger (*Plant Dis. Reptr*, 1957, **41**, 156—159).—Two applications of maneb (2 lb. per 100 gal.) to lima bean plants within a month of harvest gave excellent control of downy mildew and increased the yields of beans. "Tribasic Cu" (4 lb. per 100 gal.) also gave good control, whilst streptomycin (100 p.p.m.) in 1% glycerol was unsatisfactory. A. H. Cornfield.

Evaluation of insecticides for control of the European pine shoot moth under spring and summer conditions. G. Guyer, W. F. Morofsky and W. Lemmien (*Mich. agric. Exp. Sta. Quart. Bull.*, 1957, **39**, 432—437).—Of the insecticides tested DDT gave the best control of the moth. For summer application emulsifiable and wettable powder formulations were equally effective : in spring the former were somewhat superior. A. G. Pollard.

Aircraft applications of insecticides in East Africa. X. Investigation of coarse aerosol clouds in woodland. G. F. Burnet and B. W. Thompson. **XI. Applications of a coarse aerosol to control *Glossina morsitans* Westw, and *G. pallipides* Aust. in Lango County, Uganda.** K. S. Hocking and D. Yeo (*Bull. ent. Res.*, 1956, **47**, 495—524, 631—644).—X. In trials with DDT in oil, satisfactory kills of caged flies were obtained in open country, but in thin woodland, kills in cages upwind of twigs and trees were reduced by a screening effect. Mortality was comparatively low in continuous woodland. The effects of atm. turbulence and clearings in woodland are examined. (10 references.)

XI. An experiment on woodland savannah with the use of DDT (0·2 lb.) or of γ-$C_6H_6Cl_6$ (0·03 lb. per acre) reduced a population of *G. morsitans* to 0·05% of the original, and eradicated *G. pallipides*. The lasting effects of the treatment are due to the effective isolation of the treated area. (10 references.) P. S. Arup.

Fungicides mixed with covering soil for control of the cotton seedling-disease complex. L. S. Bird, C. D. Ranney and G. M. Watkins (*Plant Dis. Reptr*, 1957, **41**, 165—173).—Tests with a large no. of fungicides, singly or in combination, on a sandy and a clay soil are reported. Some of the materials were partially effective in controlling the seedling-disease complex (caused by several soil-borne fungi) but results varies with soil type and year of testing. A. H. Cornfield.

Large-scale spraying of cotton in the Gash delta in Eastern Sudan. R. J. V. Joyce (*Bull. ent. Res.*, 1956, **47**, 399—413).—Spraying with DDT gave good control of the leaf-destroying cotton-jassid and -thrips, but little lasting control of the flea-beetle. The benefits obtained by spraying were, however, lost by reduced yields due to increased boll-worm (*Diparopsis watersi*) attack on the sprayed (especially the twice sprayed) fields. P. S. Arup.

Treatment of tobacco plant beds with methyl bromide. F. A. Todd and G. B. Lucas (*N. Carolina agric. Exp. Sta.*, 1956, Bull. 399, 20 pp.).—Tests at several locations showed that methyl bromide (9 lb. per 100 sq. yd.) gave excellent control of black shank, root knot and weeds. The material was ineffective when applied to wet soil. January treatments were as effective as autumn treatments. Supports which held a cover (sisalkraft or polythene sheeting) at least 2 in. above ground level were satisfactory. The chemical was equally satisfactory when 1 to 9 points of application were made over 100 sq. yd. A. H. Cornfield.

Biology and control of sugar-cane chafer beetles in Tanganyika. W. F. Jepson (*Bull. ent. Res.*, 1956, **47**, 377—397).—Descriptions are given of the morphology, life history and infestation habits of

the red cane beetle, *Cochliotis melolonthoides* (Gerst.) (Melolonthoidæ). The growing of certain resistant varieties of cane with suitable methods of cultural control is suggested. The possibilities of parasitical biological control are considered. Promising results have been obtained by application of γ-$C_6H_6Cl_6$ to the soil.
P. S. ARUP.

Control of chafer grubs (*Schizonycha* sp. *Coleoptera, Melolonthinæ*) in the Sudan. D. G. Pollard (*Bull. ent. Res.*, 1956, **47**, 347—360).—Yields of lubia (*Dolichos lablab*) are greatly improved by application to the soil, during the sowing, of γ-$C_6H_6Cl_6$ at 10—40 g. per acre. The action of the insecticide is repellent, but not lethal to the grub. No action is observed on the pupa. Good lubia crops have a beneficial effect on subsequent cotton crops. (10 references.)
P. S. ARUP.

Control of brown rot, *Alternaria passifloræ*, of passion fruit. Anon. (*Agric. Gaz. N.S.W.*, 1956, **67**, 490—493).—The disease was controlled by spraying with Bordeaux mixture (4–4–50) every 2–4 weeks during spring and summer and every eight weeks until the next pruning. A. H. CORNFIELD.

Control of damping off of safflower with antibiotics. M. L. Gattani (*Plant Dis. Reptr*, 1957, **41**, 160—164).—Filipin, patulin and actidione controlled five root-rotting fungi *in vivo*, but only filipin was non-toxic to safflower seed. Seed treatment with filipin (500—5000 p.p.m.) did not control damping-off. Soaking 7-day old seedlings in filipin (50 p.p.m.) for 72 hr. prior to planting into *Pythium*-infected soil controlled the disease for four weeks. Soil treatment with filipin (50 p.p.m.) for three days controlled the disease in safflower seedlings for three weeks. A. H. CORNFIELD.

Control of ants. E. H. Zeck (*Agric. Gaz. N.S.W.*, 1956, **67**, 494—497).—Chemical methods of controlling black, brown and Argentine ants are described. A. H. CORNFIELD.

Control of *Crematogaster* ants as a means of controlling the mealybugs transmitting the swollen shoot virus disease of cacao in the Gold Coast. A. D. Hanna, E. Judenko and W. Heatherington (*Bull. ent. Res.*, 1956, **47**, 219—226).—The existence of the mealybug (*Pseudococcus njalensis*) depends on attendance by the ants. Attempts at eliminating the ants with the use of insecticides on the trees were unsuccessful unless supplemented by the laborious operation of cutting out the ants' nests from the dead wood of the trees.
P. S. ARUP.

Control of quackgrass, *Agropyron repens*, L. R. S. Dunham, K. P. Buckholz, L. A. Derscheid, B. H. Grigsby, E. A. Helgeson and D. W. Staniforth (*Minn. agric. Exp. Sta.*, 1956, *Bull.* 434, 12 pp.).—Quackgrass is controlled by application of $NaClO_3$ (480—800 lb. per acre in autumn), Na trichloroacetate (" TCA ", 20—40 lb. in late summer), Monuron (3-*p*-chlorophenyl-1 : 1-dimethylurea, 20—40 lb. in spring or autumn), maleic hydrazide (4—6 lb. in spring or summer) or Delapon (2 : 2-dichloropropionic acid, 5—10 lb. in autumn or spring). A. H. CORNFIELD.

Effects of 2 : 4-dichlorophenoxyacetic acid and maleic hydrazide on free amino-acids and proteins in potato, sugar-beet and bean tops. J. L. Fults and M. G. Payne (*Bot. Gaz.*, 1956, **118**, 130—133).—Sprayings with 2 : 4-D (Na salt) or with maleic hydrazide (Na salt) causes significant increases in amino-acid content in sugar-beet and potato tops, but not in bean tops. With 2 : 4-D, a decrease occurs in bean tops. With the combined sprays the effect on the amino-acid content is additive in potato tops, but competitive in beet and bean tops. As regards protein content, the trends are towards increases in potato, and decreases in bean tops ; the effects on beet tops are negligible. The sprayings cause qual. changes in the proteins. P. S. ARUP.

Animal Husbandry

Digestibility of various rations by steers as influenced by the length of the preliminary feeding period. J. W. G. Nicholson, E. H. Haynes, R. G. Warner and J. K. Loosli (*J. Anim. Sci.*, 1956, **15**, 1172—1179).—In digestibility trials with steers, if the change of ration from the preliminary to the experimental period involved a considerable alteration in the ratio hay : grain a preliminary period of 16—30 days was necessary. When the ratio was substantially unchanged but the protein supplement was altered a 7-day preliminary period sufficed. A. G. POLLARD.

Factors influencing *in vitro* rumen cellulose digestion. C. N. Huhtanen and R. F. Elliott (*J. Anim. Sci.*, 1956, **15**, 1180—1187).—The ease of digestion by rumen organisms of the cellulose of lucerne, timothy and Solka Floc decreased in the order named. The organisms attacked only about 50% of the lucerne cellulose whatever the proportion present. The digestion was unaffected by addition of *n*- or *iso*-valeric acid (5—250 μg. per ml.) to the rumen fluid. Feeding chlortetracycline to sheep did not influence the digestion of lucerne cellulose by rumen organisms. A. G. POLLARD.

Use of the œsophageal fistula for the determination of consumption and digestibility of pasture forage by sheep. R. L. Bath, W. C. Weir and D. T. Torell (*J. Anim. Sci.*, 1956, **15**, 1166—1171).—The method described and supported by experimental data is based on sampling the ingested forage by the fistula and on the analysis of fæces from normal animals. Little change occurs in the forage during passage through the fistula. Assuming all the sheep in the trial eat the same quality forage use of the lignin-ratio technique permits calculation of the dry matter intake, total digestible nutrients and the digestible protein in the forage. A. G. POLLARD.

Nutrient consumption and utilization from lucerne pasture soilage and hay. G. P. Lofgreen, J. H. Meyer and M. L. Peterson (*J. Anim. Sci.*, 1956, **15**, 1158—1165).—The intake of dry matter and total digestible nutrients (TDN) by grazing steers was measured by the pre- and post-clipping method and by the chromogen technique. The former method over-estimated the values. The dry matter intake of steers grazing lucerne was 30% smaller than of those fed lucerne soilage but the increases in live-wt. were the same. The net energy of the TDN in forage selected by grazing animals probably exceeds that of the same crop harvested fresh or as hay.
A. G. POLLARD.

Grain equivalent value of pre-bud lucerne hay and lucerne–rye-grass silage in respect of milk production. C. F. Huffman, C. W. Duncan and R. M. Grimes (*Mich. agric. Exp. Sta., Quart. Bull.*, 1956, **39**, 241—247).—In comparisons of pre-bud and flowering lucerne with the silage, 27·4 lb. of pre-bud lucerne hay + 4·2 lb. of maize gave better yields of fat-corrected milk than did 24·5 lb. of mature lucerne hay with 10·2 lb. of maize. A. G. POLLARD.

Sodium bisulphite as a preservative for grass silage. R. L. Cowan, J. W. Bratzler, E. Keck, jun., R. W. Swift, G. Alderman and J. B. Washko (*J. Anim. Sci.*, 1956, **15**, 1188—1198).—The heavy losses occurring during the ensilage without a preservative, of pre-blossom lucerne are lessened and the poor quality of the product much improved by the prior addition of $NaHSO_3$ at the rate of 8 lb. per ton of unwilted green matter (cf. Bratzler *et al.*, *ibid.*, 1955, **15**, 163). When the lucerne was cut at the early-blossom stage (one-tenth in flower) $NaHSO_3$ did not affect the yield to the same extent but greatly improved the quality of the silage. Addition of dried beet pulp or ground maize cobs with $NaHSO_3$ to early-blossom lucerne diminished nutrient losses and increased the feeding value of the silage. Lucerne cut at full-bloom produced inferior silage regardless of the use of $NaHSO_3$. A. G. POLLARD.

Factors influencing iodine content of pasture herbage. G. W. Butler and J. M. Johnson (*Nature, Lond.*, 1957, **179**, 216—217).—The I content of herbage is independent of that in soil and is a strongly inherited characteristic of each strain. J. S. C.

Growth factors in milky extract from immature maize. G. Beauchesne (*C. R. Acad. Sci., Paris*, 1957, **244**, 112—115).—Three groups of growth factors present in the milky extract of maize were distinguished using cultures of tissues of Jerusalem artichoke. Of these groups, two activate cell division and a third promotes cell elongation. J. S. C.

Preliminary report on the nutritional significance of bound gossypol in cottonseed meal. B. P. Baliga and C. M. Lyman (*J. Amer. Oil Chem. Soc.*, 1957, **34**, 21—24).—In the processing of cottonseed meal to reduce the gossypol content to a level sufficient to avoid adverse physiological effects, part of the gossypol may have become inactivated by forming an inert gossypol–protein complex. The relation between such bound gossypol and the nutritional value of cottonseed meal has been examined. Procedures for removing bound gossypol without recourse to heating are described. One procedure involves adding fatty acid amines of 8–9 C to the hexane commercially used for extracting the oil. The nutritional value of cottonseed meal for rats and chickens is greatly increased by removing bound gossypol. Gossypol–protein complex was prepared without " injury " to the protein by adding a methanol solution of gossypol to an aq. suspension of protein. The repletion value of the gossypol–protein complex was one-third of that of the original protein and the lysine availability was halved ; the N solubility fell from 89·0 to 48·3%. G. HELMS.

Dicalcium phosphate and " soft " phosphate with colloidal clay as sources of phosphorus for beef heifers. T. A. Long, A. D. Tillman, A. B. Nelson, B. Davis and W. D. Gallup (*J. Anim. Sci.*, 1956, **15**, 1112—1118).—In a 98-day trial " soft " phosphate was inferior to $CaHPO_4$. Animals given the clay phosphate showed pica, coprophagy, enlarged joints, difficulty in walking and low ash content in bones. They did not recover when turned out to pasture.
A. G. POLLARD.

Growth performance and blood- and liver-copper values in Hereford calves offered certain mineral elements free-choice. W. E. Dent, H. B. Howell, F. W. Adams and J. P. Mehlig (*J. Anim. Sci.*, 1956, **15**, 1103—1111).—Heifer calves given a silage–grain–hay–$CaHPO_4$ ration showed increased growth and food intake following free-choice feeding of Cu, Co and Mg. The daily intake of the three elements averaged respectively 17·3, 0·6 and 60 mg. No consistent relationship was apparent between whole blood- or plasma-Cu and liver-Cu although initially the extreme high and low values in blood coincided with the corresponding extremes in liver. The amounts of Fe and Cu stored in the liver were inversely related. Animals with low liver-Cu did not tend to correct this by free-choice Cu feeding. A. G. POLLARD.

Errors in drying silage and fæces for protein and energy determinations. Improved procedures. N. F. Colovos, H. A. Keener and H. A. Davis (*J. Dairy Sci.*, 1957, **50**, 173—179).—All silages and fæces lost significant amounts of protein during drying prior to analysis. Silage preserved with molasses, SO_2 or $Na_2S_2O_5$ was more stable during drying than were maize or unpreserved grass–legume silages. Loss of crude protein during drying might be used to assess the effectiveness of silage preservatives. An improved procedure for determining energy values on fresh materials is described. S. C. JOLLY.

Stability of supplements and feeding stuffs containing penicillin. R. Brunner, G. Machek and H. Margreiter (*Mitt. VersSta. Gärungsgew.*, 1957, **11**, 4—9).—The grinding (during 6 hr.) of procaine-penicillin supplements, with dried penicillin mycelium as carrier, causes considerable losses in activity (25—90%) on storage during 1 week—1 year. The loss by the unground material after 1 year is ~10%. The loss is not caused by comminution of the mycelium, excessive moisture, or by intentional infection with penicillinase or with penicillinase-producing organisms, but must be attributed to the effect of grinding on the procaine-penicillin itself. P. S. ARUP.

Intracellular distribution of radio-iodine-labelled lactogenic hormone in the rabbit mammary gland. W. F. Williams and C. W. Turner (*Missouri agric. Exp. Sta.*, 1956, Res. Bull. 598, 52 pp.).—The distribution of ^{131}I-labelled lactogenic hormone after intraductal injection into the rabbit is reported. A. H. CORNFIELD.

Assay of acetyl-(*p*-nitrophenyl)sulphanilamide in feeds. J. W. Cavett (*J. Ass. off. agric. Chem. Wash.*, 1956, **39**, 964—967).—A cooled methanol extract of the feed is treated with $ZnSO_4$ solution and a few drops of aq. NaOH. After 2 min at 100° the mixture is cooled, diluted and filtered; an aliquot is acidified (HCl) and heated at 100° for 1 hr., then cooled and diluted. An aliquot is placed in each of two colorimeter tubes; to one is added aq. *N*-1-naphthylethylenediamine dihydrochloride, whilst to the other is added $NaNO_2$ solution. After 3 min. ammonium sulphamate is added, followed by *N*-1-naphthylethylenediamine dihydrochloride. The extinction of each is read in a photoelectric colorimeter and the acetyl-(*p*-nitrophenyl)sulphanilamide content is read from a standard curve. Arsanilic acid or sulpha-drugs cause high results. A. A. ELDRIDGE.

Colorimetric method for the determination of 4-nitrophenylarsonic acid in feeds. J. W. Cavett (*J. Ass. off. agric. Chem., Wash.*, 1956, **39**, 967—969).—The 4-nitrophenylarsonic acid is extracted from the feed by hot methanol and reduced with $Na_2S_2O_4$ to the amino-compound which is then diazotized and coupled with *N*-1-naphthylethylenediamine, the extinction of the red colour being determined in a photoelectric colorimeter and used with standard curves to determine the quantity present. A. A. ELDRIDGE.

Assay for dinitrodiphenylsulphonylethylenediamine in feed. J. W. Cavett (*J. Ass. off. agric. Chem., Wash.*, 1956, **39**, 969—972).—Acetyl-*p*-nitrophenylsulphanilamide (**I**) and dinitrodiphenylsulphonylethylenediamine (**II**) are extracted with hot methanol. **I** is deacetylated and **II** is reduced by heating with $Na_2S_2O_4$ and NaOH and the absorbance due to both after treatment with $NaNO_2$, ammonium sulphamate and *N*-1-naphthylethylenediamine is determined with a photoelectric colorimeter. The absorbance due to the quantity of **I** usually present is determined and subtracted, the result being read from standard curves. Other "sulfas" and arsenicals, if present, cause high results. Certain interfering substances can be removed by chromatography. The method is suitable for routine control. A. A. ELDRIDGE.

Fertility of bovine spermatozoa stored at − 79° for one week and for seventeen weeks. R. W. Bratton, J. C. Flood, R. H. Foote, S. Wearden and H. O. Dunn (*J. Dairy Sci.*, 1957, **50**, 154—162).—Confirmation is presented, based on a larger no. of breedings, of a previous report (Bratton *et al.*, *ibid.*, 1955, **38**, 40) that the fertility of bovine semen stored for 103 days at − 79° and thawed immediately before use was equal to that of unfrozen semen used within 24—36 hr. of collection and dilution. A kit capable of maintaining semen at − 79° for six days at the expense of 2 lb. of "dry ice" is described. S. C. JOLLY.

Metabolic behaviour of bovine spermatozoa as influenced by bacteria. M. S. Trueblood, M. L. Hopwood, S. M. Morrison and H. J. Hill (*J. Dairy Sci.*, 1957, **50**, 149—153).—The O_2 uptake of semen was significantly increased by the presence of *Bacillus* and *Pseudomonas* spp., due to ability of spermatozoa to utilize bacterial intermediate metabolites or to a stimulatory effect of the metabolites on spermatozoa metabolism; seminal plasma had little or no O_2 uptake. Dihydrostreptomycin sulphate (500 μg. per ml.) had little or no effect on the metabolism of spermatozoa or *Pseudomonas*, but inhibited respiration of *Bacillus* to a significant extent. S. C. JOLLY.

Bovine semen metabolism. I. Methods for expressing fructolytic activity. J. P. Mixner, R. E. Mather and M. Freund (*J. Dairy Sci.*, 1957, **50**, 142—148).—The ability of spermatozoa to utilize fructose, independent of the initial substrate concn. and no. of spermatozoa, may be expressed by the regression coeff. of a first-order reaction based on fructose utilization in successive equal periods of incubation. S. C. JOLLY.

Environmental physiology and shelter engineering, with special reference to the domestic animal. XXXVII. Moisture vaporization by Jersey and Holstein cows during diurnal temperature cycles, as measured with a hygrometric tent. R. G. Yeck and H. H. Kibler. **XXXVIII. Influence of diurnal temperature cycles on heat production and cardiorespiratory activities in Holstein and Jersey cows.** H. H. Kibler and S. Brody. **XXXIX. Environmental temperature and blood volume.** H. E. Dale, G. J. Burge and S. Brody (*Missouri agric. Exp. Sta.*, 1956, Res. Bull. 600, 19 pp.; Res. Bull. 601, 28 pp.; Res. Bull. 608, 20 pp.).—Results are presented. A. H. CORNFIELD.

Utilization of urea nitrogen by young dairy calves. L. D. Brown, C. A. Lassiter, J. P. Everett and J. W. Rust (*J. Anim. Sci.*, 1956, **15**, 1125—1132).—When half of the N of a conventional starter ration for calves (>6 weeks old) was supplied as urea there was no significant change in growth rate or feed efficiency. Digestion of crude fibre was less in the case of urea-fed calves. A. G. POLLARD.

Value of lucerne silage in the diet of the young dairy calf. G. H. Porter and E. M. Kesler (*J. Dairy Sci.*, 1957, **50**, 163—172).—Growth of Holstein calves fed good quality lucerne silage (*S*) *ad lib.* as the sole source of roughage during the first 16 weeks of life was equal to that of calves fed either lucerne hay (*H*) or a combination of *S* and *H*; similar results were obtained with Guernseys. With *H* or *S* and *H* in the ration, growth and total-digestible-nutrient consumption tended to parallel each other, but with *S* alone consumption of dry matter and total digestible nutrients was relatively low. Growth similar to the Ragsdale standard was obtained by restricting consumption of starter to 3 lb. daily with high-quality roughage *ad lib.* Feeding *S* did not cause any digestive disturbances. S. C. JOLLY.

Effect of streptomycin on growth of young calves. L. L. Rusoff, F. T. Landagora and Barney Harris, jun. (*J. Dairy Sci.*, 1957, **50**, 119—122).—The growth of dairy calves was increased by 13% at 12 weeks of age by feeding 50 mg. of streptomycin sulphate (**I**) daily from birth; with 30 mg. of **I** daily, growth stimulation ceased after four weeks. Leucocyte counts of calves receiving **I** at the higher rate were significantly lower than those of controls after four weeks; those of calves on the lower rate were lower, but not significantly so. S. C. JOLLY.

Compensatory growth of beef cattle: effect of protein supplements. V. R. Bohman and C. Torell (*J. Anim. Sci.*, 1956, **15**, 1089—1096).—Although calves in winter on hay harvested at an early stage of maturity gained more wt. than those receiving hay cut at a later stage, the latter group gained faster in the following summer and the final wt. of the two groups were the same. Feeding protein supplements tended to mask the effect of differences in hay quality. Summer growth was influenced by the type of protein supplement given in the previous winter, lucerne giving an increase greater than and cottonseed meal less than that of control animals given no protein supplement. The wt. of animals receiving either of the protein supplements in winter exceed those of controls in the following autumn. A. G. POLLARD.

Effect of wintering plane of nutrition on subsequent gains of beef yearling steers on irrigated pastures. W. W. Heinemann and R. W. Van Keuren (*J. Anim. Sci.*, 1956, **15**, 1097—1102).—Calves receiving high, medium or low levels of winter feed (155 days), when turned out to pasture showed increases in wt. inversely related to the plane of winter nutrition. In all cases the rate of gain was greater on a grass–clover than on a grass pasture. A. G. POLLARD.

Effect of plane of nutrition of beef cows on the depletion of liver vitamin A during gestation and on carotene requirements during lactation. D. C. Church, L. S. Pope and R. MacVicar (*J. Anim. Sci.*, 1956, **15**, 1078—1088).—During gestation cows were given either a normal adequate ration or one low in protein and energy, the carotene level being low in both cases. Depletion of liver vitamin A was unaffected by the plane of nutrition in one trial but was delayed by the low-energy ration in another. During lactation also depletion was slower with the low-energy ration. No relationships were apparent between the carotene and vitamin A levels in cows and those in their calves. Supplementary feeding of carotene during lactation (126—150 mg. per head daily) retarded depletion and raised the plasma-carotene and A levels although deficiency symptoms were still apparent in the calves. Cows receiving low-carotene rations during the latter half of gestation required <20 mg. of carotene per 100 lb. live-wt. daily during lactation irrespective of the plane of nutrition. A. G. POLLARD.

Effect of level of grain feeding upon efficiency of milk production. E. L. Thomason, jun., M. Ronning and E. R. Berousek (*Oklahoma agric. Exp. Sta.*, 1956, Bull. 483, 8 pp.).—Cows fed 1 lb. of grain for each 8 lb. of milk produced showed a greater return over feed costs per 100 lb. of 4% milk than did cows fed 1 lb. of grain for each 3 or 5 lb. of milk produced. A. H. CORNFIELD.

Utilization of dicyanodiamide and urea by lactating dairy cows. J. W. Rust, C. A. Lassiter, C. Davis, L. D. Brown and D. M. Seath. (*J. Anim. Sci.*, 1956, **15**, 1133—1140).—Urea, dicyanodiamide (**I**) and soya-bean meal were compared as N sources in a low-protein concentrate mixture for cows, the protein content of the common hay-grain ration being limited to 90—100% of the Morrison standard min. Yields of fat-corrected milk over 196 days were the same for all three rations. Body wt. showed a slight gain with soya-bean meal but losses with urea and, especially, with **I** in the concentrate. Coeff. of apparent digestibility of all nutrient fractions except ether extract were lower for the **I** than for the other rations. The **I**-N was less efficiently utilized. A. G. POLLARD.

Value of maize distillers dried grains, coconut oil meal and maize gluten feed for milk production. R. G. Warner, J. K. Loosli and R. F. Davis (*J. Dairy Sci.*, 1957, **50**, 123—127).—The total milk production of cows fed a concentrate mixture containing 40% of maize distillers' dried grains was significantly higher than that of cows receiving maize gluten feed or coconut oil meal in the mixture. The fat content of milk from cows on the latter feed was, however, significantly higher. S. C. JOLLY.

Short-term use of iodinated casein for milk production. J. W. Thomas, D. V. Kopland, E. A. Keyes and L. A. Moore (*J. Dairy Sci.*, 1957, **50**, 128—141).—The large and rapid decline in milk production that follows withdrawal of thyroprotein from the ration of cows was not significantly different whether the withdrawal was gradual or abrupt. Monetary advantage was gained by thyroprotein feeding only if the response in milk production was large. S. C. JOLLY.

Effect of progesterone–œstradiol implants and stilbœstrol feeding on feed-lot performance and carcass characteristics of steers. R. J. Deans, W. J. Van Arsdell, E. P. Reineke and L. J. Bratzler (*J. Anim. Sci.*, 1956, **15**, 1020—1028).—Oral administration of Et_2 stilbœstrol (10 mg. per head daily) increased the growth rate of steers and implantation in combination with progesterone (1·5 g.) and œstradiol (50 mg.) had a still greater effect. Carcass grades were unaffected. A. G. POLLARD.

Influence of fat, chlortetracycline and stilbœstrol on vitamin A and carotene storage in the liver of beef steers. E. S. Erwin, I. A. Dyer and M. E. Ensminger (*J. Anim. Sci.*, 1956, **15**, 1147—1153).—Storage of carotene but not that of vitamin A in the livers was increased by feeding inedible animal fat (" bleachable fancy tallow ") at the rate of 7% of the ration. Liver-carotene and -vitamin A were unaffected by inclusion of stilbœstrol (0·45 mg.) or chlortetracycline (5 mg. per lb.) in the ration. A. G. POLLARD.

Parasitism in beef yearlings as related to forage availability and levels of protein feeding. H. H. Vegors, D. M. Baird, O. E. Sell and T. B. Stewart (*J. Anim. Sci.*, 1956, **15**, 1199—1206).—In calves on full pasture the no. of parasitic larvæ were more numerous and adult worms less numerous than in those on part pasture or dry fed. Under the latter conditions high protein feeding was associated with fewer larval stomach worms. A. G. POLLARD.

Utilization of a high level of lucerne by growing-fattening swine. J. F. Kidwell and J. E. Hunter (*J. Anim. Sci.*, 1956, **15**, 1067—1071).—Comparison of pigs fed a conventional ration with those given a ration which included 50% of lucerne, showed that at this level of supply 1 lb. of lucerne replaced 0·5 lb. of barley + protein supplement (soya-bean oil meal + meat scrap). Carcasses from the lucerne-fed pigs had a smaller dressing %, lower proportions of fat cuts and a higher % of lean and lean cuts. A. G. POLLARD.

Swine feeding with cooked residential garbage. H. Heitman, jun., C. A. Perry and L. K. Gamboa (*J. Anim. Sci.*, 1956, **15**, 1072—1077).—Feeding the unsupplemented garbage to pigs produced 39—80 lb. of pork per ton of feed. At the rate of 10% of the garbage 1 lb. of barley replaced 9—10 lb. of garbage. Addition of chlortetracycline to the garbage, with or without barley, increased the rate of growth and the % utilization of the feed. A. G. POLLARD.

Use of stabilized tallow in swine rations. H. Heitman, jun. (*J. Anim. Sci.*, 1956, **15**, 1046—1051).—Supplements (5—10%) of stabilized tallow in a pig ration accelerated growth rates. Stabilized lard had no significant effect. Feed efficiency and consumption varied with the protein content and nutritive ratio of the ration. No off-flavour in tallow-fed pork was apparent. A. G. POLLARD.

Effect of high copper supplements on growth of fattening pigs. A. Schürch (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 458—463).—Supplementation of a ration already containing aureomycin (10 p.p.m.) with 230 p.p.m. (dry basis) of Cu (as $CuSO_4.5H_2O$) promoted growth and nutrient utilization. The favourable outcome was probably largely due to an attack of influenza which affected the control animals during the 6th to the 8th week and against which the Cu apparently afforded protection. P. S. ARUP.

Protein levels with and without an antibiotic for growing-finishing swine fed on legume pasture. V. C. Speer, G. E. Combs, jun., G. C. Ashton and D. V. Catron (*J. Anim. Sci.*, 1956, **15**, 1052—1058).—Pastured pigs were fed, from 30 to 200 lb. live-wt., on rations of varied protein content (8—18%). Growth rates, feed intake and feed efficiency were directly related to dietary protein levels with an optimum at 14% of protein. Supplements of chlortetracycline hydrochloride (5 mg. per lb. of feed) did not affect growth rates or feed efficiency. A. G. POLLARD.

Effect of high-level antibiotic supplementation during part or all of the growing-fattening period of swine. R. C. Wahlstrom (*J. Anim. Sci.*, 1956, **15**, 1059—1066).—Administration of a 1 : 3 mixture of penicillin and streptomycin (25—100 g. per ton of feed) or of chlortetracycline (100 g. per ton) increased the rate of gain in wt. of weanling pigs. At all rates of feeding the mixed antibiotic produced similar gains in wt. but with the higher (50 and 100 g.) rates the feed consumption and feed intake per unit gain in wt. were lowered. Growth rates were lowered when the mixed antibiotic was discontinued at the stage of 100 lb. live-wt. but not when the dosage was lowered to 10 g. per ton at this stage. Omission or diminution of the chlortetracycline supplement at 100 lb. live-wt. depressed the feed intake and the average daily gain in wt. during the growth period from 100 to 200 lb. live-wt. A. G. POLLARD.

Factors affecting growth, feed efficiency and carcass quality in swine. L. F. Tribble, W. H. Pfander, J. F. Lasley, S. E. Zobrisky and D. E. Brady (*Missouri agric. Exp. Sta.*, 1956, Res. Bull. 609, 46 pp.).—From weaning to 100 lb., wt. gains and feed efficiency were higher on a 16%- than on a 12%-protein diet, but the reverse was true from 100 lb. to 200 lb. Addition of chlortetracycline (0·018 g. per lb. of feed) to the diet gave more rapid wt. gains up to 200 lb. and increased feed efficiency up to 100 lb. Restricting the feed intake to 85% of full feed resulted in slower wt. gains. The effects of the treatments on carcass quality are also reported. A. H. CORNFIELD.

Effect of oral administration of methyl testosterone on swine growth and development. P. R. Noland and M. J. Burris (*J. Anim. Sci.*, 1956, **15**, 1014—1019).—Methyl testosterone (1·5 mg. per kg. body wt. daily) slightly lowered the growth rates of female but not of male pigs. Carcasses of treated animals were leaner than those of controls. There was marked suppression of spermatogenesis in treated boars. A. G. POLLARD.

Parakeratosis in swine. J. W. Stevenson and I. P. Earle (*J. Anim. Sci.*, 1956, **15**, 1036—1045).—Feeding trials described indicate in pig rations containing up to 1% of Ca, Zn (40—80 p.p.m.) is needed to prevent parakeratosis. A. G. POLLARD.

Effect of varying levels of protein and cerelose on the utilization of mature timothy hay by sheep. W. R. Woods, C. M. Thompson and R. B. Grainger (*J. Anim. Sci.*, 1956, **15**, 1141—1146).—Rations of low-quality timothy hay were supplemented with varying levels of soya-bean meal and of cerelose. Additions of soya-bean meal to give 6·9—13·6% of protein in the ration increased the apparent digestibility of dry matter, org. matter, protein, N-free extract and energy. Additions of cerelose to form 3·3 and 6·2% of the ration increased the apparent digestibility of the N-free extract without affecting that of the other fractions. With 6% of cerelose protein digestibility diminished. A. G. POLLARD.

NN′-Diphenyl-p-phenylenediamine in the prevention of vitamin-E deficiency in the lamb. H. H. Draper (*J. Anim. Sci.*, 1956, **15**, 1154—1157).—Addition of this compound (0·10% of the dry matter) to an artificial milk diet containing tocopherol-free lard as source of fat prevented vitamin-E deficiency symptoms in lambs. A. G. POLLARD.

Feeding high-efficiency rations to laying hens. R. H. Thayer and D. L. Brooks (*Oklahoma agric. Exp. Sta.*, 1956, Bull. 480, 19 pp.).—Feed formulæ and an explanation of the feeding systems are described. A. H. CORNFIELD.

Hydrolysed poultry feathers and poultry by-product meal in broiler rations. E. L. Stephenson, A. A. Jimenez, W. Dean and C. Treat (*Arkansas Farm. Res.*, 1957, **6**, No. 1, 3).—Part of the protein of a broiler ration was replaced in varying proportions (2·5—15%) on an equi-protein basis) of the two products separately and in combination without causing change in body wt. of chicks. A. G. POLLARD.

Effect of vitamin B_{12} on egg production and hatchability. L. Kállai, B. Aros, F. Biszkup and U. P. Kralovánsky (*Acta agron. hung.*, 1956, **5**, 441—449).—Feeding vitamin B_{12} to ducks or geese (25 μg. per kg. of ration) increased the output of eggs. Injection of the vitamin into the yolks of hens' eggs improved hatchability and increased the wt. of chicks hatched. When supplied to hens (25 μg. per kg. of feed) vitamin B_{12} was transferred to the eggs in amounts sufficient to increase the proportion of chicks hatched by 10%. A. G. POLLARD.

Antibiotics in poultry feeding. M. W. McDonald (*Agric. Gaz. N.S.W.*, 1956, **67**, 531—536).—A general account. A. H. CORNFIELD.

Antibiotic supplements at high levels in poultry foods. W. M. McKay (*Agric. Rev., Lond.*, 1957, **3**, 17—20).—In intensive poultry keeping the use of small dosages of antibiotics from an early age is preferable. Factors influencing the choice of continuous *versus* intermittent use and high *versus* low levels of antibiotics are discussed. A. G. POLLARD.

Storage of fodder beet. Koninklijke Industrieele Maats. (B.P. 752,176, 15.9.54. Neth., 13.5.54).—Storage properties of the beet are improved by treating with a mixture of chloronitrobenzene (2—10), an insecticide, e.g., Gammexane (0·01—1%), and a carrier. F. R. BASFORD.

Preparation of salt of S-aryl-thiosulphuric acids. American Cyanamid Co. (B.P. 751,889, 12.4.54. U.S., 14.5.53).—Compounds $R{\cdot}S{\cdot}SO_3M$ (R is aryl, M is cation), especially S-m-*nitrophenylthiosulphuric acid* (**I**) and its salts, useful in the treatment of coccidiosis in chickens, are economically made by interaction of $R{\cdot}S{\cdot}X$ (X is amido group) with aq. SO_2. Thus, SO_2 is passed during 1 hr. into a suspension of crude *m*-nitrobenzene sulphen-*NN*-diethylamide in water and EtOH, then after heating to 50—60° the solution is clarified, and evaporated, to leave the $NHEt_2$ *salt* of **I**, m.p. 89—90°. F. R. BASFORD.

Fungicides. N.V. Philips' Gloeilampen-Fabrieken (B.P. 752,255, 26.4.54. Neth., 29.4.53).—*Trichloromethyl* p-*acetamidobenzene-thiosulphonate*, m.p. 150—155°, useful as a fungicide of low phytotoxicity, is prepared by interaction of perchloromethyl mercaptan with an alkali metal salt of *p*-acetamidobenzenesulphonic acid. With a suitable diluent it is prepared for use as an oil, aërosol, spray powder, lycopodium powder, etc. F. R. BASFORD.

Insecticidal composition. Compania General de Insecticidas S.A. (B.P. 752,487, 15.4.54. Mex., 21.4.53).—A homogeneous, water-dispersible, liquid insecticidal composition, *d* 1·1, pH 7·4, which can be used on plants without burning thereof, comprises mineral oil (34), abietic acid (25), α-pinene (25), 47% aq. NaOH (7), EtOAc (7), and a chlorinated insecticide, e.g., DDT, Gammexane, chlordane, chlorinated camphor, dieldrin or aldrin (13 pt.). F. R. BASFORD.

ε-Acyllysines. Farbenfabriken Bayer A.-G. (B.P. 751,524, 19.3.54. Ger., 19.3 and 4.7.53).—The compounds useful as additives in cattle- and poultry-feed, are obtained economically by saponifying hexolactam, acylating the resulting ε-aminocaproic acid (with fatty acid anhydride or chloride), chlorinating the acyl derivative (with thionyl and/or sulphuryl chloride), and treating the ε-acylamine α-chloro-caproic acid with ≯50% aq. NH_3 (in absence of iron). The yield of ε-acetyllysine by this process is 75—80% (or 70% only in Fe equipment). F. R. BASFORD.

2.—FOODS

Elucidation of [process of] formation of bitter substance in cereal products. M. Rothe (*Ernährunsgforschung*, 1956, **1**, 315—324).—Experimental evidence shows that the development of bitter taste in oat grist depends on the simultaneous presence of the following three factors: (*a*) the presence of a fatty oil, the effect of the oil being roughly proportional to its degree of unsaturation, and independent of the terminal ester-group, (*b*) an antioxidant, partly removable by extraction with light petroleum, and (*c*) a water-sol. enzyme which is adsorbed by Al_2O_3 and destructible by steaming or treatment with EtOH at the b.p. The special factor distinguishing oats from other cereals is the presence of the antioxidant which diverts the normal auto-oxidation of the oil into a course resulting in the production of the bitter substance. A working hypothesis is presented to account for the above observations. (13 references.) P. S. ARUP.

Cereal starches. I. Flow curves of rice starch pastes. T. Tani, S. Chikubu and H. Horiuchi (*J. agric. chem. Soc. Japan*, 1956, **30**, 179—182).—Starch pastes from domestic rice (*Oryza sativa japonica*) and Burmese rice (*O. sativa indica*) were examined by a Stormer viscometer and were found to show plastic flow at 1—10% from the curves of *S* (shearing stress, g.) *versus D* (deformation rate as the rate of shear, r.p.m.). Irrespective of temp., concn. and cooking time, the starch pastes of *indica* rice had stronger torque than those of *japonica* rice. Measurement of the strength of starch gels by a Tarr-Baker jelly tester showed that *indica* rice was stronger than *japonica*. The *japonica* rice starch was more viscous than the *indica*, when measured by a B-type viscometer (similar to the Brookfield viscometer). S. KAWAMURA.

Quality control programme for the processing of sweet maize. A. Kornetsky and A. Kramer (*Food Technol.*, 1957, **11**, 188—192).—The application is reported of statistical quality control (SQC) techniques, with which quality factors in sweet maize might be readily evaluated objectively, to two plants (cream style and whole kernel packs). (22 references.) E. M. J.

Development and evaluation of new wheat varieties. R. H. Harris, L. D. Sibbitt and G. M. Scott (*Food Technol.*, 1957, **11**, 166—169).—The quality evaluation of selected progeny from a cross between a red vitreous wheat (Rushmore) and a white starchy wheat (Kenya 338 AC) is discussed and the methods used in the breeding of these hybrids are described. The progeny varied greatly in milling, baking and physical dough properties and the range in quality characteristics generally tended to exceed that between the parent types. E. M. J.

Attempts at improvement of gluten quality of wheat by ultrasonic and infra-red irradiation. E. Anders (*Ernährungsforschung*, 1956, **1**, 379—385).—In preliminary trials, no appreciable improvement in baking quality is attained by ultrasonic irradiation of the grain. The quality of inferior wheat can be improved by i.r. irradiation. P. S. ARUP.

Determination of potassium bromate in flour. A. W. Armstrong (*Analyst*, 1956, **81**, 616).—When KI is added to the acidified filtrate obtained after treatment of flour with aq. $ZnSO_4$ and aq. NaOH, iodine is liberated if the flour has been bleached with NO_2. Consequently determinations of bromate added to such flours by the method previously reported (Brit. Abstr. C, 1953, 28) yield high results and the error may reach 1·5 g. per sack. This error can be eliminated completely by addition to the filtrate of specified amounts of aq. ammonium sulphamate after, or of hydroxylamine before, acidification. In either procedure the mixture should be set aside for 2 min. before continuing the analysis. A. O. JONES.

Peroxidase-type reaction of wheaten flour: sensitivity to rate of extraction and to treatment with chlorine dioxide. E. E. McDermott and J. Pace (*Chem. & Ind.*, 1957, 234—235, 429).—The bluish-purple colour formed by the peroxidase reaction between H_2O_2 and benzidine added to flour was found to be less intense in flour treated with ClO_2 than in untreated flour. A precise method of observing such reactions is described and the use of the peroxidase test for grading flours discussed. In an addendum it is stated that, in addition to benzidine, thymol is also essential to the operation of the test. J. S. C.

Consequences of mechanical disturbance of starch grains. M. Ulmann (*Ernährungsforschung*, 1956, **1**, 284—303).—The effects produced by grinding in a ball mill which has in addition to the rotatory motion a strong intermittent oscillatory movement, on potato starch are slight in comparison with those produced on cellulose and are not increased by prolonged grinding. Grinding distorts the starch granules and greatly facilitates both the dissolution of the starch in cold water, and the chromatographic separation (on Al_2O_3) of the amylose and amylopectin components. The Al_2O_3-chromatogram, on treatment with aq. I fails to show the reddish-brown band due to dextrin or the dark-blue-violet band associated with the higher amylopectins. The blue amylose band is correspondingly widened. Apart from the depolymerization of the higher

amylopectins (which causes a decrease in the η of the solution), the main effect of the grinding is the dissociation of mol. aggregates. P. S. ARUP.

Changes in the water-soluble carbohydrates of chapaties during ageing. N. Nath, S. Singh and H. P. Nath (*Food Res.*, 1957, **22**, 25—31).—There is decrease in water-sol. reducing sugars and polysaccharides of aq. extract of chapaties after storage for 24 hr. under different conditions; reducing sugar is variable; after storage for six months, there is increase in the reducing sugars (glucose and fructose) and appreciable decrease in polysaccharides. (11 references.) E. M. J.

Bran extract for increasing nutrient value of [rye] bread? B. Thomas and K. Fuchs (*Ernährungsforschung*, 1956, **1**, 304—314).—The addition to rye meal dough of rye bran extract prepared by the Friese process does not enrich the bread with B vitamins or other nutrients to any significant extent. P. S. ARUP.

Simple method for determination of inner surface (capillarity) of [rye] bread crumb. H. Rönnebeck (*Ernährungsforschung*, 1956, **1**, 372—378).—The method of Meljnikow is unsatisfactory. Reproducible results are obtained by suspending freshly cut 3-mm.-thick strips of the bread with the ends dipping into red-coloured (with Sudan III) xylene, and noting the height to which the xylene has risen after 12 min. Each strip is suspended from the stopper of a tap-funnel, and the xylene is introduced and kept at constant level through the tap. The results show wide variations in the porosity of different samples of rye bread, and do not vary with the ageing (during 1—3 days) of the bread. P. S. ARUP.

Rôle of lipins in baking. IV. Some further properties of flour lipins and defatted flours. M. A. Cookson, M. L. Ritchie and J. B. M. Coppock (*J. Sci. Food Agric.*, 1957, **8**, 105—116).—Factors affecting the characteristics of flour are discussed. Variation in the bread-making quality of defatted flours is attributed to differences in condition of wheat constituting the grist and to flour extraction rate. Methods of fractionating flour lipins and effects on the bread properties of various fractions are described. Dough and baking tests on undefatted and defatted flours in which the extracted lipins have been replaced indicated that the original nature of the flour lipin cannot be restored. U.v. absorption spectra of the lipins of milling products and breads might be used (*a*) to indicate the bran content of a flour and (*b*) whether the flour had been treated, with ClO_2 or $KBrO_3$. (15 references.) E. M. J.

Isolation and identification of aromatic constituents of rye bread. B. Thomas and M. Rothe (*Ernährungsforschung*, 1956, **1**, 362—371).—The total aromatic constituents can be extracted from the bread by means of ether, and separated into a main water-sol. aldehydic or ketonic fraction, and a minor water-insol. fraction. Hydroxymethylfurfural is probably present in the main fraction. Chromatographic analysis of the 2 : 4-dinitrophenylhydrazones obtained from the two fractions reveals the presence of four different substances containing carbonyl groups. The yield of aromatic material is very small owing (partly) to its instability. Improved yields can be obtained by drawing a current of air over the hot bread and condensing the volatile matter in an ice-cooled U-tube. A convenient colorimetric method for determining the aldehydic and/or ketonic material in the condensate can probably be devised. P. S. ARUP.

Effect of γ-irradiation on cake mixes at high and low moisture levels. H. E. Bauman, H. Baeder, J. A. Stein, C. G. Harrel and R. A. Larsen (*Food Technol.*, 1957, **11**, 193—196).—At irradiation levels which would decrease bacterial no., the colour, odour and baking characteristics of cake batters were markedly changed. At levels of 3×10^4 and 5×10^4 rep there were changes in colour and odour on subsequent storage. Up to 2×10^6 rep there was no effect on α-amylase activity, but the gelatinization properties of the starch were markedly changed. At no level of irradiation used was there any change noted in total or reducing sugar, fat, I value of the extracted fat, monoglycerides, acidity or pH of the batters. Up to 1×10^5 rep dry cake mix made acceptable cakes; at 5×10^5 rep the cake was slightly off colour and odour; 1,000,000 rep resulted in off colour, off odour, gelatinous cakes. No change in α-amylase activity was noted in the dry cake mixes at any of the irradiation levels used, but the gelatinization characteristics of the starch were changed at high levels. There was no change initially in the total or reducing sugar, pH, fat, I value of the extracted fat, monoglyceride, or acidity of the dry cake mixes. (11 references.) E. M. J.

Unfermentable sugars. XVII. Disaccharides in koji extract and saké. 4. Isolation and identification of αα- and αβ-trehalose. K. Matsuda (*J. agric. chem. Soc. Japan*, 1956, **30**, 119—123).—Both αα- and αβ-trehalose were isolated from koji extract as their cryst. octa-acetates, and the latter was identified by mixed m.p. with the synthesized specimen prepared by the method of F. Micheel and K. O. Hagel (*Chem. Ber.*, 1952, **85**, 1087). Separation of these sugars from *iso*maltose was performed by removing *iso*maltose as its water-insol. phenylosazone. There are recorded different physical constants for αβ-trehalose in the literature. Those obtained by Matsuda are m.p. 150—153°, m.p. of octa-acetate 140—141°, [α] of octa-acetate +82°. Kojibiose also gave no phenylosazone and was presumed to be 1, 2-α-linked disaccharide, but neither the free sugar nor its octa-acetate could be isolated in cryst form. (17 references.) S. KAWAMURA.

Quantitative determination of reducing sugars and sucrose separated by paper chromatography. R. S. Shellenberger and R. G. Moores (*Analyt. Chem.*, 1957, **29**, 27—29).—The sugars are separated and detected by normal chromatographic procedure and are determined by treating the "cut out" spots with Nelson's reagent (*J. biol. Chem.*, 1944, **153**, 375) and measuring the absorbance at 500 mμ. Sucrose is determined in the same way after hydrolysis with invertase. The standard deviation is <5 μg. in the range 10 to 200 μg. The method is applicable to the analysis of plants and foods. G. P. COOK.

Rapid chromatographic method for sugars using glass paper impregnated with silicic acid. J. W. Dieckert and W. J. Morris (*Analyt. Chem.*, 1957, **29**, 31—32).—Sucrose, raffinose, D-glucose, L-fructose, D-xylose and L-rhamnose were successfully separated, singly and as a mixture. The developing solvent was a mixture of ethyl ether, phenol, acetone and water and the spray reagents used were conc. H_2SO_4 and *p*-anisidine phosphate. G. P. COOK.

Quantitative chromatographic procedure for determining dextrose in sugar mixtures. E. J. McDonald (*Analyt. Chem.*, 1957, **29**, 32—34).—Dextrose is separated from other sugars by paper chromatography and is then transferred from the paper to glass fibre paper. The dextrose is measured in the presence of the fibre by the method of Somogyi (*J. biol. Chem.*, 1945, **160**, 61). Application to honey samples gave good reproducibility. G. P. COOK.

Simple photometric determination of pentosan in wood. Y. Sakai (*J. agric. chem. Soc. Japan*, 1956, **30**, 256—259).—The colorimetric determination of pentosan with benzidine (**I**) (McCance, *Biochem. J.*, 1926, **20**, 1111) was modified for rapid analysis with a spectrophotometer. The colour produced between furfural (**II**) and **I** reaches max. intensity 20 min after the addition of the reagent and remains unchanged for 5 min., obeying Beer's law for 0·1 to 0·4 mg. of **II** per 5 ml. Conversion of xylose into **II** in 13% HCl was not affected by presence of H_2SO_4. The sample is digested with 72% H_2SO_4, diluted and a portion is treated with HCl in a boiling water bath. The product is cooled, extracted with benzene and the org. layer treated with **I** in a mixture of ethanol and acetic acid for the extinction to be measured at 570 mμ. K. SAITO.

Jelly strength grading of pectins for use in jam manufacture. M. Olliver, P. Wade and K. P. Dent (*J. Sci. Food Agric.*, 1957, **8**, 188—196).—Gels prepared from rapid set powder pectins, but not generally from slow-set powder- or liquid-pectins, by the "acid-in-boil" procedure may vary in strength as measured by the Ridgelimeter, according to the type of sugar used. Modification of the method by the addition of a suitable quantity of a surface-active agent (e.g. Teepol, 5 ml. of a 10% v/v solution per kg. of gel) gives reproducible results approximating to those obtained in the presence of fruit juices. The grade is calculated from the sag of the resultant gel on the basis that 0·705% of 100-grade pectin in the gel would produce a sag of 23·5%, giving results in line with the "acid-in-glass" procedure. E. M. J.

Chemistry of gelatinization of pectins. G. Feldmann and K. Täufel (*Ernährungsforschung.*, 1956, **1**, 260—270).—A theoretical review covering possible modifications in the basic pectin structure which adapt it for functioning as an intermediary in cellulose synthesis, a cementing medium, ion-exchanger, and regulator of water-economy. The mechanism of mol. chain and (consequent) gel-formation is specially considered. P. S. ARUP.

Pectins of sunflower heads. T. K. Gaponenkov and Z. I. Protsenko (*Zh. prikl. Khim.*, 1956, **29**, 1444—1447).—The dry material of the de-seeded heads contains 49% of substances sol. in water at 100°, cellulose 24·3, pentosans 16·3, pectins 25·1, lignin 8·8, protein 6·4, and ash 11·8%. The pectin resembles that from apples. R. TRUSCOE.

Recovery of pectin from orange peel extracts as aluminium pectinate. G. De Luca and M. A. Joslyn (*Food Technol.*, 1957, **11**, 137—141).—In the recovery of pectin, $AlCl_3$ gave better recovery and formed pectinates of lower ash content than did $Al_2(SO_4)_3$ at pH 3·8. Pptn. at 70° resulted in higher recovery with both Al salts than at 30°. (19 references.) E. M. J.

Volatile water-soluble and oil constituents of Valencia orange juice. J. G. Kirchner and J. M. Miller (*J. agric. Food Chem.*, 1957, **5**, 283—

291).—On examination of fresh, freshly canned and stored canned California Valencia orange juices, acetic, propionic, *iso*valeric and traces of butyric acid were found in the stored juice. There was a decreased acetaldehyde content and there were traces of diacetyl. An unidentified acid ($C_8H_8O_2$) was found in canned and stored juices. Data on 29 compounds from the oil fraction in each of the three juices are given. Changes in the oil fraction result in loss in total volatile oil, conversion of hydrocarbons to alcohols and loss in esters, aldehydes and terpene aliphatic alcohols. An off-flavour is derived from the non-volatile portion of the juice. (33 references.) E. M. J.

Rôle of amino-acids in the browning of orange juice. M. A. Joslyn (*Food Res.*, 1957, **22**, 1—14).—The browning of foods and particularly of orange juice is reviewed. Results of storage of Valencia orange juice in air for four years at room temp. indicate that of the amino-acids present, lysine and glutamic acid are more reactive. There is some evidence that decrease in amount observed is the result of reaction with sugars, and for the presence of ninhydrin-positive amino-acid compounds. In the oxidative browning of ascorbic acid–amino-acid–sugar systems ascorbic acid is the most reactive component; glucose and fructose inhibit its browning. The concn. of ascorbic acid originally present has a marked effect on the rate and extent of browning of ascorbic acid solutions. (44 references.) E. M. J.

Colour changes in acerola juice on pasteurization and canning. R. Santini, jun. and A. Huyke (*J. Agric. Puerto Rico*, 1956, **40**, 171—178).—The bright red colour of acerola juice turned to yellow on pasteurization and then to brown after keeping for two months at room temp., with production of CO_2. Stored frozen juice retained its red colour. The anthocyanin malvin was isolated from the juice. Changes in colour are probably due to two types of non-enzymic reactions. A. H. CORNFIELD.

Effect of syrup composition on flavour and texture of canned clingstone peaches. M. A. Joslyn, S. Leonard, E. Hinreiner and B. Filice (*Food Technol.*, 1957, **11**, 170—176).—The effect of replacing sucrose by maize syrup on flavour and texture of the peaches was observed during five canning seasons. Variability between halves in the same can, between cans of the same lot was noted in texture and composition; there were seasonal and varietal differences. Replacement of sucrose by 25% by wt. of maize syrup solids caused increase in firmness, owing to the Ca content of the syrup, and increases in the Ca content of the fruit (10 p.p.m.). Flavour differences observed at the 25% replacement, increased at higher replacement levels. (18 references.) E. M. J.

Organic acid metabolism of apple fruits: changes in individual acids during growth on the tree. A. C. Hulme and L. S. C. Wooltorton (*J. Sci. Food Agric.*, 1957, **8**, 117—122).—Individual org. acids present in the pulp and peel of apple tissue were examined by ion-exchange separation and silica gel chromatography. The concn. of quinic acid falls in both tissues as the fruit reaches maturity, but is greater in the peel than in the pulp; that of malic acid increases and reaches a peak at the end of June; citric acid, present in smaller proportion at concn. $\not>$ 10 mg. per 100 g. of tissue, changes considerably in amount and concn. (18 references.) E. M. J.

Polyethylene film box liners for reducing weight losses and shrivelling of Golden Delicious apples in storage. R. E. Hardenburg (*Proc. Amer. Soc. hort. Sci.*, 1956, **67**, 82—90).—Polyethylene box linings (sealed or unsealed) prevented weight loss and shrivelling in Golden Delicious apples stored at −0·5° for six months. In paperboard boxes O_2 and CO_2 concn. were similar to those of ordinary air. With unsealed linings the O_2 fell slightly after six months at −0·5° (17·6%) whilst the CO_2 concn. rose to as much as 2·9%. For boxes with sealed linings corresponding values were 2—3% of O_2 and 5—6% of CO_2. These effects were enhanced if the unopened boxes were kept at 21° for six days after removal from the cold store unless the polyethylene lining was punctured. L. G. G. WARNE.

Cold storage of pears. H. Kessler and K. Stoll (*Landw. Jahrb. Schweiz.*, 1956, **5**, 259–282).—Data for 24 varieties of pears are recorded, the best times of harvesting, problems of after-ripening and the most suitable temp. and effective periods of storage being examined. A. G. POLLARD.

Metallic components of fruit juices. I. Copper as a factor affecting sedimentation in bottled apple juices. M. E. Kieser, A. Pollard and C. F. Timberlake. **II. Nature of copper complexes in apple juice.** C. F. Timberlake (*J. Sci. Food Agric.*, 1957, **8**, 151—158, 159—168).—I. The characteristic sedimentation occurring in stored pasteurized apple juice results from the pptn. of partly oxidized phenolic compounds with nitrogenous components of a protein nature, and deposition is increased by the presence of a metal, especially Cu. The process takes place in the absence of enzymes and the deposits in apple juice may be compared with the non-biological hazes of beer. The apple phenolic compounds concerned are the *leuco*-anthocyanins and *epi*-catechin; these undergo degradation in presence of Cu and are precipitated. (18 references.)

II. The interaction of Cu^{++} with the org. acid, amino-acid and phenolic components of apple juices was studied in juices and model systems. Cu is mainly complexed with malic acid and the dissociation constant of Cu malate has been calculated; the proportion of Cu complexed with amino-acids is small. With the phenolic components, complexing of Cu^{++} results in the formation of Cu^{+} and oxidized phenolics; the Cu^{+} would be re-oxidized to Cu^{++} in presence of air and so catalyse the further oxidation and condensation of the phenolics. The deposition of condensed phenolic material (mainly *leuco*-anthocyanin) in stored apple juices containing Cu (< 2 p.p.m.) is in agreement with these suppositions. Apple juice storage deposits, however, contain in addition to phenolic materials appreciable quantities of protein and Cu. (41 references.) E. M. J.

Amino-acids of apple juices and ciders. L. F. Burroughs (*J. Sci. Food Agric.*, 1957, **8**, 122—131).—The free amino-acids of apple juices and ciders were examined chromatographically. In juices, asparagine, aspartic and glutamic acids were the principal amino-acids, serine, α-alanine, γ-aminobutyric acid, valine, *iso*leucine and methylhydroxyproline occurred in small, and ten other amino-acids were sometimes present in trace amounts. The ciders had very low N-content only part of which was contained in amino-acids such as aspartic, glutamic and methylhydroxyproline and traces of other amino-acids and of peptides. The effect of yeast autolysis on the amino-acid content of ciders and the presence of nucleotide material in juices and ciders are discussed. (21 references.) E. M. J.

1-Aminocyclopropane-1-carboxylic acid: a new amino-acid in perry pears and cider apples. L. F. Burroughs (*Nature, Lond.*, 1957, **179**, 360—361).—Chromatographic investigation of cider apple and perry pear juices showed the presence of a hitherto unidentified amino-acid. Its investigation by ion-exchange resin techniques, analysis and i.r. spectrum is described. The probable structure was 1-aminocyclopropane-1-carboxylic acid (**I**). Synthesis of **I** was therefore effected and the resulting product found to be identical, in its chromatographic behaviour, reactions and i.r. spectrum, with the natural product. J. S. C.

Fruit of *Diospyros kaki*. I. Composition of white powder of the dried kaki fruit. K. Asô, T. Watanabe, M. Okubo and T. Yamazaki. **II. Total sugar determination of the samples containing fructose.** K. Asô and T. Watanabe (*J. agric. chem. Soc. Japan*, 1956, **30**, 187—191, 191—195).—I. The white powder produced on the surface of dried fruit of *D. kaki* contained glucose and fructose in the ratio of 9 : 1. Contrary to mention in the literature mannitol was not detected. In the flesh of the dried fruit glucose and fructose occurred in the ratio of 6 : 4; the fructose content increased during drying, while the glucose content decreased. By paper chromatography, in the fresh fruit glucose, fructose, sucrose, another oligosaccharide containing ketose, and malic acid as the sole org. acid, were found, but in the dried fruit sucrose and the other oligosaccharide were not found. (12 references.)

II. In the course of analysis of the sugar contents of *kaki* fruit, the so-called total sugar determined after boiling with 2·5% HCl for 2·5 hr. gave lower value than the invert sugar or reducing sugar. The object of this study was to find the cause of this discrepancy. Fructose underwent no change under the conditions of hydrolysis for determining invert sugar (heating with 0·1% HCl for 30 min.), but about 83% of fructose was decomposed nearly completely to form hydroxymethylfurfural under the conditions for determining total sugar. Hydroxymethylfurfural had lower reducing power than fructose in the Bertrand method and behaved as an aldose in the Willstätter–Schudel method for determining aldose and ketose. Thus samples containing fructose would give lower total sugar content and lower fructose content. Glucose, xylose and arabinose were not affected by the same conditions of hydrolysis. H_2SO_4 had lower effect (less than a half) on the decomp. of fructose than HCl. S. KAWAMURA.

Apparatus designed for the rapid electrochemical estimation of flavours in vegetables. J. D. Hartman and W. E. Tolle (*Food Technol.*, 1957, **11**, 130—132).—Rapid and reproducible responses to many volatile flavouring constituents of vegetables were obtained with an apparatus of the above type which is described. E. M. J.

Preservation of collections of vegetables using air distribution plates. F. Desprez (*C. R. Acad. Agric. Fr.*, 1957, **43**, 87—88).—A method is described in which air injected into the base of a heap of, e.g., potatoes by a central canal is distributed in horizontal chimneys maintained at 0—5°. Advantages, including cost, compared with preservation in silo are discussed. E. M. J.

Quality in baked sweet potatoes affected by varieties and post-harvest treatments. W. F. Jenkins and M. Gieger (*Food Res.*, 1957, **22**, 32—36).—Of two varieties studied both increased in reducing and non-reducing sugars during the first six days of the curing period, but changed little afterwards in curing and storage. Baking increased the reducing sugar content from about 0·1% to 2 or 3% in the roots of each, reduced the starch content to about half and dry matter changed little on storage or baking. Quality in baked roots was dependent on the variety and length of curing and storage times employed. E. M. J.

Determination of dry matter and starch content of potatoes, with special reference to relation between specific gravity and starch content. A. Metzger (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 344—350).—Methods for sampling potatoes for analysis are examined. Truly representative samples can be obtained only by the direct sampling of the paste formed by treating ≤2 kg. of the chipped potatoes in a high-speed mixer. The linear graphs obtained by plotting dry matter and starch content, respectively, against the sp. gr. of the potatoes diverge with increasing sp. gr., and disagree (especially for low starch %) with previously published data. With increasing sp. gr., the starch increases more rapidly than the non-starch content. Statistical analysis of the results throws some doubt on the reliability of the sp. gr. as a criterion of the influence of cultural conditions on the composition of different varieties of potatoes. P. S. Arup.

Determination of density and evaluation of quality in peas preserved by freezing. R. U. Makower (*Food Technol.*, 1957, **11**, 126—129).—Data on the density of frozen peas (68 samples) determined by displacement in sucrose solution (8%) were analysed statistically. Density increases with maturity, and results were compared with organoleptic evaluation of maturity (cotyledon texture scores) and with % of alcohol-insol. solids. (16 references.) E. M. J.

Flavorous substances in soy sauce. XIV. Flavorous substances in heated soy sauce. 1. T. Yokotsuka and K. Takimoto (*J. agric. chem. Soc. Japan*, 1956, **30**, 66—71).—Raw brewed soy sauce (*shôyu*) was heated at 80° for 35 hr. and the samples were analysed at 5-hr. intervals. Total titratable acidic substances, N-containing acidic substances sol. in ether, both in the free and combined forms, ether-sol. acids in the free form, phenolic substances in the combined form, and the extinction values at 470, 530 and 610 mμ. (red, yellow and blue colours) increased by heating. The ether-sol. acids in the combined form and total N did not change. Amino-N and reducing sugar decreased. The phenolic, acidic and neutral fractions of the ether extract of soy sauce were examined for u.v. absorption spectra. The max. absorption of the phenolic fraction shifted from 265 to 280 mμ. by heating, the intensity increased and then decreased by prolonged heating. This may have been related to a change in 1-hydroxy-2-methoxy-4-ethylbenzene (an important flavorous ingredient of soy sauce described previously). The acidic fraction showed max. absorption at 265 mμ. presumably corresponding to vanillic acid. The neutral fraction showed max. absorption at 290 mμ. seemingly corresponding to furan components, the presence of which, however, could not be indicated by colour reactions. The degree of heating of soy sauce might be judged by the above changes of some constituents. (16 references.) S. Kawamura.

Sauerkraut and sauerkraut juice. O. Wyler (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 424—431).—Data are given for the ascorbic acid and saline content of sauerkraut and the by-products of its manufacture. The supernatant liquor accumulating during pressing is valueless and unsuitable for consumption, but the fresh press-juice has appreciable dietetic value. Under reasonable conditions of storage and culinary prep., the ascorbic acid shows very satisfactory stability, probably due to the protective influence of the acid medium. P. S. Arup.

Colour evaluation of canned tomato juice with natural and artificial illumination. J. B. Wegener, E. R. Thompson and L. S. Fenn (*Food Technol.*, 1957, **11**, 196—199).—The colour of canned tomato juices was compared visually under daylight and artificial sources of illumination with spinning Munsell colour discs and then assigned numerical scores. The colour values of all samples were read on a Hunter Colour and Colour Difference meter. Results indicated that it would be feasible to substitute a source of artificial illumination (7000° Kelvin) for natural light in the evaluation of colour for canned tomato juice. E. M. J.

Antioxidant properties of tomato lipin. R. E. Henge and F. M. Quackenbush (*J. Amer. Oil Chem. Soc.*, 1957, **34**, 1—4).—The light-petroleum extract (3·1%) of dried ground tomatoes contained 22·4% of a fraction sol. in abs. MeOH (M fraction), which had good antioxidant properties to lard. Phasic separation of M between 92% MeOH and light petroleum gave an ME fraction and a light-petroleum fraction (PE), which separately had very low, but together had high, protection factors. This synergistic effect was attributed to tocopherol in ME and a phosphatide in PE. The effect could be simulated by mixtures of tocopherol and H_3PO_4. When added to lard with peroxide values of 20—130, addition of M resulted in an immediate drop in the peroxide value. G. Helms.

Modern techniques for examination of mineral waters. O. Gübeli (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 305—332).—A review covering methods for determining the gaseous and mineral constituents of medicinal (bathing and drinking) spring waters, with analyses (38) of Swiss waters. P. S. Arup.

Growth of wine yeast cultures in continuous process. A. Fiechter (*Schweiz. Brauerei Rdsch.*, 1957, **68**, 15—19).—The literature is reviewed. With the wine yeast "Herrliberg" grown in grape juice in presence of air, increase in age of the culture was accompanied by decrease in fermenting power (as measured by the Warburg technique) and increase in dry substance and quantity of yeast. The fermenting power of the starter culture was ~700 μl./hr. × 20 mg. of wet yeast. A series of photomicrographs indicates the composition of a suspension in stages of increasing age. Samples of yeast cell suspensions taken from a continuous-process liquid fermentation, with individual old cells appearing yellow coloured, when examined under the phase contrast microscope, have a fermentation speed of 70—80% of the max. possible value. (45 references.) E. M. J.

Simplified process for determination of glycerin and butylene glycol in wine. H. Rebelein (*Z. LebensmittUntersuch.*, 1957, **105**, 296—311).—Details are given of the method which requires only small quantities of material, permits the determination of glycerin and butylene glycol simultaneously, within an hour, or a series of 15 to 20 determinations in 8 hr. In the separation of sugar with $Ba(OH)_2$ in the sugar-containing wines the resulting loss of glycerin is accounted for by a correction factor determined by experiment. The method depends on the prep. of the wine sample and the development of colour tests, with phloroglucinol for glycerin, and Na nitroprusside and piperidine for butylene glycol. E. M. J.

Leucocyanidol in red wines. P. Ribereau-Gayon (*C. R. Acad. Agric Fr.*, 1957, **43**, 197—199).—On chromatographic examination of the colouring matter of grapes and of young red wine, the composition is similar, but in older wine part of the colouring matter passes into colloidal form. Red wine contains *leuco*-cyanidol (of the same order as that of the anthocyanins) which has vitamin P activity and confers an astringent quality to the wine. (11 references.) E. M. J.

Removal of iron from wine by means of phytates. E. Capt (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 431—441).—The use of phytates is unsatisfactory, due to the lack of any definite relationship between the amounts of phytate added and Fe removed. Considerable concn. of residual Fe (10—20 mg. per l.) are frequently found, even after previous aëration of the wine. Residual phytate can cause turbidity on subsequent contamination of the wine with traces of Fe. (26 references.) P. S. Arup.

Organoleptic wine-quality evaluation. I. Standards of quality and scoring vs. rating scales. II. Performance of judges. F. Filipello (*Food Technol.*, 1957, **11**, 47—51, 51—53).—Standards for quality were established by paired preference comparison. Using Burgundy and sherry wines of defined quality, grades in quality were produced in terms of thresholds of quality difference. A paired comparison analysis was used to substantiate the results derived from the paired preference study. In the Burgundy series, incomplete block presentation of three samples yielded significantly higher correlation coeff. than four samples when used with a rating method. When more than one wine of the same quality grade was present in the block, there was a significantly better correlation using the rating, as compared with the scoring method. (25 references.)

II. Of two panels, one of wine judges and the other non-judges, the wine-judges were significantly better in grading a series of quality-different Burgundy wine blends. For the rating scale, incomplete blocks of three samples yields higher correlation coeff. than for blocks of four samples. E. M. J.

Evaluation of brewing water. A. Hansen (*Brauwelt*, 1957, **97**, **B**, 479—484).—A review covering the effects on the brewing processes of the several ions of the principal mineral constituents of water supplies. P. S. Arup.

Influence of chemical composition of brewing water on quality of wort and beer. V. Salač, M. Kortrlá-Haplová and M. Vančura (*Brauwissenschaft*, 1957, **10**, 34—45).—The results of experimental brewings with several types of water in which the ratio of permanent to temporary hardness was varied by adjustment, show the optimum ratio as regards the organoleptic quality of the beer to be 1 : 1; in comparison with this factor, the actual degree of total hardness

is of minor importance. Excessive temporary hardness has an adverse effect on hop-boiling, causing a harsh bitter flavour in the beer. A high ratio (e.g., 1 : 4) of $Mg(HCO_3)_2$ to $Ca(HCO_3)_2$ is an adverse factor, even in waters showing a satisfactory Kolbach " residual alkalinity " value. The adverse effect of $Mg(HCO_3)_2$ is largely neutralized in waters rich in Cl' and SO_4''. The harmful effect of excessive NO_3' and $CaSO_4$ is confirmed; their effects on fermentation are discussed. A favourable effect is exerted by $CaCl_2$. (15 references.) P. S. ARUP.

Composition of protein of brewing barley in amino-acids in regard to species and habitat. W. Postel. (*Mschr. Brauerei wissen. Beil.*, 1957, **10**, 3—10).—A survey is made of species of brewing barleys grown in England, Ireland and various European countries including several different parts of Germany and data are presented on the proportions of the following amino-acids occurring: valine, leucine, *iso*leucine, threonine, arginine, histidine, lysine, phenylalanine, tryptophan, methionine, and cystine, and their total N-content. Climate affects the protein content and with rising content of raw protein, the proportions of certain amino-acids, e.g., lysine, decrease, some remain the same, e.g., tryptophan, and the proportion of phenylalanine increases. Species also affects the protein content. (46 references.) E. M. J.

Measurement of enzymic amylolytic activity. Group A. IV. Methods depending on iodine starch reaction. (The following of the course of starch decomposition by means of changes in the iodine reaction.) iii. contd. H. Wildner and G. Wildner (*Brauwissenschaft*, 1957, **10**, 46—52; cf. J.S.F.A. Abstr., 1957, i. 192).—Descriptive reviews are given of the principles and practice of colorimetric methods and their application to the examination of flour-extracts, honey and urine. P. S. ARUP.

Coagulable proteins in beer and in wort during brewing. P. Kolbach (*Brauwelt*, 1957, **97, B**, 464).—Experiments in which the times of boiling of wort, with or without hops, are varied demonstrate that the small amounts of coagulable proteins found in wort increase both head-retention and cold-trub in the beer. Prolonged boiling gradually removes these proteins with negative results on the qualities mentioned. When wort is boiled with hops, the loss of the coagulable proteins is counterbalanced by the formation of other substances which promote head-retention and the formation of cold-trub. Expedients by which this information might be turned to practical advantage are considered. P. S. ARUP.

Rapid determination of carbon dioxide in lager-tank beer. S. Rischbieter (*Brauwelt*, 1957, **97, B**, 459—461).—The apparatus used consists of a glass cylinder, closed above and below with brass caps, each fitted with a tap. A pressure-gauge is mounted on the top cap, and a thermometer is fitted inside the cylinder. After the air in the apparatus has been replaced by CO_2, and a pressure (greater than the pressure in the lager-tank) of CO_2 has been established in the apparatus, sufficient of the sample to occupy ~96% of the capacity of the cylinder is drawn in, first by opening the lower tap under the surface of the beer, and then by applying suction through the upper tap. After closing both taps and gently agitating the apparatus, the pressure over the beer and the temp. of the beer are read and converted into % by wt. of CO_2 by reference to a table which is attached to the cylinder. The results obtained agree satisfactorily with the results of chemical determinations. P..S. ARUP.

Invertase activity of yeast in presence of acids and salts. M. M. Biswas (*J. Indian chem. Soc.*, 1956, **33**, 815—817).—Invertase activity of brewer's yeast is found to maintain a practically constant level in the presence of constituent ions of different acids (HCl, H_2SO_4, citric, oxalic and acetic acid) at the optimum pH (4·5). The activity of yeast invertase, purified by dialysis and precipitation with alcohol at the optimum pH, is appreciably influenced by different salts at the same pH. The salts studied are: KCl, NaCl, Na_2SO_4, K_2SO_4, $CaCl_2$, $BaCl_2$, $AlCl_3$ and $K_4Fe(CN)_6$. I. JONES.

Effect of brewer's yeast strain on *Flavobacterium proteus* contaminants of brewery fermentations. F. B. Strandskov and J. B. Bockelmann (*J. agric. Food Chem.*, 1956, **4**, 945—947).—The growth of *Flavobacterium proteus* during a brewery fermentation is affected by the strain of yeast used in the fermentation and is apparently inversely related to the rate at which the yeast deaminates certain amino-acids. None of the yeasts could attack lysine, histidine, phenylalanine, tyrosine, tryptophan, cysteine or hydroxyproline. (10 references.) S. C. JOLLY.

Chromatographic determination of trace amounts of sucrose in beer. D. A. M. Mackay and R. L. Evans (*J. agric. Food Chem.*, 1957, **5**, 298—300).—In the examination of beer by paper chromatographic method, sucrose was not found in bottled beer samples, but it occurred in quantity of 20 p.p.m. in draught beer. Concn. of 5 p.p.m. could be detected in a sample of beer, but at lower concn. materials present in the filter paper interfered. (25 references.) E. M. J.

Mineral composition of cloudiness in beer. H. Le Corvaisier (*Brasserie*, 1957, **12**, 30—34).—In samples of beer one brewed in 1950 (**I**) and the other in 1952 (**II**) the deposit at the bottom of the bottles was isolated by centrifugation; data are presented and compared with those in the literature. **I** had a deposit of 110 mg./l. **II** had 90 mg./l. Comparison of the ash of the deposit and the ash of the beer indicates little change in P content of the beer, slight diminution of the SiO_2 content, changes in contents of Ca and Cu and total elimination of Fe from the beer. The catalytic action of Cu in the auto-oxidation of beer, the effects of the systems ferrous–ferric and stannous–stannic, the fact that Mg remains more easily in solution than Ca, etc. are discussed. (20 references.) E. M. J.

Kieselguhr filtration of beer. J. Gloetzl (*Brauereitechniker*, 1957, **9**, 65—67).—The properties of kieselguhr and the fundamental requirements for its use as a filtering agent in breweries are discussed; data on its chemical composition are given. The process of kieselguhr—is compared with that of Masse filtration. The introduction of kieselguhr filters in the brewery signifies a decided advance in the filtration technique. The advantage lies in the special adaptability to the filter process, the good performance and economic service, costing on average about half as much in contrast with Masse filtration. E. M. J.

Progress of dairy science: dairy chemistry. I. Milk proteins and enzymes. B. Aschaffenburg. **II. Composition of milk and dairy products and methods of analysis.** E. R. Ling (*J. Dairy Res.*, 1956, **23**, 134—143, 144—157).—I. Recent work on casein, whey protein, β-lactoglobulin, phosphatase, rennin and other enzymes is reviewed. (43 references.)

II. A review. The effects of roughage, metals, milk products, insecticides on milk composition and changes associated with cheese making and with flavour defects are considered together with relevant analytical methods. (179 references.) A. G. POLLARD.

Reviews of the progress of dairy science. Section D. Nutritive value of milk and milk products. W. A. McGillivray and J. W. G. Porter (*J. Dairy Res.*, 1956, **23**, 283—299).—A review with 105 references. S. C. JOLLY.

Thermal resistance of micrococci in milk. A. N. Myhr and J. C. Olson, jun. (*J. Dairy Sci.*, 1956, **39**, 1635—1643).—Of 39 cultures of micrococci isolated from laboratory-pasteurized samples, 13 survived heating at 143°F. for 30 min.; all of the 12 cultures isolated from raw milk drawn aseptically from cows were destroyed within 5 min. at this temp. Heat resistance of cultures varied at different experiments. The slopes of thermal death time curves (*z* value) for 1 sp. of micrococcus were higher than that generally considered to be characteristic of non-spore-forming bacteria, and might account for the lower efficiency of the high-temp. short-time pasteurization process compared with the holder method. S. C. JOLLY.

Acetone taint in milk due to *Bacterium cloacæ*. G. E. Jones (*J. Dairy Res.*, 1956, **23**, 21—23).—The taint was introduced by inoculating milk with a strain of *B. cloacæ*. Other strains of the bacterium and other organisms of the coli-aërogenes group failed to produce this effect. A. G. POLLARD.

Characterization of some lactobacilli found in milk. R. G. Jensen and J. E. Edmondson (*J. Dairy Sci.*, 1957, **40**, 180—186).—Of 102 lactobacilli isolated from milk and cream, *L. casei* constituted 50, *L. plantarum* 19·6, *L. fermenti* 7·8, *L. acidophilus* 6·9, *L. brevis* 2·9, *L. lactis* 2, *L. bulgaricus* 1, unidentified heterofermenters 7·9 and unidentified homofermenters 2%; *L. plantarum* was the most widely distributed species. The best sources of lacto-bacilli were raw milk and sour cream. Isolation techniques are described, and data on resistance at 61·8° are presented. S. C. JOLLY.

Heat resistance of mould spores in cardboard. H. Baumgärtner (*Ernährungsforschung*, 1956, **1**, 325—329).—The development of mould on the surface of separated milk after autoclaving in closed bottles at 116° during 16 min. was traced to the survival of mould spores in the interior of the cardboard discs used (in conjunction with metal caps) for closure. Preparatory spraying of the discs with disinfectants or soaking in sterile water failed to suppress the infection. Autoclaving under the above conditions destroyed the spores in aq. suspensions. P. S. ARUP.

Oxidation of vanillin by unheated milk. S. Kuramoto, R. Jenness and S. T. Coulter (*J. Dairy Sci.*, 1957, **40**, 187—191).—The action of raw milk on vanillin, rendering it reactive with 2 : 6-dibromoquinonechloroimide (**I**), is apparently due to oxidation of vanillin to vanillic acid by xanthine oxidase. Oxidation products of other

phenolic aldehydes do not react with **I**, or the reaction is suppressed in milk. This reaction appears suitable for determining xanthine oxidase activity and for assessing heat treatment of high-heat skim milk powder intended for baking purposes. S. C. JOLLY.

Colour changes in heated and unheated milk. V. Effects of temperature of measurement, pH, and the addition of certain ions on the reflectance of separated milk. H. Burton (*J. Dairy Res.*, 1956, **23,** 92—104).—The reflectance of separated milk decreased with rise in pH >6·6, increased on addition of Ca^{++} and decreased on that of citrate or $PO_4^{'''}$. These effects are ascribed to changes in the size of the casein particles. A. G. POLLARD.

Variations in alkaline phosphatase activity of milk. W. Haab and L. M. Smith (*J. Dairy Sci.*, 1956, **39,** 1644—1650).—The phosphatase activity of milk from individual cows varied during a complete lactation from 119 to 4380 phenol equiv. (μg. of phenol liberated from Na_2 phenyl phosphate by the enzyme in 0·5 ml. of milk in 1 hr.); min. and max. concn. and max. phosphatase production per milking occurred ~1 week, 28 and 24 weeks, respectively, after parturition. Phosphatase concn. was related inversely to milk yield, but not to breed, fat % or feed. Phosphatase activity in pooled milk from 500 cows ranged from 950 to 1700 phenol equiv. during one year, with a max. during early winter. Variations in phosphatase activity could influence heat treatment required to inactivate the enzyme. S. C. JOLLY.

Reactivation of milk phosphatase following heat treatment. IV. Influence of certain metallic ions. R. C. Wright and J. Tramer (*J. Dairy Res.*, 1956, **23,** 248—256).—Reactivation of the alkaline phosphatase of milk following heat treatment is increased by the presence of Mg^{++}, Zn^{++} and Mn^{++}, and inhibited by Cu^{++}, Ni^{++} and Co^{++}. Mg-induced, but not Zn-induced, reactivation is inhibited by Cu^{++}. Addition of Mg^{++}, but not of Zn^{++} causes reactivation in washed cream to which boiled whey treated with cation-exchange resin is added. Based on the action of edetic acid (EDTA), metallic ions are involved in both alkaline phosphatase activity and its reactivation. Mg^{++} and/or Zn^{++} may be involved in reactivation. S. C. JOLLY.

Precipitation of calcium caseinate by heat and subsequent reversal. C. A. Zittle, E. S. Dellamonica and J. H. Custer (*J. Dairy Sci.*, 1956, **39,** 1651—1659).—Addition of $CaCl_2$ to 2% aq. Na or Ca caseinate decreases the η of the solution, causing opalescence and possibly aggregation. When the total concn. of Ca is ≯0·012M, η is not affected by heating at 90° for 1 hr., but opalescence increases and, at the lower Ca concn., decreases again on cooling. With Ca concn. >0·012M, η increases on heating and partially reverses in 1—3 hr. at lower temp.; η changes are due to the formation of a colloidal ppt. of casein which partially redissolves on cooling, the amount of ppt. increasing with increasing Ca concn. and decreasing pH. Period of heating, stirring and temp. after heating also affect η changes; cream decreases re-solution rate. S. C. JOLLY.

Protein production in the bovine. Breed and individual variations in the specific protein constituents of milk. G. D. Rolleri, B. L. Larson and R. W. Touchberry (*J. Dairy Sci.*, 1956, **39,** 1683—1689).—Variations in total casein α- (**I**), β- (**II**) and γ- (**III**) casein and α- (**IV**) and β- (**V**) lactoglobulin among five breeds of cows are determined by electrophoretic methods. The amounts of **I, II, IV** and **V,** and to a lesser extent the immune globulins, in skim milk are highly correlated, but little correlation occurs between these constituents and **III** and serum-albumin. S. C. JOLLY.

Variations in the chemical composition of milk, with particular reference to solids-not-fat. I. Effect of stage of lactation, season of year and age of cow. R. Waite, J. C. D. White and A. Robertson. **II. Effect of heredity.** A. Robertson, R. Waite and J. C. D. White (*J. Dairy Res.*, 1956, **23,** 65—81, 82—91).—I. Milk samples from 814 cows showed a diminution in total solids, solids-not-fat and crude protein contents up to 45 days (time of max. milk yield) of lactation. All values subsequently increased progressively with a sharper rise after 200 days. Changes in lactose content were of the reverse order. Seasonal changes in composition were much smaller than those due to period of lactation.

II. Dam-daughter relationships in respect of milk yield and contents of fat, solids-not-fat, crude protein, casein and lactose in milk are examined statistically and their practical significance in breeding is discussed. No significant correlations between yield and chemical constituents in milk were found. A. G. POLLARD.

Determination of lactose in milk. F. H. Grimbleby (*J. Dairy Res.*, 1956, **23,** 229—237).—Protein-free serum necessary for the determination of lactose in milk by methods other than the direct titrimetric method of Lane and Eynon is best prepared by use of a clearing agent containing Zn acetate, phosphotungstic acid and acetic acid. Lactose is subsequently determined by polarimetry. S. C. JOLLY.

Size of fat globules in the milk of diseased cows. J. O. L. King (*J. Dairy Res.*, 1956, **23,** 105—110).—In animals (diseased or post-operational) having body temp. >102·4—104°F. the rise in body temp. was associated with a decline in milk yield and increases in % of fat and average diam. of fat globules in the milk. When the body temp. was ≯102·4°F., a subsequent return to normal was accompanied by a significant rise in yield but changes in fat content and globule size were not significant. A. G. POLLARD.

Effect of commercial sterilization on the nutritive value of milk as determined in experiments with rats and baby pigs. A. P. de Groot and C. Engel (*J. Dairy Res.*, 1956, **23,** 257—268).—The nutritive value of milk is not reduced significantly on sterilization by the Stork procedure (heating at 135° for ~15 sec., bottling and capping, and then heating again at 113° for a longer period). S. C. JOLLY.

Destruction of *Mycobacterium tuberculosis* and phosphatase in heat-treated cream. R. Aschaffenburg, C. A. E. Briggs, E. L. Crossley and J. Rothwell (*J. Dairy Res.*, 1956, **23,** 24—29).—*M. tuberculosis*, artificially introduced into cream, was inactivated in the high-temp. short-time pasteurization process under the same conditions as were required for milk. A. G. POLLARD.

Steam-stripping of taints from liquids. IV. Continuous counter-flow equipment. J. K. Scott (*J. Dairy Res.*, 1956, **23,** 30—47).—The physical and engineering factors concerned in this process are examined. A. G. POLLARD.

Steam distillation of taints from cream. F. H. McDowall (*J. Dairy Res.*, 1956, **23,** 48—64).—The effects of structural and operational details of the Vacreator pasteurizing unit on the efficiency of removal of diacetyl and acetoin (as reference substances) from water and cream are examined. A. G. POLLARD.

Use of synthetic β-carotene for colouring butter. R. R. Riel and C. K. Johns (*J. Dairy Sci.*, 1957, **40,** 192—199).—The stability and flavour of butter coloured with synthetic β-carotene (0·35 g. per 100 lb. of fat) were comparable with those of butter coloured with coal-tar dye (1 oz. per 100 lb. of fat). The colour of carotene-dyed butter was slightly darker and its vitamin-A content was increased by 3800 i.u. per lb., both factors being unaffected by storage. S. C. JOLLY.

Comparative study of Junge's and an electrical apparatus for melting-point determination in the phytosteryl acetate test on ghee (butter fat). B. S. Dane and O. Prakash (*J. Proc. Oil Technol. Ass., India*, 1956, **12,** 29—40).—An electrically-heated apparatus was compared with Junge's apparatus for determining m.p. of sterol acetate from 135 ghee samples and the two sets of results did not agree. Suggestions are made for improving the electrical apparatus. J. S. C.

Water content determination of butter depending on the working method with or without dispersion means. A. Seuss, F. Kiermeier and F. J. Brunner (*Z. LebensmittUntersuch.*, 1957, **105,** 89—98).—The methods of determination of water in butter are statistically examined including the use of NaCl in five grades of purity, Baltic Sea sand, a drying cupboard method without distributing agent and the rapid method. Gravimetric methods (drying in 1-hr. intervals at 103—105° in a drying cupboard to constant wt.) with and without distributing agents are compared in relation to accuracy and time expenditure. (19 references.) E. M. J.

Variations in carotene and vitamin A contents of herd butterfats throughout lactation. A. K. R. McDowell (*J. Dairy Res.*, 1956, **23,** 111—119).—For periods up to 10—12 days after parturition the vitamin A and carotene contents of milk may exceed normal ranges. The carotene contents of butterfat from small herds was dependent on the amount of carotene in the pasture rather than on lactational effects. The vitamin A content of such butterfat was generally low throughout the lactation and occasional autumn increases were due to seasonal conditions and not to the stage of lactation. A. G. POLLARD.

Host–phage relationship of cheese-starter organisms. I. Interaction of phage races with a strain of *Streptococcus lactis*. and to lysogenic and resistant derivatives. J. Czulak and J. Naylor. **II. Effect of phage activity on heterologous strains of lactic streptococci.** J. Naylor and J. Czulak. **III. Significance in selection and maintenance of starter cultures in commercial use.** J. Czulak and J. Naylor (*J. Dairy Res.*, 1956, **23,** 120—125, 126—130, 131—133). A. G. POLLARD.

Determination of the coagulating power of commercial rennet extracts using an automatic tester. F. C. Storrs (*J. Dairy Res.*, 1956, **23,** 269—276).—The prep. of freeze-dried rennet reference standards and the construction and use of an automatic apparatus for measuring rennet coagulation time are described. A substrate which will give reproducible results under standardized conditions has been developed using ordinary spray-dried skim milk. Max. experimental error with the method is ±2%. S. C. JOLLY.

Flavour of untreated, oiled and thermostabilized shell eggs after storage at 34°F. S. F. Banwart, A. F. Carlin and O. J. Cotterill (*Food Technol.*, 1957, **11**, 200—204).—Untreated, oiled or thermostabilized eggs stored at 34°F. up to eight months all declined in internal quality as measured by Haugh units; each of the two treatments retarded losses in internal quality. A highly significant, negative correlation existed between length of storage and decrease in flavour scores of both treated and untreated eggs. After six months the flavour of the stored untreated was superior to that of the stored treated eggs when scrambled or soft cooked. (14 references.) E. M. J.

Isolation of proline from egg-white hydrolysate with 2 : 4 : 6-trinitrobenzoic acid. J. Kapfhammer and G. Mohn (*Hoppe-Seyl. Z.*, 1956, **306**, 76—83).—The new method of Kapfhammer (*ibid.*, 1953, **295**, 413) for the pptn. of proline using 2 : 4 : 6-trinitrobenzoic acid yields good results in conjunction with the old pptn. method with Reinecke salt. The most suitable starting materials are proteins which contain only small amounts of hydroxyproline and basic amino-acids, but 70% yields are obtained. The isolation of pure proline, containing <0·2% of impurities, can be shown only by paper chromatography. C. A. FINCH.

Phospholipins. IV. Composition of hen's egg phospholipins. D. N. Rhodes and C. H. Lea (*Biochem. J.*, 1957, **65**, 526—533).—A mild extraction and purification procedure is described for hen's egg phospholipins; it yields 97% of the total material. The crude phospholipin is separated by chromatography on Al_2O_3 into choline-containing and non-choline-containing fractions. The phospholipin examined contained phosphatidylcholine 73·0, lysophosphatidylcholine 5·8, sphingomyelin 2·5, phosphatidylethanolamine 15·0, lysophosphatidylethanolamine 2·1 and inositol phospholipin 0·6 mol.-%. The plasmalogen content of the whole phospholipin was 0·9%; most of it was in the lecithin fraction. The phosphatidylethanolamine fraction contained a small amount of an amino-acid-containing phospholipin. J. N. ASHLEY.

Ten years' work on meat at the Low Temperature Research Station, Cambridge. E. H. Callow (*Food Sci. Abstr.*, 1957, **29**, 101—112).—A review. Subjects discussed include the composition of carcasses and tissues, post-morten changes in muscular tissues and the microbiology, processing, storage and analysis of meat. (90 references.) A. G. POLLARD.

Calorimetric investigation of freezing of meat. L. Riedel (*Kältetechnik*, 1957, **9**, 38—40).—Using the method and the adiabatic calorimeter of the earlier investigation on sea fish (*Kaltetechnik*, 1956, **8**, 374) a similar investigation is now made of the freezing process for meats. Determinations are made of enthalpy of several kinds of meat over temp. range −60 to +20°, and calculations are made of the amounts of water frozen and of water remaining unfrozen at the different temp. The results follow very closely those obtained with marine fish. With meats the amount of water remaining unfrozen even at very low temp. is ~0·35 kg. per kg. of dry meat, corresponding to 0·4 kg. of water per kg. of albumin or 2 mol. of water per amino-acid of the protein. H. L. WHITEHEAD.

Discrimination techniques in meat acceptance studies. H. D. Naumann, V. J. Rhodes, D. E. Brady and E. R. Kiehl (*Food Technol.*, 1957, **11**, 123—125).—A discussion of the potential utility of laboratory-, small urban- and metropolitan consumer panels and of techniques involved. (14 references.) E. M. J.

Use of organic reductants in the canning of luncheon meat. P. W. Hardy, J. S. Blair and G. T. Krueger (*Food Technol.*, 1957, **11**, 148—151).—The discoloration which sometimes occurs on meat (commercially packed in 6- or 8-lb. cans) along the side seam of the can and the elimination of the effect by the insertion at one end of the can of an Al anode are discussed. The meat pigment is oxidized by nitrite; Fe ions catalyse the reaction. In the presence of reducing agents, e.g., ascorbic acid or reductone, a meat product when canned is much less likely to develop localized discoloration. (12 references.) E. M. J.

Effect of storage conditions on loss of colour and free sulphydryl groups in cured meat. A. M. Erdman and B. M. Watts (*Food Technol.*, 1957, **11**, 183—185).—Loss of sulphydryl groups was considerably faster at room temp. than at refrigerator temp. in cured meats stored in darkness; application of a 2% nitrite solution increased greatly the speed of loss. Light and type of wrapping material had little effect on SH losses. Colour fading was affected most markedly by light. E. M. J.

Effect of inorganic phosphate on animal protein. VI. Influence of polyphosphate on the properties of sausages and hams. K. Möhler and F. Kiermeier (*Z. LebensmittUntersuch.*, 1957, **105**, 265—274).—Experimental sausages prepared from freshly killed and stored meat, with and without fat, and with the addition of phosphate were examined. There was no improvement in the consistency resulting from addition of phosphate but changes in pH affect cooking losses. The water-binding capacity of meat proteins immediately after slaughter and on storage and the acceleration of the change from collagen to gelatin by the addition of phosphate (2—5%) are discussed. (16 references.) E. M. J.

Purine bases in meat and yeast extracts and in protein hydrolysates. P. Tempus (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 351—368).—The purine-N as determined by the modified Micko method (described) amounts to >94% of the total N in the above products. A method for the isolation of the purine bases for (radial) paper-chromatographic separation is described. The sum of the eluted adenine, guanine, hypoxanthine and xanthine thus obtained and determined spectrophotometrically agrees well with the purine-N as determined by the modified method. (21 references.) P. S. ARUP.

Edible fats. J. P. Sisley (*Olii min.*, 1956, **33**, 423—432).—Advances in the following fields are reviewed: extraction, refining, demucilagination, deacidification, decolorization, deodorization, solvent segregation, hydrogenation, shortenings, inter-esterification, antioxidants, synergists and sequestering agents. (45 references.) L. A. O'NEILL.

Extraction of oil-seeds with special reference to alcohols as solvents. K. Barthel and K. Täufel (*Ernährungsforschung*, 1956, **1**, 344—353).—Graphs are given for phase-separation temp. observed during the slow cooling (in a glass autoclave) of the clear miscellæ containing various edible oils (5—95%) with 91 and 99% (w/w) EtOH or 91 and 99% Pr^{i}OH as solvent. With EtOH, the results are particularly sensitive to small changes in the water, and it is frequently necessary to exceed the b.p. in order to obtain clear miscellæ; these disadvantages can be avoided with the use of Pr^{i}OH. Laboratory experiments on the countercurrent extraction of soya-bean and poppy-seed oils by 95% Pr^{i}OH indicate the feasibility of large-scale extraction of >99% of the oil from the unpressed meats with the use of three charges of fresh solvent. The oil-phase of the cooled miscellæ contains ~70% of the total oil and 9—15% of the solvent. The supernatant phase contains (in addition to oil) the bulk of the fatty acids, colouring matters, phospholipins and other minor constituents. The oils of the oil-phase are, however, only slightly less coloured than oils extracted by means of light petroleum. P. S. ARUP.

Detection of soya-bean oil based on its content of δ-tocopherol. K. W. Biefer and H. Hadorn (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 445—455).—The method of Brown and of Eggit *et al.* is used for the isolation, purification and chromatographic separation of the tocopherols. The separation is improved by coating the papers with a petroleum fraction (230—270°) instead of with vaseline. Tocopherol spots are conveniently located by the blue coloration given with aq. $Fe_2(SO_4)_3$ + K ferricyanide. Chromatographic data are given for the tocopherol contents of 11 edible oils. Soya-bean and mustard-seed oils are distinguished from the other oils by their content of δ-tocopherol. In the absence of erucic acid, the presence of soya-bean oil can be inferred. The presence of 10% of soya-bean oil in the other oils can be detected. (19 references.) P. S. ARUP.

Improvement of food flavour, fat stability and nutritional values with sesame products. E. F. Glabe, P. W. Anderson and A. F. Holtorff (*Food Technol.*, 1957, **11**, 185—188).—A description is given of the cultivation and characteristics of the sesame plant in U.S.A. including the presence in the seed of antioxidant compounds, sesamoline and sesamol. This sesamol–sesamoline system is tightly bound in the cellular structure inside the seed envelope. A new type of sesame product is obtained by removal of the seed coat followed by heat treatment. Grinding this heat-processed seed releases the oil and yields a flavour-rich product. Antioxidant value is high. (13 references.) E. M. J.

Development of oil in groundnut. B. G. Prasad and B. Biswas (*J. Proc. Oil Technol. Ass., India*, 1956, **12**, 23—28).—As the groundnut seed ages, moisture decreases and oil content increases to reach a max. between 65 and 70 days after the opening of the flower. This is therefore the best stage for harvesting. Free fatty acid content decreases with maturity of seed. The I val. of the oil is slightly lower in the initial stage and increases with age, indicating formation of unsaturated fatty acids. Max. starch content is found in the early growth period and decreases with age. (16 references.) J. S. C.

Chromatography of phospholipins and related compounds on glass [-fibre] paper impregnated with silicic acid. M. Brown, D. A. Yeadon, L. A. Goldblatt and J. W. Dieckert (*Analyt. Chem.*, 1957, **29**,

30—31).—Some phospholipins and their hydrolytic cleavage products were separated on glass-fibre paper, impregnated with silicic acid. Choline-containing compounds were detected with a phosphomolybdic acid–$SnCl_2$ reagent or with a modified Dragendorff reagent (K iodobismuthate). Compounds containing a primary amine group were detected by a ninhydrin spray and substances that char by a conc. H_2SO_4 spray followed by heating. R_F values for 12 compounds are listed. G. P. COOK.

Integrated quality control [of food]. W. W. Prouty (*Food Technol.*, 1957, **11**, 152—155).—A discussion of food quality control from the raw materials to public relations. E. M. J.

Separation and determination of cyclic imino-acids. K. A. Piez, F. Irreverre and H. L. Wolff (*J. biol. Chem.*, 1956, **223**, 687—697).—Two colorimetric methods are described for determination of cyclic imino-acids in the effluent fractions from an ion-exchange column. Both need ninhydrin in acetic acid. When the reaction is effected at room temp. the method is suitable for proline, hydroxyproline and *allo*-hydroxyproline. Heating at 100° allows determination of pipecolic acid, 5-hydroxypipecolic acid, proline and baikiain (4 : 5-dehydropipecolic acid). The separation of these compounds by ion-exchange and paper chromatography is described. The diastereoisomers of hydroxyproline are separated by ion exchange and paper chromatography, and those of 5-hydroxypipecolic acid by paper chromatography. The methods are used for the identification and determination of proline, hydroxyproline, pipecolic acid and baikiain in dates. J. N. ASHLEY.

[A] Sterilization of dry gelatin. [B] Sterilization of dried egg-powder by means of ethylene oxide. G. Mayr and H. Kaemmerer (*Mitt. VersSta. Gärungsgew.*, 1957, **11**, 9, 9—11).—[A] Sterilization of dry gelatin can be accomplished by exposure during 6 hr. under reduced pressure, according to the Degesch vac. process, to an atm. containing ethylene oxide and MeBr (1000 g. per cu. m.) at 25—30° and R.H. 55—70%.

[B] Dried egg-powder can be sterilized by the Degesch process with the use of ethylene oxide (750 g. per cu. m.). Chromatographic investigations show the treatment to be without effect on the amino-acid or fatty acid constituents of the proteins or the egg-oil, respectively. P. S. ARUP.

Column chromatography of anthocyanins. T. Endo (*Nature, Lond.*, 1957, **179**, 378—379).—A simple experimental procedure for anthocyanin separation on cellulose powder columns is described : the quality of the cellulose powder used was found to be one of the most important factors. J. S. C.

Use and analysis of synthetic carotenoids in foods and feeding stuffs. O. Isler, S. Nobile and P. Zeller (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 422—423).—The probability of the dietetic value of O-containing carotenoids is suggested. Five synthetic carotenoids containing hydroxy-, carboxy- or keto-groups have each been found to promote egg-yolk coloration in poultry feeding experiments. Spectrophotometric data for carotenoids containing the same double-bond system are not influenced by the presence of hydroxy-groups, but hydroxy- in contradistinction to hydrocarbon-carotenoids, are adsorbed from light petroleum solution by Al_2O_3. The adsorbed carotenoids can be eluted by the addition to the solvent of Et_2O. P. S. ARUP.

Apparatus for multiple determination of vitamin B_1 by column chromatography. I. Antener (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 415—421).—In the battery assemblage described, four or eight solutions for vitamin B_1 determination can be simultaneously purified under uniform conditions by adsorption at room temp. on a Decalso column, washing with water at 100°, and elution at room temp. with acid aq. KCl. Equally satisfactory results by the thiochrome and by two biological methods are obtained for purified and unpurified solutions of vitamin prep., but when dealing with extracts from vitaminized infants' food or cocoa, purification is essential. P. S. ARUP.

Factors influencing acidity and confidence limits of pantothenic acid estimation. Microbiological assay with *Lactobacillus casei*. M. F. Clarke (*Analyt. Chem.*, 1957, **29**, 135—139).—In the microbiological assay of pantothenate using *L. casei* in peptone media, there is a linear relationship between the logarithm of pantothenate per tube and the amount of acid formed upon incubation. Change of the media from peptone to enzyme-hydrolysed casein results in a straight line of much greater slope. Factors influencing this slope were examined and the most favourable conditions for assay occurred when 3% glucose and heavier inoculum were employed and when dosage corresponded to 0·125 to 0·25 μg. of Ca pantothenate. G. P. COOK.

Determination of ascorbic acid in foodstuffs by paper chromatography. G. Cerutti (*Chim. e Industr.*, 1957, **39**, 14—16).—Ascorbic acid, e.g., in wines, beers or syrups, is separated by paper chromatography (ascending) using BuOH as solvent, and the band corresponding to the R_F value of the acid (0·30 to 0·40) is titrated after stabilization with oxalic acid. Determination from the area of the spots, revealed by treatment with dichlorophenol-indophenol or by long exposure to air, is less accurate. L. A. O'NEILL.

Extraction and chromatography on aluminium oxide of various colouring matters. M. Mottier (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 372—386).—Owing to their presence in the acidic form, water-sol. dyes extracted with the use of quinoline and an acid (AcOH) buffer (as previously described) fail to travel satisfactorily in neutral solvents on Al_2O_3 plates. Satisfactory movement of the dyes as Na salts can be ensured either by neutralization (with N-NaOH) of the solutions of the extracted dyes, or by impregnation of the Al_2O_3 in the vicinity of the starting-point with N-NaOH, followed by drying before use. The behaviour during extraction of 50 water-sol. and 10 fat-sol. dyes is examined, and directions are given for dealing with difficult cases. A method for the prep. of Al_2O_3 plates, based on the technique of Matthias and of Schwerdtfeger is described. (11 references.) P. S. ARUP.

Paper-chromatographic separation and detection of food-colouring matters permitted in East Germany. K. Müller and K. Täufel (*Ernährungsforschung*, 1956, **1**, 354—361).—Directions are given for the isolation and chromatographic (ascending and radial) identification of 16 colouring matters. Tabulated data include R_F values and details concerning the appearance of the spots in daylight and u.v. light before and after spraying with N-NaOH or N-HCl. P. S. ARUP.

Colour of capsicum spices. II. Extraction of colour. J. B. Moster and A. N. Prater (*Food Technol.*, 1957, **11**, 146—148).—Extracts were prepared by treating 0·100 g. of capsicum spice with 50 ml. of 99% *iso*propanol for 3 hr. at 70° ± 1°. Colour was measured by the Gentry method and the Gentry units were converted to linear colour units. E. M. J.

Properties of the synthetic sweetening agent, cyclamate. K. M. Beck (*Food Technol.*, 1957, **11**, 156—158).—Cyclamate is a strong electrolyte-salt ~30 times as sweet as sucrose ; stable to heat, acids and bases. (17 references.) E. M. J.

Rapid spectrophotometric technique for evaluation of vanilla extracts. R. Pomerantz, S. A. Goldblith and B. E. Proctor (*J. agric. Food Chem.*, 1957, **5**, 292—293).—The use of the rapid scanning spectrophotometer to differentiate between pure and artificial vanilla solutions (60%) in water by typical transmittance (at 700 mμ.) curves is discussed. E. M. J.

Specific effect of high-frequency alternating dielectric fields on micro-organisms. K. Siebert and H. Seidler (*Ernährunsforschung*, 1956, **1**, 330—343).—The literature of the subject is reviewed. In experiments in which aq. bacterial suspensions had been gently dried on absorbent paper-discs, preparatory to exposure to the field, *Escherichia coli*, *Bacterium proteus* and micrococci were killed by exposures lasting for <30 min., whilst *Bacillus mesentericus* and *Bacillus subtilis* remained viable. (52 references.) P. S. ARUP.

Use of high-voltage cathode rays to destroy bacteria of the *Salmonella* group in liquid and frozen egg white and egg-white solids. J. T. R. Nickerson, S. E. Charm, R. G. Brogle, E. E. Lockhart, B. E. Proctor and H. Lineweaver (*Food Technol.*, 1957, **11**, 159—166).—*Salmonella* was more resistant to irradiation in dried egg-white products than in liquid or frozen egg white. The average dose of high-voltage cathode rays (3 mev.) required to cause a 10^7-fold destruction of *S. typhimurium* and *S. senftenberg* in unsugared egg-white solids was ~700,000 rep, in sugared ~1,000,000 rep ; with reconstituted (liquid) egg white, ~300,000 rep for *S. typhimurium* and ~230,000 for *S. senftenberg* ; with frozen egg white ~260,000 rep for *S. typhimuriam* and for *S. senftenberg* ~150,000. Such irradiation dosages caused only minor deteriorations in the functional and organoleptic properties of these products. (19 references.) E. M. J.

Treatment of foods with ionizing radiation. H. Mohler (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 387—408).—A review covering theoretical and technical aspects, with recommendations applicable to Switzerland. (42 references.) P. S. ARUP.

Detection of free radicals in irradiated food constituents by electron paramagnetic resonance. J. P. O'Meara and T. M. Shaw (*Food Technol.*, 1957, **11**, 132—136).—The technique of electron paramagnetic resonance (EPR) for detecting free radicals *in situ* based on the fact that free radicals contain at least one unpaired electron per radical, and that unpaired electrons have properties detectable by magnetic resonance technique, is discussed. Preliminary results on irradiated foodstuffs and food constituents are presented. E. M. J.

Inspection frequencies and sample numbers for raw materials procured for food processing. A. Kramer (*Food Technol.*, 1957, **11**, 176—179).—Factors are indicated which influence the choice of specific sampling plans for specific purposes, and some rapid statistical procedures are outlined by which these sampling plans may be constructed. E. M. J.

Flavour permeability in food packaging and its evaluation. M. Karel, B. E. Proctor and A. Cornell (*Food Technol.*, 1957, **11**, 141—145).—A procedure for the estimation of permeability of packaging materials to org. vapours (ethanol, acetone, trimethylamine), based on the $KMnO_4$ process of Lang *et al.*, was developed and tested. Results indicated that the procedure offers a rapid and accurate method for comparison of different materials from the view-point of permeability to org. vapours. E. M. J.

Properties and food applications of a new packaging film. H. Nagel and J. P. Wilkins (*Food Technol.*, 1957, **11**, 180—182).—The mechanical, thermal, chemical and packaging properties of "Mylar," an oriented film of polyethylene terephthalate, are discussed. The film offers excellent protection to frozen foods; is similar in behaviour to window glass with respect to u.v. light transmission; has exhibited excellent resistance to bacterial and fungal degradation and is acceptable for packaging in food and drug applications for temp. up to 250°F. E. M. J.

Plastics in the packaging of foodstuffs. L. Robinson-Görnhardt (*Kunststoffe*, 1957, **47**, 54—58).—Toxic and non-toxic plastic materials, compounding ingredients, and additives of plastics of possible use in the food packing industries are described in the light of current knowledge, as a basis for future discussion. C. A. Finch.

Baked wafer products. L. Marsden (B.P. 751,948, 2.12.54).—A device is described which ensures that shrinkage of the biscuit on cooling raises it from the mould without breakage. K. Ridgway.

Separation of nitrogenous compounds [glutamic acid, etc.] from molasses by means of ion-exchange. N.V. Centrale Suiker Maats. (B.P. 751,963, 22.1.54. Neth., 30.1. and 25.6.53).—The molasses is diluted with a miscible org. liquid (0·6—2 kg. per kg.), e.g., MeOH, then an acid capable of forming sparingly sol. salts with the cations present in the molasses is added, e.g., H_2SO_4, aq.SO_2, or H_3PO_4, to pH 2—3·5 (3). The resulting ppt., preferably formed at 35—80 (40—50)° is separated, and the filtered liquor is concentrated, then passed through one or more ion-exchangers. F. R. Basford.

Processing of whole fruit. Food Machinery and Chemical-Corp. (B.P. 753,988, 1.2.54. U.S. 24.2.53.—Citrus fruits are processed in a machine which has two sets of meshing compression fingers mounted over a central tube. Juice is forced into the tube with pulp, and emerges through perforations. The peel is lifted upwardly and scraped by knives to take off the rind and release the peel oil. Thus the fruit is automatically divided into peel, peel oil, juice and pulp, without loss or mingling of the four products. K. Ridgway.

Preparation of citrus fruit liquors and beverages. Northern Dairy Engineers, Ltd. (Inventor: J. W. Shearman) (B.P. 751,766, 15.1.54).—Oranges or other citrus fruits are cut in halves and passed between shaped rollers under a continuous flow of sugar syrup. One roller carries numerous small points and rotates at a slow speed, puncturing the oil cells and releasing aromatic oil into the syrup. The other roller rotates at high speed and reams out the fruit cells leaving the albido intact. K. Ridgway.

Protein hydrolysates from potatoes and potato waste. Wise Potato Chip Co. (Inventor: P. A. Xander) (B.P. 752,886, 11.1.54).—Potato material is slurried in water and the liquid fraction separated. To this is added an alkaline earth oxide or hydroxide (CaO) to precipitate sugars. After filtration, the liquid is hydrolysed with a mineral acid (HCl) to convert protein to amino-acids. Acid is removed on an anion-exchange resin and the Na salts of the amino-acids formed by passage through a cation-exchange resin (Na form). The solution is finally evaporated and the residue dried. J. S. C.

Improvements in preserving milk. W. Böhm and H. Böhm (B.P. 752,680, 23.4.54. Ger., 28.4.53).—Milk is heat-sterilized, introduced into containers while still at a temp. high enough to ensure a sterile filling (~100°), so that the containers are completely filled. The containers are then closed and cooled. The process renders unnecessary sterilization of the filled containers and is claimed to give a shelf life of several months. J. S. C.

Treatment of milk and soft curd milk produced thereby. Armour & Co. (B.P. 752,922, 17.3.54. U.S., 18.3.53).—Trypsin, substantially free of other pancreatic enzymes (e.g., the cryst. material), in an amount equivalent to 5—30 tyrosine units of "tryptic activity" is added to milk at 100°F. to convert it to soft curd milk, which may then be subjected to flash pasteurization. The "tryptic activity" is the wt. in mg. of a trypsin unit of trypsin prep. which when added to 1 l. of untreated skimmed milk liberates 1 mg. of tyrosine after incubation for 1 hr. at 100°F. J. S. C.

[Preparation of] dried milk product. Instant Milk Co. (B.P. 753,600, 22.12.53. U.S., 27.7.53).—A milk powder which will dissolve rapidly and easily in water is prepared from ordinary 80-mesh skim milk powder. It is fed by an air stream into a hydrator where at a temp. of 90—120°F. a spray of atomized water raises the moisture content of the particles to 10—20%, whereby aggregates are formed. The aggregated powder is dried in a shaker drier to 3—5% moisture content, and classified by screening or air elutination. The fines are reprocessed. The product consists of loose aggregates of particles of $>74\mu$. size, which owing to capillary action will take up water extremely rapidly. K. Ridgway.

Beverages. Mortlock's Modern Dairies, Ltd. (Inventors: A. M. Laidlaw, J. L. Mortlock and A. P. Unthank) (B.P. 751,900, 29.5.54).—A bottled or sealed up milk-based beverage, which will remain in good condition in the container for a considerable time, is made by (*a*) adding a pre-made mixture of an alcoholic spirit and a sweetening material (honey) in pre-arranged proportion to milk (1·125 fluid oz. of sweetened mixture to 2·5 fluid oz. of full-cream milk), the milk having been previously heated at 165—175°F. for ~20 sec., (*b*) sealing the resulting mixture in bottles or other containers, and (*c*) heating the contents to ensure full sterilization (e.g., at 190—212°F. for 2—10 min. for a 3·75-oz. bottle). H. L. Whitehead.

Beverages. Mortlock's Modern Dairies, Ltd. (Inventors: A. P. Unthank and A. M. Laidlaw) (B.P. 753,747, 2.3.54).—Full-cream milk is kept at 74—99° during $\geq$22 sec., then homogenized, admixed (8) with fruit syrup (1·25 pints) comprising, e.g., sugar 30, glucose 60, and fruit juice 10 wt.-%, and after stirring (during $\geq$22 min. at 70—80°) the mixture is fed into bottles and sterilized, to give a milk base beverage. F. F. Basford.

Coffee-making device. Brevetti Gaggia S.v.l. (B.P. 751,687, 5.8.54. It., 14.10.53).—A coffee machine for coping with sudden demands consists of a jacket containing a heating element, into which cold water is supplied by a nozzle on to a thermo-sensitive element. From the jacket it enters a central cylinder from where it is ejected by a plunger through a filter containing coffee. K. Ridgway.

Cooking of meat. H. H. Tanner (B.P. 752,239, 19.2.54).—A base plate has one upturned end and carries a vertical plate. A rolled boned joint (e.g., gammon) is placed on the base plate and pressed with the plate against the upturned end. String is then lashed around it using projecting lugs on the base plate. The meat can be cooked without distortion or loss of juices due to pressing. K. Ridgway.

Treatment of newly shot whales. Alf Kaare Kristoffersen (B.P. 753,290, 24.8.54. Nor., 28.8.53).—Air, carrying an atomized preservative, e.g. formalin, is pumped into the fresh whale carcass, to prevent decomposition of the interior before the whale can be processed. J. S. C.

Manufacture of margarine. Rootry Exploitatie Maats.N.V. (B.P. 753,040, 3.3.54. Neth., 3.3.53).—A margarine of extremely low bacterial count is obtained by preparing an oil-in-water dispersion with $\geq$16% water from the oil and water phases. The dispersion is then pasteurized, followed immediately by working on cooling means to reverse it into a water-in-oil emulsion. J. S. C.

Laminated aluminium foil packaging material for butter, margarine, etc. O. J. Bruun (B.P. 753,717, 16.7.54).—Improved laminated material for wrapping butter and other edible fats is made by laminating a layer of thin Al foil to a sheet of common sulphite paper by means of an adhesive (an aq. emulsion of polyvinyl acetate), embossing the laminated material with a close pattern of relief undulations (to impart flexibility), and then coating the paper side of the embossed laminate with a layer of wax. Preferably the wax is applied by roller from a molten bath at such temp. as to ensure that the wax is sterile. To preserve the sterile properties of the wax coating (which will contact with the butter, etc.) and to enable a sterile product to be used in the ultimate packaging of the fat, the coated material, after cooling, may be directly wound up tightly to prevent access of air and moisture. H. L. Whitehead.

Food products stabilized with antioxidant compositions. Universal Oil Products Co. (B.P. 753,794, 20.7.54. U.S., 7.10.53).—Food is stabilized against deterioration by incorporation of 0·001—0·1% of

a mixture of butylated hydroxyanisole (30—70) and a 2 : 4 : 6-trialkylphenol (30—70%), e.g., 4-methyl-2 : 6-di-*tert*-butylphenol. F. R. BASFORD.

Separation of proteins. L. Rane and L. R. Newhouser (B.P. 753,148, 23.2.54. U.S., 2.3.53).—Pptn. of protein from a solution containing it, e.g., animal fluid (blood) or vegetable fluid (maize extract, etc.), is effected by adding Na tetrametaphosphate, with prior, simultaneous or subsequent adjustment to pH 3·5. F. R. BASFORD.

Synthetic pepper compositions. T. Hasselstrom, E. J. Hewitt, K. S. Konigsbacher, and J. J. Ritter (B.P. 751,476, 12.5.53. U.S., 14.5.52).—The composition comprises pungent material (piperine, chavicine, piperettine, capsaicin, etc.); resin (synthetic resin, e.g., polyvinyl acetate, Staybelite, etc., or natural resin, e.g., pine oleoresin); a carrier (heat-processed grain product, grain flakes, potato peels, etc.); and (synthetic) flavours, viz., woody, aromatic, or citrus, phenolic, floral, and/or putrid flavour (skatole etc.). Many examples of suitable flavours are given. F. R. BASFORD.

3.—SANITATION

Trials of residual insecticides in window-trap huts against Malayan mosquitoes. J. A. Reid and R. H. Wharton (*Bull. ent. Res.*, 1956, **47**, 433—468).—The kills obtained by the window-trap-hut method (described) are a good guide to those to be expected in treated houses. The effects of DDT, BHC and dieldrin on the biting and resting behaviour of the principal species of mosquitos are studied with respect to individual susceptibility to the insecticide, and the duration and frequency of contact. DDT is irritant and persistent, but not toxic enough except to the most susceptible spp. Fresh BHC is irritant and highly toxic, but on ageing loses toxicity considerably. Dieldrin acts more slowly than DDT or BHC, but is non-irritant, very toxic, and persistent over longer periods. (37 references.) P. S. ARUP.

Insecticidal fogs against tsetse flies on trains. R. Fairclough (*Bull. ent. Res.*, 1956, **47**, 193—196).—Experiments were carried out on trains at rest in infected areas with the use of DDT and pyrethrum in " Swingfog " machines in order to test the possibility of preventing the transport of the flies from infested to non-infested areas. The operations resulted in reductions of 60—70% in the catches of flies after the trains had left the infested areas. In view of the cost of the operations, therapeutic control of trypanosomiasis in the affected regions would prove more economical. P. S. ARUP.

Exceptional presence of nitrites in drinking water. E. Matthey and S. Gay (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 368—371).—Examples are cited of the presence in satisfactory drinking waters of NO_2' (0·02—0·06 p.p.m.) produced by u.v. irradiation or contamination with Zn, or naturally present in water containing little Fe and O_2, and no Mn, drawn from certain subterranean accumulations. P. S. ARUP.

Calculation of oxygen percentage saturation of water. R. Burkard (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 409—414).—The " O_2-Calculator " consists in a circular nomogram operating on the slide-rule principle, and provided with scales for ascertaining the concn. of the dissolved O_2 from analytical data, and the % saturation of O_2 from the concn. of O_2 and the altitude and temp. of the source. The conversion of O_2 concn. to % saturation is based on data given by Truesdale *et al.* The calculator can be applied to data for sea-water. (13 references.) P. S. ARUP.

Intermittent sand filters and their biology. W. T. Calaway (*Sewage industr. Wastes*, 1957, **29**, 1—5).—While filtration and adsorption play an important part in sewage purification by intermittent sand filters, the main action is biological oxidation. Zoogleal organisms are the primary agents in the process and are prevented from clogging the filter by protozoa and metazoa. Of all the animal forms present, the oligochæte worms appear to be the most important in consuming sludges and slimes, thereby keeping the bed open and active. J. S. C.

Volatile organic acids of tobacco smoke. D. A. Buyske, P. Wilder jun., and M. E. Hobbs (*Analyt. Chem.*, 1957, **29**, 105—108).—The identification and quant. determination of the steam-volatile monocarboxylic acids present in the smoke from three major types of tobacco are reported. The methods used involved partition chromatography, paper chromatography and i.r. and u.v. spectrometry. Acetic acid accounted for 50% and formic acid for 25% of the total acidity found and *iso*butyric, *iso*valeric, benzoic and an unknown acid were determined. The concn. of the homologous series from propionic to capric varied with the type of tobacco. About 90% of the acids were accounted for and the remaining 10% included at least five additional acids. (18 references.) G. P. COOK.

Substituted 4-hydroxycoumarins. Norddeutsche Affinerie, P. Spiess and W. Spiess (Inventor: K. Knoevenagel) (B.P. 752,528, 30.8.54).—The compounds, useful as rodenticides, are obtained by condensation of 4-hydroxycoumarin (**I**) with $CHR{:}CAc{\cdot}CO_2R'$ (R is phenyl, R' is alkyl), optionally in an inert solvent. Thus, a mixture of Et α-benzylidineacetoacetate, **I**, Na_3PO_4 and water is boiled during 4 hr., to give a 25% yield of *Et α-acetyl-β-phenyl-β-(4-hydroxy-coumarin-3-yl)propionate*, m.p. 149—151°. F. R. BASFORD.

Separation of press water obtained from cooking of marine animals. A.-B. Separator (B.P. 752,052, 16.9.54. Sw., 15.10.53).—Glue-water from the separator is arranged to contain most of the oil remaining in the glue-water phase, and the oil contains 3 to 10% of glue water. The mixed emulsion is broken by autoclaving at >100°; the glue-water from the secondary separating plant is returned into the press water coming into the plant so that the heat from the autoclaving stage is largely conserved. K. RIDGWAY.

4.—APPARATUS AND UNCLASSIFIED

Accuracy and reliability of melting- and solidifying-point determinations. H. Kilchher (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 456—458).—Attention is drawn to the necessity of checking the purity of org. substances used for the determination of standard data. Examples are given showing the effect of small amounts of moisture in causing solidifying-point depressions in hygroscopic org. substances. The determinations must be carried out in closed vessels. Micro-m.p. methods are more accurate and reliable than the macro-methods. Conditions for ensuring the accuracy of micro-determinations are pointed out. P. S. ARUP.

Dialysis of small specimens in batches. E. Ben-Gershom (*Nature, Lond.*, 1957, **179**, 379).—A description is given of the dialysis of large no. of samples of protein mixtures, each of small vol., by means of a mechanically-operated rotor system, consisting of a funnel-shaped test-tube rack (of polyethylene), with radial ridges holding the centrifuge tubes between them. J. S. C.

Absorption of inorganic salts by non-ionic resins (a new absorptive mechanism). J. Kennedy and H. Small (A.E.R.E., 1956, C/R 1668, 9 pp.).—Non-ionic resins were prepared by polymerization of triallyl, diallyl ethyl and diallyl methyl phosphates. They were found to be capable of absorbing various metallic salts (e.g., uranyl nitrate, cobaltous nitrate, LiCl) from org. solvents. Absorption was reduced by water, tri-alkyl phosphates and other systems of relatively high solvation energies. A mechanism of addition complex formation between resin phosphoryl oxygen and metal is suggested. J. S. C.

Comparative study of methods for the determination of the isotopes ^{32}P, ^{45}Ca and ^{35}S in plant tissues. R. de Loose (*Chim. et Industr.*, 1956, **76**, 1291—1298).—Various methods described in the literature are summarized and their reproducibility is discussed. The mean standard deviations are: 2·07% for ^{32}P, by a modification of the method of McKenzie and Dean (*Analyt. Chem.*, 1948, **20**, 559 and 1950, **22**, 489), 1·82% for ^{45}Ca, by a modification of the method of Norris and Lawrence (*Analyt. Chem.*, 1953, **25**, 956) and 1·93% for ^{35}S by pptn. as $BaSO_4$, followed by homogeneous distribution of the ppt. on Al plates, a method based on that employed for the determination of ^{14}C by Govaerts (*Bol. de Radioactividad*, 1953, **25**, 104). J. M. JACOBS.

Chromatographic method of analysis for thiols. C. A. Price and C. W. Campbell (*Biochem. J.*, 1957, **65**, 512—516).—A method is described for the determination of thiols using paper chromatography. The thiol is treated on the paper with *N*(4-hydroxy-1-naphthyl)*iso*maleimide and after development the condensation product is detected with tetrazotized di-*o*-anisidine to give an intensely coloured dye. The method is applicable to plant material and to a variety of thiols, but is not yet suitable for alkylthiols. The method will detect 0·3 μm.-mole of thiol, and under optimum conditions 0·1 μm.-mole is visible under u.v. light. J. N. ASHLEY.

Journal of Applied Chemistry

The following p[a]per appearing in the August, 1957, issue of the *Journal of Applied Chemistry*

The autothermic cracking of light hydrocarbons
By P. H. Calderbank, V. P. Hovnanian and F. O. F. Talbot

Solubility studies on arachin
By Kirsteen O. Kelly and R. H. K. Thomson

Ternary liquid equilibria : methanol-water-esters
By R. Jagannadha Rao and C. Venkata Rao

Dinuclear novolaks from *m*- and *p*-cresol
By L. E. A. Godfrey, E. G. E. Hawkins and P. A. Jenkins

Preparation of 2 : 4-dinitrobenzenesulphonyl chloride
By E

A direct method for calculating the hydrodynamic parameters of explosives
By (Mrs.) C. J. Eekhoff

The influence of rubber on the brittleness and viscosity of bituminous materials
By P. Mason, E. N. Thrower and L. M. S

The microdetermination of di[s]
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