TANNINS—THEIR OCCURRENCE AND SIGNIFICANCE*

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The extent to which tannin extract production is itself an agricultural operation is indicated and the distribution of tannins in the plant kingdom discussed. Present knowledge of the nature of the tannins is summarized and illustrated, by reference to specific extracts. The problem of the biological origin and function of tannins is discussed, particular reference being made to the effect of these substances on viruses. Brief mention is made of the effect of tannins on soils which they may disperse or aggregate to a marked extent.

UNDER present-day conditions, not only are tannins becoming of increasing importance as raw materials for industrial chemical processing in their own right, but their production also represents an agricultural operation of appreciable magnitude. The present world annual production of tannin extracts is of the order of 500,000 tons. Four extracts represent the bulk of this and are available in reasonably standardized form:

(i) Quebracho extract, from the heartwood of *Schinopsis lorentzii* or the related *balansae*—the annual production ranging from 200,000 to 250,000 tons.
(ii) Wattle (mimosa) extract, from the bark of *Acacia mollissima*—annual production approximately 120,000 tons.
(iii) Chestnut extract, from heartwood and sapwood of *Castanea sativa* and *dentata*—annual production something under 100,000 tons.
(iv) Myrabolam extract—from the dried fruits of *Terminalia chebula* and related species—annual production probably above 20,000 tons.

A variety of other extracts are also commercially available in smaller quantities.

Of the four mentioned, only wattle extract is produced by an organized agricultural operation in that the source tree is grown to a standard cycle in plantations planned and planted as such. Some 850,000 acres of African soil are under wattle cultivation, mainly in the Union of S. Africa, and the industry has its own Growers Research Association co-operating closely with the research staffs of the major extract-producing firms. All aspects of growth, cultivation, genetics etc. are receiving the same intensive scientific attention as is customary with the world's major food crops.

Quebracho extract is produced from natural stands of timber in the Argentine and Brazil, and although collection of the trees is organized, cultivation is not. The Forestal operations represent some 50% of the total and involve the ownership of about 2,500,000 acres of land. Corresponding figures for the acreage of European chestnut forests and of the Indian forests in which *Terminalia* spp. occur as a component are not available, but would certainly be quite large.

Definition of 'tannins'

A rigid definition of what constitutes a tannin is not easy to give. The term was first used by Seguin[1] in 1796 to indicate the material present in oak gall nuts, responsible for the formation of leather when hide was treated with aqueous infusions of the galls. Before this time tannage had been regarded as a physical process which caused shrinkage of raw hide and depended on an ill-defined astringent property of certain plant infusions—the fact that chemical substances were involved had not been realized. The origin and implication of the term 'tannin' is therefore quite clear—it implies a material which produces leather from hide. This point is stressed because the term 'tannin' is often used to indicate polyphenolic materials which give some of the diagnostic reactions of the tannins such as precipitation of gelatin, development of colour with

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iron salts, oxidation by permanganate, but which are still not capable of tanning. Thus much of the botanical data on the occurrence of tannins in plants is of doubtful validity since it is based on tests which are insufficiently specific. All the vegetable tannins are phenolic, but they constitute only a proportion of the polyphenols (i.e. polyhydric phenols) present in plants. Because of earlier confusion about this, one can say little more about the distribution of tannins in the vegetable kingdom than that they are of little importance in fungi, algae, mosses, liverworts, grasses and monocotyledons generally. Certain dicotyledonous families provide most of the materials used in leather manufacture, notably the Anacardiaceae (e.g. quebracho, sumach), Rhizophoraceae (mangroves), Myrtaceae (eucalypts), Leguminosae (wattle, Burma cutch), and Combretaceae (myrabolams), many of these being typically tropical or semi-tropical in habitat. Polyphenols generally are much more widespread than this. That so few families provide the bulk of the world's tannin extracts indicates only that in these the accumulation in particular tissues of the type of polyphenols which can cause tannage is remarkably high, sometimes as high as 40% of the dry weight. This accumulation may take place in any type of plant tissue—root or stem, fruit, pods, bark, wood or leaves.

Tanning

The section of polyphenols which constitutes the tannins consists of those substances which can form hydrogen bonds between phenolic groups and receptor sites on collagen molecules in such a way as to build up a reasonably stable cross-linked structure. The product, which we call leather, exhibits a greater stability to the effects of heat, water, enzymes, abrasion, etc., than the original hide. This increased stability is produced only by phenolic molecules which are large enough to link adjacent collagen sites, and which have a sufficient number of phenolic hydroxyl groups to achieve linkage at several points. If the molecule is too large, it fails to penetrate the hide under the conditions of tannage. A case-hardening effect results with the interior of the hide left untanned and susceptible to gelatinization. It is now believed that the best range of molecular weight for tannage is of the order 500 to 3000, the more significant phenolic substances present in the extracts used commercially appearing to have molecular weights about 1000 (White[2]). This is remote from the previously accepted idea that the tannins are polymeric, high-molecular-weight colloids. Low-molecular-weight polyphenols such as gallic acid, catechin, etc., cannot be regarded as tannins since, although they are adsorbed by hide, they do not cross-link collagen, and hence do not tan.

Post-war work has also shown clearly that the so-called tannins are much more complex in composition than was previously realized. Except when referring to specific substances isolated in pure crystalline form, it is preferable to use the term ' tannin extract ', rather than ' tannin '. This became clear when two-way paper chromatography[3] showed each of the normal extracts to consist of a mixture of individual substances, mainly phenolic, but not necessarily otherwise related. A two-way chromatogram of myrabolam extract (Fig. 1), for example, shows up the presence of some forty substances of which eight are now of known structure and represent some 50% of the polyphenolic material present. As such materials are produced by aqueous extraction of plant tissues, the complexity is only to be expected.

Classification of tannins

Classification of tannins, other than by reference to the source plant, is not straightforward. The most acceptable major division is into hydrolysable and condensed tannins as suggested by Freudenberg,[4] but neither term should now be read as implying uniformity of type within the group. The main distinction between the groups is that simple treatment of the hydrolysable tannins with acid or alkali splits them into sugars and recognizable phenolic carboxylic acids. The condensed tannins do not break down in this manner, nor do sugars contribute to their molecular structures.

From the leather chemist's point of view the two groups merely illustrate two ways in which plants can build up polyphenolic molecules large enough, and possessing a sufficient number of phenolic groups, to form cross-links with collagen. In hydrolysable tannin molecules a central

FIG. 1.—*' Myrabolam ' extract from* Terminalia chebula *chromatographed* (1) *with* tert.*-amyl alcohol/acetic acid/ water* (6 : 0·1 : 94) *followed* (2) *with water-saturated* sec.*-butanol. Sprayed with ferric chloride/ferricyanide reagent.*

1. Chebulic acid
2. Glucogallin
3. Gallic acid
4. Corilagin
5. 3 : 6-digalloyl glucose
6. Chebulagic acid
7. Ellagic acid (by fluorescence)
8. Chebulinic acid

carbohydrate core serves as a poly-alcohol carrying a number of phenolic carboxylic acids bound by ester linkages. The molecule is therefore surrounded by phenolic hydroxyls available for hydrogen bonding on to collagen. In the condensed tannins the same end is achieved by linking together a number of di- and trihydric phenol nuclei (e.g. catechol, phloroglucinol, pyrogallol, resorcinol) via carbon atoms or chains, ether linkages, or aliphatic heterocyclic structures of the furan, pyran and possibly other types. Both methods obviously allow of very considerable structural variation and the primary problem of tannin chemistry is to identify what individual molecular structures are present as components of typical tannin extracts.

Hydrolysable tannins

In the case of the hydrolysable tannins, Schmidt and his colleagues[5] have made reasonable progress in this direction. Fig. 2 shows the structures so far identified as extract components of myrabolams. The basis of the structure is polyesterification of glucose by gallic acid, or by phenolic acids clearly derivable from gallic acid by processes of condensation, ring-opening, oxidation, etc.

The importance of gallic acid in this group is also indicated in Fig. 3, which shows the structures of phenolic bodies isolated from other hydrolysable extracts.

The central core of a hydrolysable tannin molecule is not necessarily a monosaccharide. In the case of the classical tannic acid (*Rhus semialata*, galls) it has been shown[2, 6] that the ' gallotannic ' which constitutes the major component of the extract has a structure quite different from the penta-*m*-digalloyl glucose structure assigned to it by Fischer.[7] There is evidence that the sugar portion is at least a trisaccharide with two hydroxyls unsubstituted, while the others carry mainly galloyl groups and only a small number of di- and possibly trigalloyl groups.

Grassmann *et al.*[8] similarly postulate a tetrasaccharide core for the characteristic major tannin present in the related extract of stagshorn sumach (*Rhus typhinus*). Ideas of structure in this group of tannins are therefore changing rapidly.

Condensed tannins

Structural information on the condensed tannins is sadly lacking. The extracts known as gambier (*Uncaria gambier*) and cutch (*Acacia catechu*) have long been known to be rich in catechin. Once Freudenberg[4] had worked out the structure of this C_{15} polyphenol, it became customary to regard the catechins as the sole basis of condensed tannin chemistry and to assume that the extracts were reasonably pure substances. In the literature there are numerous catechin-based structural formulae for ' tannins ' which are now known to be highly complex mixtures of many substances.

Work such as that of Roberts[9] on the many isomeric catechins, gallocatechins, catechin and

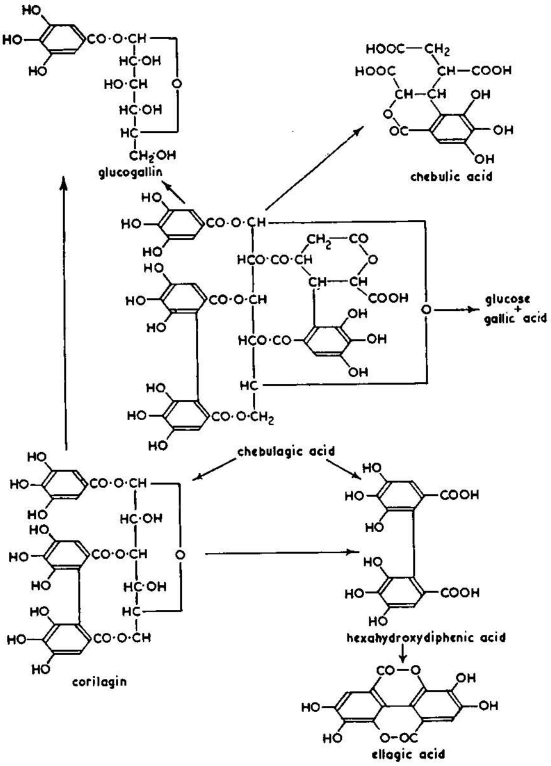

Fig. 2.

gallocatechin gallates, *leuco*-anthocyanidins and other polyphenols present in tea-leaf juice and infusions, and the attention devoted to wattle and quebracho tannin extracts, has taken us well past this phase. So far structures have been assigned to only minor non-tannin components of condensed tannin extracts, and no individual tannin has yet been isolated in sufficiently pure condition for determination of its structure. Studies in quebracho extract were directed first to the characteristic yellow fluorescent components present, all of them flavonoid in type.[10] At least sixteen such compounds are present, four of them primary substances, both by virtue of their initial presence and in that the remaining twelve are converted to them on hydrolysis. They proved to be robinetin (III), fisetin (II), monomethoxyfisetin (IV), and the related 3 : 7 : 4'-trihydroxyflavone (I) (see Fig. 4), a mono-, di- and tri-hydroxy relationship reminiscent of that found in anthocyanidins and in the aldehydic derivatives of lignin oxidation. Non-fluorescent substances have since been identified which give fisetin and monomethoxyfisetin on treating first with alkali then with acid—possibly dihydroflavonols of the fustin and methoxyfustin type. These are only minor components but they may prove to be of significance in relation to the chemistry of the actual tannins present, once these have been obtained in sufficiently pure form to permit work on their structures.

The work of Roux,[11] Hillis,[12] and King[6, 13] at Harpenden on wattle extract is rapidly elucidating the nature of the minor phenolic components, now known to contain fisetin, robinetin, gallic acid, resorcinol, syringic acid, catechin, *epi*-catechin and traces of *leuco*-anthocyanidins capable of conversion to cyanidin, delphinidin and pelargonidin. The relationship between these latter and the *leuco*-anthocyanidin, first isolated from wattle sap-wood and since crystallized by Keppler,[14] is still to be determined. The wattle sapwood compound has been shown to have the structure, V 3 : 4(*cis*)-7 : 3′ : 4′-pentahydroxyflavan.

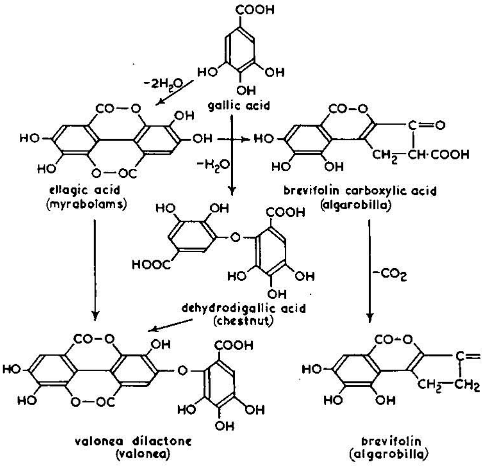

FIG. 3.

In view of the presence of so many minor phenolic components of different types, it can only be said with safety that the evidence about the molecular weight of the true tannins indicates that if they are derived from C_{15}-type units, they are likely to be dimers, trimers or tetramers with characteristic end-groups, which it should be possible to recognize or even split off. Attempts to characterize such end-groups in quebracho and mimosa extract have so far been unsuccessful and seem to indicate the absence of end groups of the catechin, flavonoid and *leuco*-anthocyanidin types.

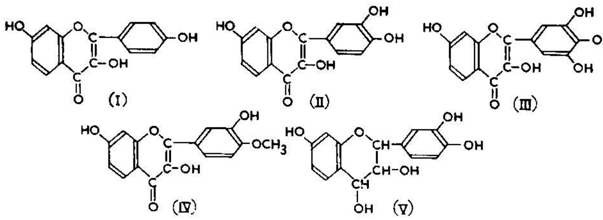

FIG. 4.

Heating mangrove and eucalypt extracts (*Rhizophoraceae* : *Myrtaceae*) with butanol–HCl gives as much as 10–15% of recognizable anthocyanidins,[6, 15] indicating that C_{15}-nuclei of the *leuco*-anthocyanidin type are of importance, either as monomeric components or as hydrolysable end-groups. It seems permissible to regard these extracts as a condensed tannin sub-group characterized by this content of anthocyanidin yielding molecules. Gambier and cutch extracts can similarly be treated as a sub-group characterized by a predominance of catechins—the other condensed tannins being so far uncharacterized, as indicated above. The group as a whole is obviously not uniform, which is to be expected in view of the heterogeneous origin of the extracts.

Origin of tannins in plants

Although our detailed knowledge of the tannins is still so vague, recent work in other fields has provided results which throw some light on the origin of tannins in plants. Davis,[16] using a variety of microbial mutants, each deficient in a single enzyme, has shown that the metabolic path which produces aromatic amino-acids in micro-organisms proceeds as in Fig. 5.

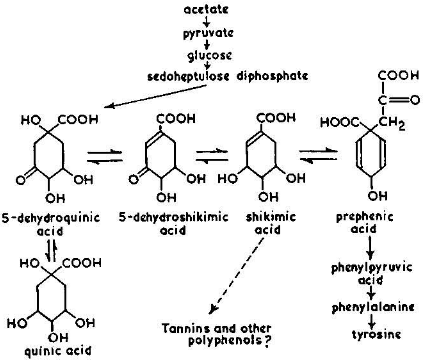

FIG. 5.

Robinson[17] has pointed out that the stages from acetate to shikimic acid represent a path from initial photosynthesis to the aromatic nuclei of the essential amino-acids.

Brown & Neish[18] suggested that in view of this evidence for the mode of biosynthesis of the aromatic ring in yeasts and bacteria, the same mechanism might be operating in plants generally. They showed, by feeding radioactive shikimic acid to wheat and maple plants, that this led to the formation of radioactive lignin, suggesting that shikimic acid is a precursor of the aromatic ring of the lignin molecule. Arising from this, Burton & Nursten,[19] and Hathway,[20] suggested that this might also be the mode of biosynthesis of tannins in plants. Burton & Nursten pointed out that Catravas[21] isolated shikimic acid from sumach extract, and suggested that the origin of hydrolysable tannins lay in a switch of aromatic biosynthesis at the shikimic acid step, leading directly or indirectly to gallic acid and its derivatives. Hathway reinforced this by demonstrating the presence in myrabolam fruits of quinic and shikimic acids as the principal acids, accompanied by lesser amounts of dihydro- and 5-dehydro-shikimic acids.

Since then, Eberhardt & Schubert[22] have shown conclusively that if shikimic acid containing radioactive C_2 and C_6 atoms is supplied to the leaves of living sugar-cane plants, the lignin subsequently isolated from these plants can be oxidized to vanillin in which the C_2 and C_6 atoms of the ring are similarly and comparably radioactive. Although this may not be the only operative mechanism, there is now no doubt that shikimic acid can act as a precursor of the aromatic ring of lignin, and this may also apply to other phenols, including the tannins, in plants. Hathway[20] suggested that gallic acid arises from a C_9-acid formed from shikimic acid and the extension of this concept to C_6–C_3–C_6 derivatives generally, could account for the origin of many of the polyphenols found in plants. In connexion with the presence of gallic acid in hydrolysable tannin structures, application of the techniques of Eberhardt & Schubert[22] to members of the Rhus family should show up if shikimic acid is a direct precursor of the galloyl groups of such products as tannic acid. It seems probable that one of the metabolic paths which result in the formation of tannins in plants has now been identified. Whether the later stages of this path involve catechins,[4] *leuco*-anthocyanidins,[15] and similar C_{15}-substances is not yet settled.

Function of tannins in plants

Mason[23] has emphasized that the enzymic activities, which he terms the ‘ phenolase complex ’ responsible for the *o*-hydroxylation of phenols and the dehydrogenation of *o*-diphenols to quinones, occur very widely in plants and produce some of the most reactive metabolic intermediates found in living tissues. The phenol-phenol-oxidase system has been assigned various functions concerned with respiration, intermediary metabolism, regulation of oxidation–reduction potentials, antibiotic effect and wound-healing. Although plants contain numerous *o*-diphenols, among them the tannins, with the right configuration for phenolase activity, these substances appear to be inaccessible to phenolase until injury has occurred. Mason[23] concludes therefore that primary terminal respiration of undamaged plant cells does not proceed through the quinones formed by the phenolase complex, but rather that it is the phenol *o*-hydroxylating function of the phenolase complex which serves to catalyse oxygen consumption. The presence of a large number of typical products of this function (lignins, tannins, flavonoids, etc.) seems to him to be evidence of this.

Besides this, Mason claims that, at the intracellular level of metabolism, polyphenols are of little value to their producers, although at the level of the organism as a whole, the lignins and flavonoids have evident value, e.g. structurally, or as pigments, or in wound-healing. Similarly, the tanned surfaces of seeds, spores and barks confer on these tissues chemical and physical resilience, while the functional browning of fruits may provide the mature seed with a humus-like environment favourable to subsequent germination and growths.

These concepts of the function of tannins and other polyphenols may have some significance. It is true that many species accumulate true ' tannins ' in tissues which are no longer the seat of active metabolism, particularly in barks and heartwoods, and the concentration attained may be very high. Such accumulated tannin substance seems to protect vulnerable parts of the plant from microbiological attack by inactivating viruses and invasive extracellular enzymes by a direct tanning action. In general, tannins do not display a significant level of toxicity to fungi, bacteria, or viruses, but above approximately 1% concentration they successfully prevent spore germination and growth of various fungi and bacteria, and certainly inhibit the activities of a wide range of viruses. If the inhibited organisms or viruses are removed from the inhibitory environment, or the concentration of tannin lowered by dilution below the inhibitory level, metabolic activity recommences. The accumulation of high levels of tannins in protective and structural tissues is probably a result of this absence of specific toxicity—low levels would be ineffective and the high levels can presumably be tolerated by the plant. The extent to which viruses curtail the life of plants is probably not known with accuracy except in relation to certain food crops, but it is significant that a high proportion of long-lived trees—e.g. Californian redwood, quebracho, etc.—have a high tannin concentration in the heartwood; while many short-lived trees have little or none. Tannin accumulation in barks should be particularly effective in preventing germination of spores of attacking fungi and penetration of fungal hyphae or of such bacteria as rely on the action of extracellular cellulases to open up a pathway for the organism.

Many published researches give evidence of the above-mentioned effects and show particularly that tannins inactivate viruses by insolubilizing them in the same way as they insolubilize hide and convert it to leather. In both cases the tanning action depends on hydrogen bonding and is reversible. The inhibition of viruses can be modified or eliminated by introducing into the inhibitory system substances such as gelatin which will form hydrogen bonds with the tannin hydroxyls and thus prevent their tanning action on the virus. Alternatively, substances which do not insolubilize viruses but will combine selectively with those sites on the virus which would normally bind tannins, also interfere with the inhibitory effect and enable tannin-treated virus preparations to display their normal virus activity.

The effect of tannins in preventing spore germination was first reported by Cook & Wilson[24] in relation to the sweet chestnut parasite *Endothia parasitica* and unpublished work[25] has shown that the spores of *Cercosporella*, responsible for barley and wheat eye-spot disease, also fail to germinate in 1% solutions of quebracho extract. Evidence of the part played by tannins in enabling plants to resist fungi has come also from the work of Nienstadt[26] on chestnut blight; Rubin & Perevyoshina[27] on cotton wilt (*Verticillium*); and of a number of workers[28–31] on the dependence on polyphenols of potato resistance to fungal attack.

Similarly, the effect of tannins in inhibiting virus infection and the fact that this is dependent on a simple tanning action has been demonstrated.[32–38] This inhibition does not seem to occur in actively metabolizing tissues—probably because these latter rarely contain true tannins. It does occur if the low-molecular-weight polyphenols in such tissues are exposed to oxidation as a result of tissue injury, and this rests on the ease of oxidative condensation of such molecules to substances having a high enough molecular weight and a sufficient number of hydroxyl groups to tan.

Use of tannins in plant protection

This question has received very little attention. Work at the Cheshunt Research Station[39] indicated that spraying tomato plants with 1% tannin solutions could prevent the spread of tomato mosaic virus and a larger-scale trial is now under way to see if this has practical application. Tannin treatment of soil might be an economic method of preventing soil transmission of virus, although such transmission is not as important as vector transmission. Harrison[40] has recently published data indicating that although a variety of ringspot viruses can be vector-transmitted, it is likely that soil-borne infection is in fact the major problem.

Soil dosage with tannins would need careful attention because of the remarkable effect that some of the extracts have on clays.[41] On a cost and performance basis in connexion with lowering of the viscosity of clay suspensions used in oil-well drilling operations, quebracho extract has a greater capacity for dispersing clays than most other materials. Arising from this, study of the effects of tannins generally on clays has shown that by proper selection of the appropriate extract and control of the pH of the clay every effect from extreme aggregation to extreme dispersion of clay particles can be produced. The state of aggregation of soil particles is of importance in relation to moisture retention, root development, crop yield, etc. Naturally-occurring tannins, or their decomposition products, play a considerable part in determining the degree of dispersion or aggregation displayed by various soils, and in determining also the accessibility of various cations. The condensed tannins oxidize readily with opening-up of the catechol and pyrogallol nuclei. The products of mild oxidation seem to be very resistant to further breakdown and are remarkably similar in general behaviour to the so-called humic acids which have so long been of interest to the agricultural chemist. These products of mild oxidation again display marked effects on the physical condition of clay particles. They probably constitute a proportion of the so-called humic acids and are therefore of direct importance to anyone studying soil physics, but this aspect of tannin chemistry has received little attention relative to the importance of the problems involved.

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THE SIMPLER PHENOLIC SUBSTANCES OF PLANTS*

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THE field of the present review covers phenolic substances whose basic structure contains not more than fifteen carbon atoms, that is the flavonoids and simpler compounds. More complex phenolic substances are omitted ; they may be described as ' tannins ' without entering into a precise explanation of the term, which some workers would limit to leather tanning materials while others include the astringent compounds of leaves and fruits. The difference of attitude depends on the direction of approach, and the term ' tannin ' is perhaps all the more useful in discussion precisely for its lack of definition.

Thus limited, the phenolics are still numerous and diverse. Derivatives of mono- to pentahydroxybenzene are found, with a wide range of side-chains, attached rings and other modifications. It has been necessary to concentrate here on the common types with only brief mention of others.

Simple phenols

Simple phenols in the free state are rare. The best authenticated is hydroquinone, which is apparently always accompanied by its glucoside, arbutin, in much larger amounts. This illustrates an important point, that most naturally occurring phenolics are found in combination as glycosides or esters, rather than in the free state—the aglycone.

Although the majority of phenolics contain the catechol and phloroglucinol patterns of hydroxylation, these simple phenols are seldom found. Catechol has been recorded in *Ephedra* and onion, but evidence for phloroglucinol in the free state is inconclusive.

Phenols with a single carbon atom attached to the ring occur more widely. Examples are hydroxylated benzyl alcohol (saligenin), benzaldehydes (salicylaldehyde, vanillin) and benzoic acids (salicylic, gallic). Phenolics with two-carbon side-chains are represented by a number of hydroxyacetophenones ; their odour and solubility rank them rather with the essential oils than the other phenolics.

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*Read at Agriculture Group Symposium on Polyphenols in Soils and Plants, 9 October, 1956

The cinnamic acid group

The C_9 compounds—a phenolic benzene ring with a three-carbon side-chain—include many substances of wide distribution. Propenylbenzenes, like the acetophenone derivatives, resemble rather the essential oils ; hydroxy-derivatives of cinnamic alcohol and aldehyde are known, but it is the hydroxycinnamic acids that are common. Bate-Smith[1] has shown these to occur very widely in leaves, in a combined form from which they are easily liberated by acid hydrolysis ; while in work at Long Ashton on the phenolics of the Pomoidae, derivatives of caffeic and *p*-coumaric acids, esterified with quinic acid, have been shown to be almost universal and in some organs, e.g. apple and pear fruit and pear leaf, occur in quite large amounts (chlorogenic, *iso*-chlorogenic and the isomeric *p*-coumarylquinic acids).[2a, b] Roberts[2b] has shown that these esters also occur in tea-leaf.

Other derivatives of hydroxycinnamic acids, the nature of whose combination has not been recorded, derive from ferulic and sinapic acids (*iso*ferulic acid has not yet been found) ; the probable occurrence of 3 : 4-methylenedioxycinnamic acid in certain species has been claimed very recently,[3] but neither 3 : 4 : 5-trihydroxycinnamic acid nor any of its derivatives other than sinapic acid appears to have been recorded. It is also remarkable that no glycosidation of the phenolic hydroxyls of these acids seems to have been noted.

Related to the hydroxycinnamic acids, but not so widely distributed, are the coumarins, which can be regarded as 2-hydroxycinnamic acids which have undergone ring-closure. As with the hydroxycinnamic acids, the first position to be hydroxylated is that *para* to the side-chain. Even in the complex furano- and chromano-coumarins the *O*-attachment is at position 7. In contrast to the hydroxycinnamic acids, coumarins often occur as glycosides.

Flavonoid compounds

The most widely distributed group of phenolics consists of the great number derived from the C_{15}-skeleton, known generally as the flavonoids. This typical carbon skeleton is an extension of the C_9-type by attachment of another benzene ring to the ' free ' end of the three-carbon chain. In a few cases the chain is ' open ', but usually it is reflexed back on itself to form a phenylchromane ring system. The flavonoid compounds can be classified chemically by the state of oxidation of the middle ring. Thus we have

1.	Catechins	saturated	ring	CH_2 at 4,	OH	at 3
2.	Flavanones	saturated	,,	CO ,, ,,	no OH	,, ,,
3.	Flavanonols	saturated	,,	CO ,, ,,	OH	,, ,,
4.	Anthocyanidins	unsaturated	,,	CH ,, ,,	OH	,, ,,
5.	Flavones	unsaturated	,,	CO ,, ,,	no OH	,, ,,
6.	Flavonols	unsaturated	,,	CO ,, ,,	OH	,, ,,

There are also a number of closely related compounds with modifications of the basic skeleton :

Chalkones : an open-chain form of flavanones.
Dihydrochalkones : which are rare.
Aurones : 5-membered middle ring with same oxidation state as flavones—they are benzylidene-coumaranones.
iso*Flavones :* 3-phenylchromane derivatives, with the same oxidation level as flavones.

There are also the *leuco*-anthocyanins, a group of compounds whose precise constitution is unknown but which are related to both the catechins and the anthocyanins. Most of them, like the catechins, react with the vanillin colour reagent, and all on treatment with acid yield among other products, anthocyanidins. Their state of oxidation appears to be between that of the catechins and the anthocyanidins and they contain the C_{15}-skeleton. Two, of unusual hydroxylation pattern, have been shown to be 3 : 4-flavandiols, and a synthetic flavandiol made by borohydride reduction of the flavanonol taxifolin has many of the properties of the group.[4] Paper chromatography has shown that the common natural *leuco*-anthocyanins are a very diverse group whose finer points of structure are still unknown.

Of these groups, the flavonols, anthocyanins, *leuco*-anthocyanins and catechins seem to be the most widespread. Phenolic hydroxyls can and do occur in almost any positions, but most commonly at 5, 7, 3′, 4′, 5′. Thus ring A commonly has a phloroglucinol pattern of hydroxylation (including the pyran ring oxygen) while ring B has a pattern corresponding to the C_9-acids, 4′, 3′ : 4′, or 3′ : 4′ : 5′. Just as caffeic is the most common of the C_9 acids, so the 5 : 7 : 3′ : 4′, the cyanidin or quercetin pattern, is the most common among the flavonoids, followed by the delphinidin and pelargonidin types. While the unmethylated 3 : 4 : 5-trihydroxycinnamic acid has not been found, no such limitation occurs in the flavonoid B ring.

Glycosidation of hydroxyls occurs in most types, but never with the catechins, and doubtfully with the *leuco*-anthocyanins. It is most common in the 3, 5 and 7 positions. Methylation is less widespread.

Occurrence and distribution

The distribution of phenolics is not uniform through the various parts of a plant or the different classes of plants. In the Pomoidae, there emerge certain broad similarities between corresponding parts of the various members, but also striking differences between different genera or even different species within a genus. The similarities pertain to the more common, widely distributed phenolics ; the differences, especially the striking differences, often concern the less common ones. Thus in the Pomoidae no delphinidin types or methylated phenolics have appeared : all Pomoidae seem to contain chlorogenic acids, *p*-coumarylquinic acids, *leuco*-cyanidins, catechins and quercetin derivatives. The relative importance of these groups varies between the parts of the tree. Broadly speaking, it appears that *in leaf*, flavonols and hydroxycinnamic acids predominate, while catechins and *leuco*-anthocyanins are less important : *in bark*, catechins and *leuco*-anthocyanins predominate : flavonols are less important and hydroxycinnamic acids much less so : *wood* resembles bark, but contains much less of the phenolics : *in fruit* the hydroxycinnamic acids (isomeric chlorogenic and *p*-coumarylquinic acids) are most important, catechins and *leuco*-anthocyanins next, flavonols least.

More detailed work on the apple and pear shows how wide a variation can occur within even the varieties of a single species. All cultivated varieties of apple show little variation in either the pattern or amounts of phenolics in the leaf and bark, but wide variations in both senses in the fruit. In dessert and culinary varieties chlorogenic acid is the most important phenolic, whereas in cider varieties, not only does the amount of chlorogenic acid increase, but an even greater increase occurs in the catechins and *leuco*-anthocyanins, the amounts and proportions varying widely according to variety.[5] The *leuco*-anthocyanins are largely of the type mobile on paper chromatograms. In one variety, the amount of *p*-coumarylquinic acids increases so greatly that it provides both a useful identification of the variety and the best source of this material so far discovered.

Pear fruits also show variations. All contain chlorogenic and *p*-coumarylquinic acids : some perry pears contain in addition large amounts of *leuco*-anthocyanins, but of a different type from those in cider apples, and only traces of catechins. Whereas much of the apple *leuco*-anthocyanin will move on paper chromatograms, the perry *leuco*-anthocyanin stays fixed at the base line. It seems to be of colloidal nature, causing heavy clots and deposits in perry-making.[6]

In the leaves of the Pomoidae, the conspicuous differences are in the apple and pear, and illustrate the generalization that differences are due to uncommon constituents. Apple leaf has as its main phenolic the dihydrochalkone glucoside phloridzin, which does not occur elsewhere in the Pomoidae. Many present day text-books state that phloridzin also occurs in the pear, cherry and plum trees, but this is not so and the error appears to have been copied from de Koninck's original paper in 1835, in spite of the fact that two French workers in 1904 and 1911, and again an American in 1926, demonstrated the absence of phloridzin from the pear.[7] The pear tree contains arbutin (hydroquinone glycoside) as a major phenolic ; this again does not seem to occur elsewhere in the Pomoidae. There are certain features of interest about the distribution of these special phenolics within the tree : phloridzin does not occur in the flesh of the apple fruit, and arbutin not in the pear ; and while in the apple tree the greatest concentration of phloridzin occurs in the root bark, the reverse holds with arbutin in the pear tree ; its concentration in the root is much less than in stem, bark or leaf.

A further interesting difference has emerged from an examination of other apple (*Malus*) species. All the cultivated varieties of apple are of one species—*M. pumila*—while botanists recognize over 20 different wild species. An examination by paper chromatography of the leaf and bark of these species showed that all contain phloridzin, but in one group of four species (Sieboldianae) the phloridzin is much reduced and a new substance appears and becomes the dominant phenolic. This has been shown to be a glucoside of a hydroxy-phloretin with the cyanidin pattern of hydroxylation instead of the pelargonidin type of phloridzin. This is believed to be the first recorded occurrence of a dihydrochalkone with anything other than the phloridzin pattern of hydroxylation.[8]

The other Pomoidae do not seem to contain any such distinctive substances, but other plants may contain unusual and highly specific phenolics. A wide survey of leaves has been undertaken by Bate-Smith, who simplified the problem by examining acid hydrolysates of plant extracts and so replaced a multiplicity of derivatives by their parent aglycones. It also shows up the *leuco*-anthocyanins as their related anthocyanidins.[9]

Paper chromatography of the hydrolysed extracts allows identification of the principal aglycones with considerable confidence by ultra-violet fluorescence, confirmed in many cases by running in a second solvent, using colour-dip reagent on the chromatograms, or by specific test-tube reactions with the original extract.

Out of the mass of data so far obtained, Bate-Smith has concentrated attention on the most common aglycones : the three flavonols, kaempferol, quercetin and myricetin ; the four hydroxy-cinnamic acids, caffeic, *p*-courmaric, ferulic and sinapic ; and the two groups of *leuco*-anthocyanins related to cyanidin and delphinidin. By considering these, it is possible to see some links between the phenolic pattern of a plant and various aspects of its botanical nature and position. Thus, dicotyledons can be contrasted with monocotyledons, woody with herbaceous plants, one family with another ; here the distinction of woody and herbaceous cuts right across the others but has apparently considerable significance since there is a high correlation between woodiness and the presence of *leuco*-anthocyanins ; flavonols too occur much more frequently in woody plants.

In contrast, the monocotyledons and dicotyledons do not differ greatly in the over-all incidence of *leuco*-anthocyanins but there is distinction in the occurrence of flavonols, which appear much more frequently in the dicotyledons ; and among the *leuco*-anthocyanins and flavonols which do occur in the monocotyledons, the proportion of trihydroxy-derivatives (delphinidin and myricetin) relative to the cyanidin and quercetin types is lower than in the dicotyledons. The monocotyledons, however, show a greater incidence of methoxycinnamic acids ; this is the most obvious distinction.

At the level of families it is not possible to generalize so easily, especially as here the woody–herbaceous distinction cuts right across many families, so that consistency within a family can only be obtained by qualification with the woody–herbaceous rule. Some families do show very consistent patterns of the common phenolics, but the most interesting points in family patterns concern the rather less common ones such as the flavonols quercetagetin and gossypetin, ellagic acid and flavones such as tricin. Such occurrences may be consistent at the level of family or genus and we have thus a whole range of occurrences in phenolics from the highly specific hydroxyphloretin at species level to the almost universal quercetic and caffeic acid. This contrast is most fascinating, and especially the erratic distribution of the rare phenolics ; thus phloretin occurs in, besides the genus *Malus*, one of the Rutaceae (*Micromelum teprocarpum*), one of the Ericaceae (*Kalmia latifolia*) and one of the Liliaceae (*Smilax glycyphylla*).

Discussion of the significance of and generalizations concerning the great variations in the phenolics of plants are premature, but it can be said that the rare and specific phenolics are just as important as the widespread and common ones. Are they the products of blocks and diversions in individual enzyme reactions which are links in a more fundamental process ? The results of Geissman on the flower pigments of the antirrhinum point to the first part of this suggestion ;[10] it seems possible that the widely distributed phenolic compound lignin, which has been scarcely mentioned so far, may provide some key to the direction of the fundamental process. The wide distribution of those hydroxycinnamic acids whose substitution pattern corresponds to the lignin breakdown products is perhaps significant.

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THE POSSIBLE SIGNIFICANCE OF POLYPHENOLS IN SOIL FORMATION *

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Soils of the podzol group are characterized by the translocation of iron compounds down the profile; it is considered that the ferric oxide in the uppermost horizons of the soil is mobilized by the action of soluble organic compounds leached from the overlying tree litter.

The solution and reduction of ferric oxide by aqueous leaf extracts is apparently caused by the joint action of carboxylic acids and polyphenols.

The development of a horizon of clay accumulation is a feature of certain podzolic soils. As aqueous leaf extracts have the property of dispersing clay suspensions, it is apparent that the development of textural profiles could be promoted by the action of these extracts. In the dispersion of clays, polyphenolic compounds constitute one of the chief groups of active compounds.

Introduction

In view of the very broad scope of this subject, this paper deals only with the movement of iron compounds and of clay in connexion with the development of soils of the podzol group.

The profile of a typical podzol is divided into a number of quite sharply differentiated horizons. The first horizon is essentially organic, consisting as it does of decomposing tree litter (the ' raw humus '); this constitutes the A_0 horizon, below which comes the mineral soil, the top layer of which (the A_1 horizon) is stained black with organic matter. Next comes the ash-coloured A_2 horizon, from which sesquioxides have been leached, and then comes a zone of accumulation, the B horizons, in which iron compounds and organic matter have been redeposited.

In a podzol revealed in a road cutting in New Zealand one may see the genetic relationship between the vegetation, in this case kauri (*Agathis australis*), and the soil development, since the podzolized area does not extend laterally beyond the boundaries of the wooded area.

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The chief characteristic of these soils is the translocation of iron compounds down the profile ; in general terms this could arise as the result either of direct mobilization of ferric oxide, or by movement of the latter as a coating on clay particles. Field evidence indicates that both processes occur under certain conditions, and indeed the occurrence of horizons of clay accumulation is a feature of certain soil types, notably the Grey Wooded and Grey Brown Podzolic soils.

The most generally accepted view is that in the process of podzol formation the causative agent is colloidal humus washed down the profile from the lower part of the A_0 horizon, the sesquioxides being peptized by the humus and moving down the profile as a humus protected colloid.

No measurable mobilization of ferric oxide by completely humified raw humus has been detected by the author, although decomposing unhumified plant material will produce a very marked solution and reduction of ferric oxide,[1] as will sterile water extracts of undecomposed tree litter.[2-4] Although the sterile systems are in general less active, the effect is quite large.

Sterile water extracts of undecomposed tree litter also possess the property of dispersing clay suspensions, and so the movement of clay down a soil profile could also be effected by the agency of such extracts.[5]

Investigation of podzol-forming agent

In view of these properties of litter extracts, material leached from unhumified forest litter, rather than colloidal humus, is regarded by the author as being the podzol-forming agent. In an investigation of the process of podzol formation the action of sterile leaf and bark extracts has been studied, and what follows is a discussion of these extracts in relation to the two processes of the solution of ferric oxide and the deflocculation of clays.

It has been shown that both the drippings from a forest canopy and rain-water, collected after its passage through undisturbed fresh litter on the forest floor, possess qualitatively the same properties as laboratory-prepared water extracts of leaf material,[3, 6] so that there seems no justification for the view that biological and chemical processes in the fresh litter are sufficiently rapid or intense to prevent such extracts from entering the mineral soil.

In connexion with the solution/reduction of ferric oxide, it has been found that all of some twenty to thirty species that have been tested were active in this respect, although the specific activity varies considerably with the species. Ferrous iron may be detected in the reaction product even when the reaction has been carried out under fully aerobic conditions. The extent of solution of ferric oxide is decreased by raising the pH but the effect is still appreciable at pH 7–7·5. From this and other evidence it is apparent that complex formation is involved.

In experiments with aspen leaf extracts, it has been shown that removal of the simpler acids by an anion-exchange resin causes a considerable decrease in the extent of solution of ferric oxide. If the acids are recovered from the resin column and treated with ferric oxide (after adjustment of concentration and pH to correspond with the original extract), appreciable solution of the oxide takes place. The amount of ferric oxide dissolved by the extract before treatment with the anion-exchanger is greater than the sum of the amounts dissolved by the acid and ' acid-free ' preparation acting separately. Since the reducing properties of the extract are apparently unaffected by treatment with the anion-exchange resin, it seems that the reaction involves an additive effect between the acids and some class of reducing compounds. The discrepancy could have arisen from incomplete removal of acids from the resin, but no appreciable differences were observed between paper chromatograms of the acids in the original extract and those removed from the anion-exchange resin.

The evidence suggests that the reducing compounds concerned are polyphenols, although in the case of aspen the evidence is less definite than for other species.

Rôle of polyphenols

A sample of rimu leaves that had been kept in the laboratory for some time was found to have become virtually inactive in dissolving ferric oxide. In comparison with an active rimu sample, a water extract of this material had a very much lower reducing capacity, as measured with triphenyltetrazolium chloride, although reducing sugars were present in the two samples in essentially the same amount. Oxygen absorption by alkaline extracts, measured in a

Warburg apparatus, was considerably higher in the case of the active sample, and this suggests that the decrease in activity of the old rimu leaf was caused by oxidation of its polyphenolic constituents.

The material precipitated by chloroform from a methanol extract of the active rimu material gave various reactions consistent with its being polyphenolic (ultra-violet absorption spectrum ; colour with ferric chloride, diazotized benzidine, etc.), and addition of an aqueous solution of this precipitate to an extract of the inactive leaves caused an increase in the extent of solution of ferric oxide to a value approaching that obtained with the active material. Further, the old leaf extract/active polyphenol preparation mixture dissolved an amount of ferric oxide considerably greater than the sum of the amounts dissolved by each alone.

A similar result was obtained with a mixture of the chloroform precipitate and acids separated from the active leaf, so that there is some justification for assuming that the polyphenols play an important part in the overall solution/reduction process. The polyphenol preparations used in the preceding experiments were rather crude, and the work needs to be repeated more rigorously.

From what is known of the history of the inactive rimu leaf sample, the loss of activity may be due to the incomplete inactivation of enzymes during drying, and, possibly following absorption of moisture from the atmosphere, enzymic oxidation of the polyphenols had taken place. This suggests a possible explanation for the not very conclusive results of tests on the aspen leaf extract for polyphenols—these leaves were collected from the ground in the autumn, and it seems very probable that in such a case one is dealing with a partial oxidation product of the polyphenols. The very rapid and intense blackening undergone by fallen aspen leaves may be a result of this oxidation.

A certain amount of evidence has been obtained for the presence in several of these leaf extracts of substances which exert an inhibiting action on the solution/reduction process. This inhibition possibly arises from the sorption of certain constituents of the extracts, and/or reaction products, on the surface of the ferric oxide.[5, 7] Probably the higher-molecular-weight fraction of the polyphenols is involved in this part of the process. This suggests the possibility that preferential sorption of these constituents of the extract in the upper levels of the soil would lead to increased attack on the sesquioxides after the solution has percolated a short distance down the profile.

Action of leaf extracts on clay suspensions

With the species studied, two types of curve have been obtained by plotting dispersion of kaolinite against the concentration of leaf extract in the suspension. In one case a very rapidly increasing dispersion is obtained with increasing concentration and once maximum dispersion has been attained, no further change takes place as the concentration of leaf extract is increased (Fig. 1, curve 1). This type of effect has been obtained with only three species—an extract of the leaves of Huon pine (*Dacrydium franklinii*) and extracts of the barks of rimu and kauri. The only constituent of the Huon pine extract that is active in this respect appears to be a polysaccharide, and, in their action on kaolinite, the two barks appear to owe their properties solely to polyphenols.

The second type of dispersion/concentration curve (Fig. 1, curve 2) is of much more general occurrence. The point of maximum dispersion here is followed by reflocculation, the shape of this part of the curve varying considerably from species to species.

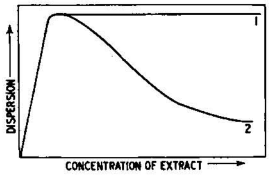

Fig. 1.—*Dispersing and flocculating effect of aqueous plant extracts on suspensions of kaolinite*

The dispersion is associated with an irreversible sorption of organic matter since kaolinite that has been reflocculated by repeated treatment with a leaf extract reverts to a dispersed state on washing, and remains in this condition despite repeated washing with water. The sorbed material cannot be removed by methanol or acetone and the clay disperses in water after such extraction. The reflocculation thus appears to be caused by the action of unsorbed, or weakly sorbed, constituents of the extract. After washing, a treated kaolinite remains dispersed down to about pH 3·5 whereas the untreated clay does not disperse below about pH 9; the effect is thus very marked. Such a treated clay appears slightly browner in colour than the untreated material and is stained blue when treated with a mixture of ferric chloride and potassium ferricyanide. Under the circumstances, therefore, it seems that the sorbed material is polyphenolic.

Two constituents which are active in deflocculation have been separated from aspen leaves. One of these is a polysaccharide, and the other possesses properties associated with polyphenols and may be the type of polyphenol oxidation product mentioned earlier.

The curious feature of this system is that while each of these constituents acting alone gives dispersion only, when both are present a degree of reflocculation approaching that given by the original leaf extract is obtained.

Confirmation of this antagonistic action has been obtained as follows. An aspen leaf extract was treated with kaolinite and the latter washed with water, the washings being combined with the treated extract. The kaolinite was now in a dispersed state, i.e. treatment with clay had the effect of removing a dispersing agent from the extract. When the effect of the treated extract on a fresh kaolinite sample was tested, it was found to possess only dispersing properties, the curve obtained being of the type illustrated by curve 1 in Fig. 1. It is apparent, therefore, that the first kaolinite treatment removes substances from the extract, presumably polyphenolic material, which, on their own, disperse the clay, but which, in the original extract, take part in causing a flocculating effect.

In preliminary experiments it has been found that if freshly picked leaves are bruised and exposed to the atmosphere, the resulting enzymic oxidation of the phenolic constituents caused marked changes in the effect of the water extract on kaolinite, so that this aspect of the problem clearly merits further study.

Effect of biological action on the properties of leaf extracts

Presumably the action of insects living in the fresh litter, coupled with the effects described by Handley,[8] would tend to decrease the amount of active material carried into the mineral soil. Once it has entered the soil, microbial action would tend to increase the extent of ferric oxide solution, but one cannot predict what changes would be induced in the deflocculating properties of the extract so far as the polyphenols are concerned. Presumably polysaccharidic material would be quickly metabolized and rendered inactive. Finally, the action of earthworms and other burrowing animals would tend to remix the soil and so tend to counteract any podzolizing tendencies. It is possibly along these lines that the answer will be found to the paradox that podzolic soils are very far from being of universal occurrence in wooded areas, although all the species so far tested are quite active in causing both deflocculation of clay and solution of the sesquioxides.

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BIOLOGICAL ACTIVITY AND CHEMICAL REACTIVITY OF ORGANOPHOSPHORUS COMPOUNDS

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Introduction

WHILE working with phosphorofluoridates in 1932, Lange & Krueger[1] accidentally discovered their toxicity. Gross proved before 1939 that certain organophosphorus compounds inhibit cholinesterase. Tri-*o*-tolyl phosphate was found by Hottinger & Bloch[2] to have a similar action. Earlier, in 1930, this compound caused paralysis in 15,000 people and a number of deaths when consumed in an alcoholic drink in the United States. The Second World War gave impetus to research on organophosphorus compounds as chemical warfare agents, both in Great Britain and Germany, and a number of extremely toxic substances had been developed. Happily these were not used as warfare agents but instead found important application in agriculture and medicine.

Since the discovery of the ability of these compounds to inhibit cholinesterase, a great volume of work on the mechanism of the inhibition has been carried out. This led indirectly to a better understanding of the nature of cholinesterases. It is with the effect of organophosphorus compounds on true cholinesterases that this discussion will be mainly concerned.

Cholinesterases and anticholinesterases

The first known anticholinesterase, eserine (or physostigmine), was extracted in 1864 from ordeal beans, used in Old Calabar in the trial of witches. The chemical structure of this alkaloid was established by Stedman & Barger[3] in 1925. The Stedmans[4] showed that eserine acted in competition with acetylcholine and proposed[5] that the hydrolytic enzyme present in horse's serum, which acted specifically on choline esters, be called ' cholinesterase '.

Two classes of cholinesterases were distinguished later:

True cholinesterases are the ones that hydrolyse acetylcholine at a faster rate than any other choline esters. They play a fundamental part in acetylcholine metabolism *in vivo*: the acetylcholine released during the passage of the electrical impulse across the neuromuscular junction, in some way causing contraction of the muscle, is eliminated almost instantaneously by the action of the enzyme, so that the nerve-muscle system is ready to respond again. *Pseudocholinesterases* are esterases that hydrolyse propionylcholine and butyrylcholine at a faster rate than acetylcholine; their function *in vivo* is obscure.

Anticholinesterases can be divided into three groups: (1) the eserine group, which has a reversible action, since the inhibition can be reversed by dialysis; (2) the direct inhibitors, such as tetraethyl pyrophosphate, whose action cannot be easily reversed and that, in common with group 1, inhibit cholinesterase *in vitro*; (3) conversion inhibitors, such as bisdimethylaminophosphonous anhydride, which are not themselves active but can be converted to active inhibitors. Thus, whereas the less stable tetraethyl pyrophosphate (TEPP) (**I**) inhibits cholinesterase *in vitro*, the much more stable bisdimethylaminophosphonous anhydride (schradan) (**II**) has to be ' metabolized ' before showing such inhibition. When the latter is incubated with liver slices, it becomes activated and converted into a direct cholinesterase inhibitor, active *in vitro*.[6] The problem of activation will be discussed later.

$$(EtO)_2P(O)\cdot O\cdot P(O)(OEt)_2 \quad \text{(I)}$$

$$(Me_2N)_2P(O)\cdot O\cdot P(O)(NMe_2)_2 \quad \text{(II)}$$

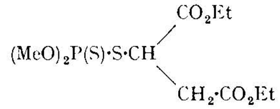
Cholinesterase inhibition and insecticidal activity

Schrader synthesized a great number of organophosphorus compounds for evaluation as insecticides. On the basis of this study he proposed the ' anhydride ' structure of the general formula (**III**) for an active compound, involving a phosphorus atom linked to oxygen or sulphur with two covalent links to alkyl, alkoxy- or alkylamino-groups, and a third, linked always to an acyl group (Schrader considered fluoridates to be anhydrides of phosphoric and hydrofluoric acids formed by the loss of the elements of water). This concept is gradually changing as more classes of physiologically active organophosphorus compounds are discovered. However, all these compounds contain the phosphoryl or thiophosphoryl grouping.

[An example that departs from Schrader's formula is the insecticide malathion (**IV**), which exhibits low *in vitro* inhibition of cholinesterase, has low mammalian toxicity and is a good insecticide, although not as potent as the compounds synthesized by Schrader.]

RR'P(O or S)Acyl

(**III**)



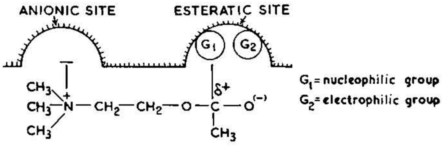
$$(MeO)_2P(S)\cdot S\cdot CH\begin{cases}CO_2Et\\ CH_2\cdot CO_2Et\end{cases}$$

(**IV**)

It is generally assumed that the lethal effect of these compounds on insects is due to their ability to inhibit cholinesterases, although Lord & Potter[7] found that certain species of insects susceptible to organophosphorus compounds of the direct inhibitor type did not appear to possess any esterases capable of being inhibited by these compounds *in vitro*. Hopf[8] found that a certain species of locust was not susceptible to mammalian cholinesterase inhibitors such as neostigmine and yet was killed by tetraethyl pyrophosphate. Also Lord & Potter[9] claimed that eggs of tomato moths could be killed by high concentrations of tetraethyl pyrophosphate. They pointed out that cholinesterases are unlikely to occur at this stage of the development of the insect, since these are enzymes associated with active nervous tissue. It seems worth considering the possibility of plasmolysis of the egg contents by the penetration of tetraethyl pyrophosphate through the cuticle of the egg, the plasticizing action of the organophosphates being well-known.

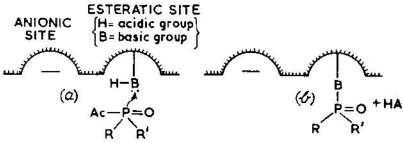
The mechanism of cholinesterase phosphorylation

Wilson & Bergman[10] proposed the following hypothetical diagram of the active groups of true cholinesterase and of their interactions with acetylcholine :



It is understood that active organophosphorus compounds occupy one of the two sites in the enzyme, i.e. the esteratic site at which the acetylcholine hydrolysis normally takes place.

The phosphorylation of the enzyme can be represented as follows : [11]



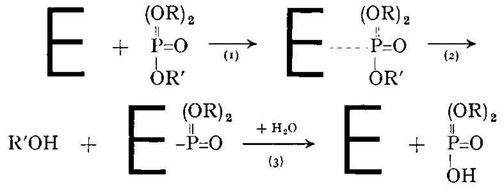
(*a*) Enzyme before phosphorylation (*b*) Phosphorylated enzyme

Cholinesterase attempts to hydrolyse the organophosphorus compound just as it does the choline esters, which are its natural substrates. When hydrolysed, the ester group remains attached to the enzyme, so preventing the natural substrate (acetylcholine) from reacting with it. Accumulation of acetylcholine causes paralysis involving the central and peripheral nervous systems.

The nature of the interaction of organophosphorus inhibitors and cholinesterase

Aldridge[12] examined the effect of temperature on the rate of reaction of erythrocyte cholinesterase with di*iso*propyl phosphorofluoridate (DFP) and with tetraethyl pyrophosphate (TEPP) and from it he calculated the energy of activation to be 10–11 kcal./mole. This could not be due to absorption, but would be consistent with occurrence of a chemical change, i.e. phosphorylation. He argued that, when inhibitors possessing the same dialkyl phosphoryl group [e.g. $(MeO)_2P(O)$—] are used, the phosphorylated enzyme is the same irrespective of the acyl group. Thus, when the acyl group was fluoride, anhydride O–P(O)$(OMe)_2$, *p*-nitrophenoxide or *p*-nitrothionophenoxide the activity of the inhibited true cholinesterase returned at the same rate. Aldridge presented the following mechanism of inhibition and reversal:

(E = enzyme ; OR′ = acyl group)



Steps (1) and (2) representing the inhibition process are comparatively fast, whereas (3), the reversal process, is slow. Aldridge concluded that if reaction (3) were faster than (1) and (2), then the enzyme would not be inhibited and the organophosphorus compound would be rapidly hydrolysed.

It is obvious that, whereas the acetylated enzyme, resulting from the reaction of true cholinesterase with its natural substrate acetylcholine, is rapidly hydrolysed by water to produce the corresponding acid and to regenerate the enzyme, the phosphorylated enzyme is very slowly attacked by water. These organophosphorus compounds are therefore inhibitors rather than substrates.

The reversibility of the inhibition

The reversibility of the inhibition (i.e. dephosphorylation of the enzyme) depends on the nature of the alkyl groups attached to the phosphorus. Thus, Barnes[13] reported that the reversal of inhibited mammalian true cholinesterase was most rapid when the alkyl groups were methyl, less rapid with ethyl and almost absent with *iso*propyl. This difference may be caused by the increasing steric hindrance effect of the alkyl groups, which would screen the phosphorus–enzyme linkage from hydrolytic attack. Burgen & Hobbiger[14] studied the duration of action of compounds having the general formula $R_2O \cdot P(O)(OR_1)_2$ (where R_1 represents an alkyl group and R_2 an aminophenyl or aminoquinolyl group whose nitrogen atom is either *tertiary* or quaternary) by injecting a single dose of the inhibitor into the test animal. They found that the action of the dimethyl homologue was short-lived and that true-cholinesterase recovery was complete after four days. On using the diethyl homologue 80–90% of the enzyme recovered in 7 days, whereas with the di*iso*propyl homologue the rate was still slower.

The activation of inhibited cholinesterases by nucleophilic reagents

Wilson & Meislich[15, 16] found that cholinesterases, combined with diethyl phosphoryl inhibitors, can be reactivated by nucleophilic reagents containing amino, hydroxyl, mercapto, guanidino, amidino, pyridyl or hydroxylamino groups ; when the inhibition was carried out with compounds containing di*iso*propyl groups (such as di*iso*propyl phosphorofluoridate) reactivation was very much slower. They pointed out that, in the process of phosphorylation of acetylcholinesterase, the anionic site of the enzyme remains uninhibited. This binds normally-alkylated quaternary amino groups, such as $Me_3\overset{+}{N}$. These workers argued that, if the two sites, the anionic and the esteratic, were attacked simultaneously by a compound containing both a hydroxylamino and a cationic amino group, reactivation of the inhibited enzyme might occur. Nicotinhydroxamic acid ethiodide proved to have these properties ; it was found to reactivate completely acetylcholinesterase inhibited even with di*iso*propyl phosphorofluoridate.

Hydrolysis rates of organophosphorus compounds as a guide to the phosphorylation rate of cholinesterases

Lipmann[17] divided biologically important phosphoric acid derivatives into two groups of compounds, those possessing ' energy-rich ' or ' high-energy ' bonds and those possessing ' energy-poor ' or ' low-energy ' bonds. Catabolic processes are thought to lead to the production of ' energy-rich ' compounds, which serve as stores of readily available energy. Anabolic processes are thought to utilize the stored energy through the agency of tri- and di-phosphates. When ' high-energy ' bonds undergo fission, they liberate about 11,000 cal. whereas the fission of a normal phosphoric ester liberates about 3000 cal. The types of compounds possessing ' energy-rich ' phosphate bonds are those containing the linkages carboxyl-P, enol-P, N–P, and P–O–P. The N–P and P–O–P concerned with the storage and utilization of phosphate bond-energy do not arise directly, but are the results of transphosphorylations. Kalckar[18] attributed the unusual thermodynamic instability of the ' high-energy ' phosphates to the resonance stabilization of the main hydrolysis product.

Brauer[19] expressed Schrader's idea of the ' anhydride ' structure in different terms and considered active cholinesterase inhibitors as compounds that might be expected from their structural arrangement to possess ' energy-rich ' bonds.

It seems reasonable to assume that phosphorylation of an enzyme is favoured by a high free-energy. This energy might be the driving force that would assist the cleavage of the P-acyl bond. There may thus be a qualitative relationship between the free-energy decrease and the velocity of the cleavage. The velocity of the hydrolysis reaction of an organophosphate (within a group of similar compounds) may therefore be a guide to the phosphorylation rate of a given enzyme.

Comparison of the reactivity of dialkyl phosphorochloridates and fluoridates

In order to exert an effect on a living system, a chemical compound must possess not only the correct physical properties, such as solubility and the correct molecular shape, but also the correct chemical reactivity, i.e. it must not be too active, as otherwise it may be wasted through non-specific interactions with various cell constituents, or even with water, before reaching the vital site of the reaction ; yet it must be active enough for the reaction to occur. An example of too high reactivity is exhibited by dialkyl phosphorochloridates (**V**), which hydrolyse with water before reaching the vital site of reaction ; the less reactive fluoridate, however, exhibits high biological activity.[20] Tetramethylphosphorodiamidic chloride (**VI**) and dimethylphosphoramidic dichloride (**VII**), though more stable to hydrolysis than the corresponding dialkyl derivatives, are still too reactive to exert an inhibiting effect. The water-stable tetramethylphosphorodiamidic fluoride (**VIII**) is a special case and will be discussed later.

$(RO)_2P(O)Cl$ (**V**)

$(Me_2N)_2P(O)Cl$ (**VI**)

$Me_2N{\cdot}P(O)Cl_2$ (**VII**)

$(Me_2N)_2P(O)F$ (**VIII**)

The action of di*iso*propyl phosphorofluoridate (DFP) on cholinesterase has received much attention. Since the enzyme is a proteinous substance, the reactivity of the fluoridate with amino-acids was studied as a basis of its phosphorylation reaction. Wagner-Jauregg *et al.*[21] could not accomplish the phosphorylation of amino-acids with this fluoridate, though obtaining a smooth reaction with the chloridate. Yet Jansen *et al.*[22] found that compounds such as $ClP(O)(OPh)_2$, $FP(S)(OEt)_2$ and tetraethyl pyrophosphate inhibited α-chymotrypsin to yield crystalline, inert proteins containing approximately one atom of phosphorus. The amount required for inhibition varied enormously. With di*iso*propyl phosphorofluoridate, for every active centre inactivated, one atom of phosphorus and two *iso*propyl groups were attached to the enzyme. In the course of inhibition, one molecule of acid was liberated and no fluorine was present in the recrystallized enzyme. The enzyme was therefore di*iso*propylphosphorylated.

Ashbolt & Rydon[23] suggested that, since the fluoridate reacted smoothly with the hydroxyl group of tyrosine, its reaction with cholinesterase would involve tyrosine. Wagner-Jauregg & Hackley,[24] on the other hand, found that the hydrolysis of di*iso*propyl phosphorofluoridate and diethyl phosphorofluoridate could be accelerated by glyoxaline, histidine, pyridine and certain derivatives of these bases ; they explained this by the formation of an intermediary quaternary complex between the *tertiary* nitrogen and the dialkyl phosphorofluoridate. They suggested that two centres may participate in the reaction of the fluoridate with an enzyme, one that functions as a phosphorylation catalyst analogous to glyoxaline and another that acts as the phosphoryl group acceptor. Pianka[25] also found that, in the presence of pyridine, hydrolysis of diethyl phosphorofluoridate to diethyl phosphoric acid occurred with water.

Activity of phosphorochloridothionates

We have seen that diethyl phosphorochloridate undergoes rapid hydrolysis with water before reaching the site of reaction and is thus wasted. However, diethyl phosphorochloridothionate $(EtO)_2P(S)Cl$, which is much more stable to hydrolysis, does possess high insecticidal efficiency, and Topley[26] reported that accidental exposure to the vapour of thiophosphoryl chloride had caused typical symptoms of cholinesterase deficiency—difficulty in breathing and constriction of the pupil of the eye.

Activation of thiono-compounds *in vivo*

The fact that the reactivity of compounds containing the P–S bond is lower than that of their phosphoryl analogues appears to be responsible for their inability to inhibit cholinesterase *in vitro.* Thus, Diggle & Gage[27] have shown that *OO*-diethyl *O*-*p*-nitrophenyl thionophosphate (**IX**) is an extremely poor inhibitor of cholinesterase *in vitro* when pure, yet the pure compound is a powerful inhibitor of cholinesterase *in vivo.* They concluded that this compound undergoes a change to an active inhibitor after injection into the animal. Gage & Payton[28] found that rat's blood may readily be inhibited by it *in vitro* in the presence of liver slices. They suggested that the thion compound became oxidized *in vivo,* thus

$$\underset{\textbf{(IX)}}{(EtO)_2P(S){\cdot}O{\cdot}C_6H_4{\cdot}NO_2\text{-}p} \xrightarrow{\text{oxidation}} \underset{\textbf{(X)}}{(EtO)_2P(O){\cdot}O{\cdot}C_6H_4{\cdot}NO_2\text{-}p}$$

They based their theory on the observation that the thiono compound (**IX**) activated by liver slices had an ultra-violet absorption spectrum and an R_F value in chromatographic analysis similar to those of compound (**X**).

Ketelaar and co-workers[29] considered that the *in vivo* activity of P=S compounds is due to their oxidation to P=O compounds.

Activation of bisdimethylaminophosphonous anhydride (schradan) and tetramethylphosphorodiamidic fluoride (dimefox) *in vivo* and by permanganate

Although diethyl phosphorofluoridate undergoes hydrolysis in the presence of pyridine and water, tetramethylphosphorodiamidic fluoride does not.[25] Jansen *et al.*[22] found that the fluoride and bisdimethylaminophosphonous anhydride had little or no effect on crystalline chymotrypsin.

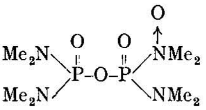
These two compounds possess dimethylamino groups exerting a large electron-donating electromeric effect, which makes them very stable in water, and it is not surprising to find that, whereas the alkyl analogues inhibit cholinesterase *in vitro,* tetramethylphosphorodiamidic fluoride and bisdimethylaminophosphonous anhydride are poor inhibitors.[30] Metcalf & March[31] found that the anhydride was non-toxic to insects on contact or by injection. Casida *et al.*[32] showed that the anhydride is converted in the plant (*in vivo* conversion) to an active cholinesterase inhibitor, and Gardiner & Kilby[30] showed this to occur also in animal tissues—an interesting example of a lethal synthesis. The activation did not occur under anaerobic conditions, so that oxygen is essential. The anhydride oxidized by permanganate[33] inhibited cholinesterase *in vitro*. Casida and co-workers[34] showed that chymotrypsin treated with the anhydride contained no phosphorus or amino groups, and no formaldehyde was evolved on acidification, whereas, when chymotrypsin was caused to react with the anhydride treated with permanganate, each moiety of the organophosphorus compound combined with one mol. of chymotrypsin. On treatment with acid, formaldehyde was liberated. These workers also obtained no reaction between tetramethylphosphorodiamidic fluoride and chymotrypsin, unless the former was treated with permanganate.

The activation of the fluoride *in vivo* may involve, as with the other thiono- and amido-organophosphates, the enzymic oxidation of the amine group to an amine oxide, which could make the P–F bond unstable. It is of interest to note that Hughes & Saunders[35] effected the fission of the C–F bond in *p*-fluoroaniline by using hydrogen peroxide and catalytic amounts of the enzyme peroxidase and obtained 2-amino-5-*p*-fluoroanilinobenzoquinone di-*p*-fluoroanil.

Spencer & O'Brien[36] found that the low *in vitro* anticholinesterase activity of bisdimethylaminophosphonous anhydride can be greatly enhanced by the introduction of an electrophilic group, such as chlorine. With increasing introduction of the electrophilic chlorine the resulting anhydride became so unstable as to undergo rapid hydrolysis. This hydrolytic instability led to a reduction in the anticholinesterase activity of the polychlorinated anhydride.

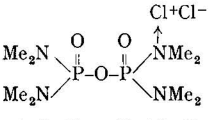
Mechanism of activation of bisdimethylaminophosphonous anhydride and of related compounds

The reactivity of bisdimethylaminophosphonous anhydride treated with permanganate or chlorine can be explained on the basis of the introduction of an electrophilic centre. With the first it may be due to the formation of an amino-oxide, thus



$$(Me_2N)_2\overset{O}{\overset{\|}{P}}\text{–O–}\overset{O}{\overset{\|}{P}}(NMe_2)(\overset{O}{\overset{\uparrow}{N}}Me_2)$$

which is then probably transformed to the $>N\cdot CH_2\cdot OH$ compound, and with the second of an amine-chloride, thus



$$(Me_2N)_2\overset{O}{\overset{\|}{P}}\text{–O–}\overset{O}{\overset{\|}{P}}(NMe_2)(\overset{Cl^+Cl^-}{\overset{\uparrow}{N}}Me_2)$$

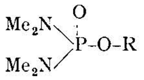
in both instances the direction of the inductive effect in the N–P bond would be reversed, the phosphorus atom becoming in its turn more electrophilic. This would facilitate the co-ordinative addition of a water molecule to the phosphorus atom. The two bonds that can undergo hydrolysis are the N–P and the P–O–P bonds. Since chymotrypsin is inactivated by the activated inhibitor, it is the P–O–P that ruptures, as otherwise inactive pyrophosphoric acid would form. Thus bisdimethylaminophosphonous anhydride and tetramethylphosphorodiamidic fluoride treated with permanganate or chlorine give rise to a reactive phosphorylation agent similar to the *in vivo* conversion product.

The remarkably high inhibition of cholinesterase exhibited by quaternary basic phosphoric acid derivatives of the general type[14, 37]

$$RR'P(O)\cdot O\cdot C_6H_4\cdot\overset{+}{N}Me_3\text{-}m$$

can be explained similarly by the presence of the positively charged group in the benzene nucleus, which is expected to enhance the electrophilic character of the phosphorus atom.

However, the compound



$$(Me_2N)_2P(O)\text{-}O\text{-}R$$

(R is 8-quinolyl)

with three groups exerting electron-donating electromeric effects, proved to be water-stable and extremely stable to alkaline hydrolysis. This compound was not insecticidal and non-toxic to mammals.[26] Even *in vivo* it could not be converted to a cholinesterase inhibitor.

Conclusions

The amount of work carried out in this field is enormous. Only certain aspects correlating the hydrolytic stability with the physiological activity of certain organophosphorus compounds have been discussed. It is, however, clear that a relationship does, in fact, exist and its consideration may be of importance when synthesizing new organophosphorus compounds with a view to their potential usefulness in clinical practice and as pesticides.

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STUDIES ON ORGANIC FLUORINE COMPOUNDS. VIII.*—*N*-Substituted Fluoroacetamides as Insecticides and Rodenticides

By **ERNST D. BERGMANN, P. MOSES** and **M. NEEMAN**

N-Mono- and *NN*-disubstituted fluoracetamides have been tested as potential larvicides, contact insecticides and rodenticides.

All monosubstituted amides were toxic to larvae of *Musca vicina* Macq. at a concentration of 100 p.p.m. Disubstituted amides were inactive or only slightly active. Only the amides monosubstituted by relatively small aryl radicals (phenyl, *p*-tolyl, *p*-fluorophenyl and *p*-chlorophenyl) showed contact toxicity to adult *Musca vicina* Macq. of a strain highly resistant to DDT.

All compounds were toxic to laboratory rats. The approximate LD_{50} values were lowest (4 to 8 mg./kg.) for *N*-mono-arylamides with small aryl radicals. Those with larger aryl substituents (α- and β-naphthyl, *p*-diphenylyl and *p*-bromophenyl) had somewhat higher LD_{50} values. The *molar* LD_{50} values of the *N*-arylamides fall within a narrow range. The possible mode of action of the toxicants is discussed.

Introduction

Gitter, Blank & Bergmann[1] have reported the surprising observation that fluoroacetamide is—on a molar basis—much less toxic to mammals than is fluoroacetic acid or its esters and is, indeed, largely excreted unchanged in the urine.† This property of the amide has since been exploited by introducing it as a rodenticide[2, 3] instead of sodium fluoroacetate. Fluoroacetonitrile has also been reported to be non-toxic.[4, 5]

It has further been stated[6] that *N*-methyl-, *N*-(β-hydroxyethyl)- and *N*-(β-chloroethyl)-fluoroacetamide are convulsant poisons for mice, but show a markedly delayed action, and that fluoroacetanilide is a contact and systemic insecticide, but is not a good rodenticide, because of its low solubility in water.[3]

It appeared, therefore, worth while to study more systematically the influence of *N*-substitution on the larvicidal, insecticidal and rodenticidal properties of fluoroacetamide.

Experimental

Materials

The compounds reported in Tables I and II have been prepared by the condensation of fluoroacetyl chloride[7] with the appropriate amine in the presence of excess pyridine. A solution of fluoroacetyl chloride (0·055 mole) in two volumes of chloroform is added, dropwise and with stirring, to a solution of the amine (0·05 mole) in pyridine (0·1 mole) at 0°. The reaction mixture is kept at room temperature for 1–2 hours, and the chloroform distilled off.

The *monosubstituted* amides (Table I) were isolated by dissolving the residue in water and cooling the solution for 12 hours, whereupon the products crystallized out. They were recrystallized from aqueous methanol, with the exception of *N*-(*p*-diphenylyl)- and *N*-(*p*-methoxyphenyl)-fluoroacetamide, which were recrystallized from glacial acetic acid and a benzene–ligroin mixture, respectively.

The NN-*disubstituted fluoroacetamides* (Table II) and *N*-(fluoroacetyl)-piperidine and -morpholine, which were water-soluble liquids, were isolated by ether extraction of the chloroform residue and purified by fractionation under reduced pressure.

Methods of biological tests

Larvicidal activity (*house flies*).—The tests were carried out on third-stage larvae of *Musca vicina* Macq. (laboratory strain exhibiting slight resistance to DDT).

One-half ml. of a 1% acetone solution of each compound was added dropwise, with stirring, to 25 ml. of distilled water containing 0·3% of the fungistatic agent Nipagin M. Sieved wheat

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* Part VII: *J. chem. Soc.*, 1956, p. 1524

† Dr. M. A. Phillips, private communication

Table I

N-*Monosubstituted fluoroacetamides*, $FCH_2{\cdot}CO{\cdot}NHR$

No.	R	Yield (%)	M.p., °C	Analysis N Calc.	Found
I	H	—	—	—	—
II	*iso*-butyl	75	35–36	10·5	10·3
III	*cyclo*-hexyl	38	93–94	8·8	8·9
IV	phenyl	78	71–72[a]	9·2	9·2
V	*p*-tolyl	95	124–126[b]	8·4	8·1
VI	*p*-methoxyphenyl	83	97–99	7·6	7·5
VII	*p*-fluorophenyl	60	114–115	8·2	8·2
VIII	*p*-chlorophenyl	96	126–127[c]	7·5	7·3
IX	*p*-bromophenyl	80	132	6·0	6·8*
X	*p*-diphenylyl	95	174–175[d]	6·1	6·2
XI	α-naphthyl	97	129–130	6·9	6·8
XII	β-naphthyl	97	105[e]	6·9	6·8
XIII	α-pyridyl	52	60–61	18·2	18·1

Notes : [a] Phillips :[3] m.p. 75–76°
[b] Price & Jackson :[8] m.p. 129–130°
[c] Phillips :[3] m.p. 131–132°
[d] Sawicki & Ray :[9] m.p. 179–180°
[e] Sawicki & Ray :[9] m.p. 106–107°
* Calc. : C, 41·4 ; H, 3·1. Found : C, 41·1 ; H, 3·5

bran (25 g.) was mixed with the resulting aqueous suspension, thus producing a toxicant concentration of 100 p.p.m. in the rearing medium. Control media were prepared similarly without toxicant. The test medium was distributed among 7 test tubes, each of which was inoculated with a 1-ml. portion from a culture of a standard bacterial flora.[10] Ten larvae, two days old, were introduced into each test tube and kept at 35 ± 1° and mortalities recorded after 48 hours. Duplicate tests were run on two different days.

Table II

NN-*Disubstituted fluoroacetamides*, $FCH_2{\cdot}CO{\cdot}NRR'$ [a]

No.	R	R′	Yield (%)	b.p., °c	/mm.	n_D^{22}	d_4^{22}	Analysis, N Calc.	Found
XIV	*n*-propyl	*n*-propyl	75	57	0·025	1·4397	1·1019	8·7	8·2
XV	*n*-butyl	*n*-butyl	34	78	0·045	1·4423	0·998	7·4	7·2
XVI	*iso*-pentyl	*iso*-pentyl	60	100	0·03	1·4433	0·887	6·4	6·0
XVII	NRR′ = piperidino		20	78	0·025	1·4740	1·169	9·7	9·7
XVIII	NRR'_2 = morpholino		13	75	0·015	1·4768	1·296	9·5	9·2

[a] *NN*-diallyl compound described by Spegiale & Hamm[9a]

Contact toxicity (house flies).—The test subjects were 2–3 days old females of *Musca vicina* Macq. of a strain highly resistant to DDT. A group of 40 flies was kept in contact for two hours at 27° with a one-day-old residual deposit of the toxicant on glass (1 g./sq. m.).[11] Knock-down counts were taken every 10 min. for the first 30 minutes, and thereafter every half hour.

Rodenticidal activity (rats).—The test subjects were male albino rats. The toxicants, in water or olive oil, were administered by stomach tube and/or by intraperitoneal injection. Symptoms of poisoning were observed, usually after 4–6 hours, and mortality counts were taken 24 hours to 6 days after the administration of the toxicant. Groups of four animals were used for each dose.

Results

The results are shown in Table III.

The observed proportional mortality values of larvae were analysed statistically by the comparison of individual means, as suggested by Tukey[12] and Hald.[13] This method permits the classification of means into groups which can be considered as belonging to a statistically homogeneous population of such means. The observed proportional mortality values of the larvae formed seven statistically homogeneous groups, as shown in Table IV.

Table III

Toxicity of N-*substituted α-fluoroacetamides*

No.[a]	Toxicity to house flies: Larvae, % mortality[b]	Toxicity to house flies: Adults, % mortality[d]	Toxicity to rats (LD_{50}, mg./kg.)[e]: Oral	Toxicity to rats (LD_{50}, mg./kg.)[e]: Intra-peritoneal
I	84	0		12 (0·16)
II	65	0	42 (0·32)	31 (0·23)
III	37			31 (0·20)
IV	70[c]	60		6 (0·04)
V	82[c]	57	7 (0·04)	4 (0·02)
VI	61		10 (0·06)	7 (0·04)
VII	83	95	2 (0·01)	5 (0·03)
VIII	75[c]	52		5 (0·03)
IX	72[c]	0		13 (0·06)
X	64	0	10 (0·04)	27 (0·12)
XI	51[c]	0		13 (0·06)
XII	59[c]	0		10 (0·05)
XIII	78	0	8 (0·05)	4 (0·03)
XIV	6	0	20 (0·12)	13 (0·08)
XV	2	0	37 (0·23)	24 (0·13)
XVI	1	20		90 (0·41)
XVII	26	0		150 (1·02)
XVIII	19	0		
XIX	74	0		2 (0·02)
Control No toxicant	1	0	0	0

[a] The numbers correspond to those in Tables I and II. I is fluoroacetamide, XIX sodium fluoroacetate

[b] 48 hours' mortality at 100 p.p.m. of the toxicant in the larval rearing medium

[c] No significant mortality of larvae at 50 p.p.m.

[d] Mortality of female house flies after two hours' contact with a residual deposit of the toxicant (1 g./sq. m.)

[e] Values in parentheses are millimoles per kg.

Table IV

Group of toxicants (No. of compounds)	Group mean percentage mortality $\bar{\bar{p}}$[a]	Confidence limits of $\bar{\bar{p}}$[b] ($P = 0{\cdot}05$)
A (I, II, IV, V, VII, VIII, IX, XIII, XIX)	76·9	72·6–82·4
B (VI, X, XII)	60·7	47·7–72·9
C (III, XI, XVII)	40·3	29·5–51·6
D (XVIII)	19·3	5·9–37·7
E (XIV, XV, XVI and control)	1·7	0·1–4·8

[a] The weighted group mean percentage mortality $\bar{\bar{p}}$ was found by averaging the percentage mortality values of all test batches

[b] Estimated by the method of Hald[13]

Discussion

The most active larvicides were the *N*-arylamides IV, V, VII, VIII, IX, the *N*-(α-pyridyl) compound XIII, and the *N*-alkylamide II. These compounds showed activity of the same order as fluoroacetamide (I) and sodium fluoroacetate (XIX). Somewhat lower larvicidal activity was shown by the *N*-arylamides VI, X, XI and XII. The above findings indicate that *N*-aryl substituents of relatively large size decrease somewhat the larvicidal activity of the amides. Compound VI caused lower mortality than would be expected from this correlation.

The three *NN*-dialkyl-fluoroacetamides (XIV, XV and XVI) were inactive and fluoroacetyl-piperidine (XVII) and -morpholine (XVIII) showed only slight activity. It appears, therefore, that at least one unsubstituted amide hydrogen is required for appreciable larvicidal activity at 100 p.p.m. This correlation applies also to the aliphatic derivative *N*-*iso*butyl-fluoroacetamide (II). However, the presence of a free amide hydrogen does not necessarily ensure high larvicidal activity, as shown by the behaviour of *N*-*cyclo*hexyl-fluoroacetamide (III).

The only compounds which showed appreciable contact toxicity to adult flies were *N*-arylamides with relatively small substituents in the *para* position (IV, V, VII, VIII). Heavier *p*-substituents, such as those present in IX, X, XI and XII, reduce the activity, perhaps by retarding penetration through the cuticula. The inactivity of XIII, as well as of fluoroacetamide (I) and of sodium fluoroacetate (XIX), may be due to their polar character which may likewise retard their cuticular penetration. The inactivity of the remaining compounds parallels in most cases their low larvicidal activity.

The toxicity to rats of the *N*-aryl-fluoroacetamides (IV to XII) and of the *N*-(α-pyridyl) amide (XIII) showed fairly uniform LD_{50} values when calculated *on a molar basis*. All but two of the molar LD_{50} values ranged from 0·02 to 0·06 mmole/kg., with those of X (intraperitoneal, 0·12 mmole/kg.) and VII (oral, 0·01 mmole/kg.) being outside this range. The LD_{50} values of these two compounds, obtained by the alternative method of administration, however, fell within the expected range (X, oral, 0·04 mmole/kg. ; VII, intraperitoneal, 0·03 mmole/kg.). Sodium fluoroacetate had a molar LD_{50} value near the lower limit of this range (0·02 mmole/kg.), while the molar LD_{50} of fluoroacetamide, in accord with previous results of Gitter *et al.*,[1] was found to be considerably higher (0·16 mmole/kg.).

The fifteen observed molar LD_{25}, LD_{50} and LD_{75} values of the *N*-aryl-amides (IV to XII) and the *N*-(α-pyridyl)amide (XIII) ranged from 0·015 mmole/kg. to 0·109 mmole/kg. Assuming that these values, as the random variable, have a normal distribution, it is possible to test the null hypothesis that the differences between these values are not significant (as $P = 0{\cdot}05$). The estimated standard deviation for this distribution was 2·37. The standardized range of these fifteen values was therefore $W_{15} = \frac{10{\cdot}9 - 1{\cdot}5}{2{\cdot}37} = 3{\cdot}96$, which is smaller than $W_{15,0{\cdot}95} = 4{\cdot}80$.

Hence the two extreme molar dose values (in the mortality range 25–75%) did not deviate significantly from the remaining 13 values.

These findings can be explained by the hypothesis that the monosubstituted amides (IV–XIII) exert their toxic action after conversion into fluoroacetate. The relative rates of penetration, hydrolysis and excretion should thus determine the overall biological response to each of these compounds.

The hypothesis appears to be supported by the constancy of the *molar* rat toxicities, although the statistical weight of the data obtained is relatively low in view of the small numbers of test animals used. Furthermore, no case seems to have been reported in which a *NN*-substituted amide is hydrolysed by an amidase,[14] and even the hydrolysis of acetamide by organ extracts is very slow.[15, 16] It has also recently been reported[17] that salicylamide is excreted after conjugation, but without hydrolysis.

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FOOD PERISHABILITY: THE DETERMINATION OF THE VULNERABILITY OF FOOD SURFACES TO BACTERIAL INFECTION

By D. H. F. CLAYSON and RUTH M. BLOOD

A technique has been devised whereby the limiting conditions of equilibrium relative humidity, and consequently of osmotic pressure, for bacterial growth can be demonstrated, using bacteria in thin films. With *Salmonella typhimurium* and *Escherichia coli* Type I, growth is prevented at and below an e.r.h. of 92% and with *Staphylococcus aureus*, at or below 85%. From the results, it may be deduced that the surface drying that occurs during baking or on exposure of moist foods to normal atmospheres does afford protection against the increase of surface bacterial contamination. The possibility of the production of variants resisting high osmotic pressure cannot be excluded, but no clear evidence of their production was obtained during the present studies.

By a modification of the technique, a heterogeneous article of food can be dissected and the vulnerability of the different parts of the growth of bacterial infection can be determined.

In an earlier paper[1] attention was drawn to the probability that bacterial growth on certain moist foods could be prevented by the formation at their surfaces of layers of high osmotic pressure by evaporation either during cooking or on exposure to atmospheres of moderate or low relative humidity. If the food has been sterilized, or rendered free from all but innocuous heat-resistant organisms, and is subject to bacterial contamination only at its surface, one might deduce that, so long as the osmotic pressure of the surface layer is too high to allow the growth of the contaminants, they will not become established in the food.

Before accepting this deduction it is necessary to establish that growth of various bacterial species does not occur above certain osmotic pressures, otherwise it is possible that contaminating bacteria may grow slowly through the surface layer of high osmotic pressure and thrive when it has been penetrated. The present paper is concerned with the development and application of an apparatus for the study of bacterial growth in nutrient films in equilibrium with atmospheres of known relative humidities, and, therefore, of osmotic pressures corresponding to those relative humidities. This technique is similar in principle to that used by various workers to establish the limiting equilibrium relative humidities (e.r.h.) for various moulds. It is considered preferable to that used by Scott[2] (who studied bacterial growth in media of which the e.r.h. were determined initially by an isopiestic method but were not controlled during the periods of growth) for the following reasons:

(*a*) Hydrolytic changes produced by bacterial growth would have some effect on the osmotic pressure and hence the e.r.h. of a given medium, although these changes may be slight at the stage at which growth can first be detected. In Scott's media, the predominating constituents from the osmotic standpoint were NaCl, KCl and Na_2SO_4, which would not be affected by metabolic changes in the medium; this probably explains why his results and the present ones are in good agreement.

(*b*) It measures growth at surfaces; the requirements for surface growth may be different from those for submerged growth.

(*c*) The calculation or determination of the relationship between e.r.h. and moisture content for each medium is avoided by bringing the media into equilibrium with NaCl solutions of known concentration.

Experimental

Apparatus and technique

The apparatus used consists of a series of units, each comprising a test-tube (150 mm. long × 14·5 ± 0·5 mm. internal diameter) and an inner glass tube (100 mm. long × 11·5 ± 0·5 mm. internal diameter) connected by two rubber bungs to one glass rod, as in Fig. 1. The inner tube carries a strip of filter paper made into a cylinder to cover most of its internal surface. The lower end of this cylinder rests in 2 ml. of NaCl solution of suitable concentration to give the e.r.h. required. These concentrations were calculated from data given in the International Critical Tables; the relationship between salt concentration and e.r.h. is shown in Fig. 2. Published figures for this relationship show some variation (up to about ± 0·3% towards 100% e.r.h.), and NaCl concentration has therefore been used as a scale of reference.

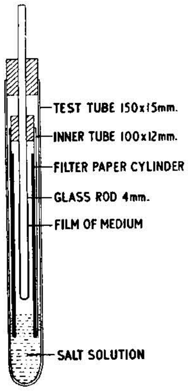

FIG. 1.—*Apparatus for measuring growth of bacteria on surfaces of controlled e.r.h.*

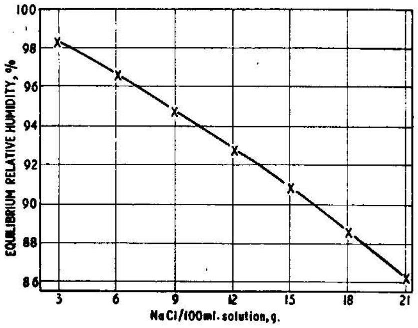

FIG. 2.—*Relation between salt concentration and e.r.h.*

The medium, suitably inoculated, is applied to the surface of the centre rod so that, on assembling the apparatus, this surface is close to the paper cylinder controlling the relative humidity. This is considered important, particularly when e.r.h. approaches 100%. The double-tube construction of the apparatus provides some insulation of the central compartment against temperature fluctuations and also allows a more liberal supply of air to be available than that contained in the centre tube alone.

At the commencement of each test it is necessary to have a known number of organisms distributed in the nutrient film, which must then be brought to equilibrium as soon as possible with the air and NaCl solution inside the apparatus. As the intention was to apply the technique to a variety of media and food materials, some of which would be solid or highly viscous when in equilibrium with the lower relative humidities to be studied, it was decided to apply the inoculated media in dilute form and to remove the excess water before growth could occur. The rods are accordingly dipped to a standard depth of 5 cm. in a dilute medium containing the inoculum and, after draining under conditions standardized for each medium, the weight of moist film is determined by dipping weighed specimen rods and re-weighing in weighed blank tubes after draining; this weight is between about 5 and 50 mg. according to the viscosity of the medium. The initial number of organisms per rod can thus be calculated from the plate count of the inoculated medium which is determined at this stage.

To prevent bacterial multiplication during the equilibration stage, one of two methods is used:

(*a*) The rods are assembled with the remaining parts of the apparatus, placed immediately in a refrigerator at 7° or less and kept there until the films reach constant weight. In some of the earlier experiments equilibrium at relative humidities down to about 94% was established in about 4 hours judging by the period required for the films to reach constant weight, but for thicker films to reach equilibrium at 90% R.H. or below, the required period was found to be 16 to 24 hours. If the assembled apparatus is transferred to the incubator before equilibrium is established, equilibration will no doubt continue during the initial stages of incubation more rapidly than it does in the refrigerator because, although e.r.h. is virtually independent of temperature, vapour pressure of solutions rises with rise in temperature. In the meantime, however, the bacterial cells of the inoculum may proliferate. On the other hand, it does not appear desirable to prolong the equilibration period unduly because the low temperature may affect the physiological state of the inoculum.

(*b*) In order to avoid the uncertainties of method (*a*), method (*b*) was evolved in which the rods, after application of the inoculated medium, are suspended in a drying chamber (see Fig. 3) and exposed to an upward current of air for a sufficient period to remove slightly more water than the loss necessary to bring the medium to equilibrium at the relative humidity of the test. For instance, it was found that films of nutrient gelatin needed to lose about 55% of their weight to reach equilibrium with an atmosphere of 94% R.H. and about 65% of their weight to reach equilibrium with an atmosphere of 90% R.H. The times required to remove these amounts of water in the drying apparatus were approximately 4 and 6 minutes respectively. To check this drying method, films inoculated with *Escherichia coli* were dried for different lengths of times and placed in the outer tubes of the apparatus without any salt solutions or filter paper cylinders. These films would have lost some water to the atmosphere within the apparatus in addition to that lost in drying, but this amount (about 0·2 mg.) was small compared with that remaining. Growth on incubation in any of these films was therefore evidence that they had not been dried sufficiently to prevent growth occurring before equilibrium was established. The results (see Table I) show that periods up to 3 minutes were inadequate, and that one film was insufficiently dried after 6 minutes and another after 9 minutes. These two films are presumed to have been above standard weight initially, owing to insufficient drainage, an uncertainty inherent in the technique employed. This uncertainty could have been avoided by checking the wet weights of the films, but the delay so caused would have introduced a further uncertainty that sub-division might occur during the weighing.

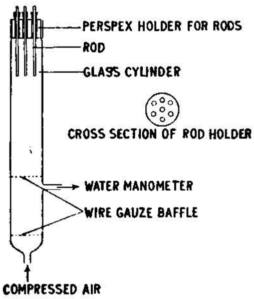

FIG. 3.—*Drying apparatus*
(Glass cylinder is 475 × 75 mm.)

To determine the numbers of bacteria on the rods after various incubation periods, the rods are transferred to small sterile test-tubes of the same dimensions as the inner glass tubes of the apparatus described above, each containing 5 ml. of sterile diluent (see later). These tubes are immersed in a water bath at 37° for 30 minutes to allow the film to disperse in the diluent, after which the suspension is shaken vigorously 50 times with a vertical movement. If the films do not readily disperse, small glass beads are included with the diluent. The subsequent stages of the plate counts follow conventional lines. It is convenient to work with not more than twelve units, which allows operations to be staggered so that the period of dispersion and plating does not exceed 40 to 45 minutes.

In initial experiments it was established that, using nutrient gelatin as medium and exposing rods inoculated with *Esch. coli*, *Salmonella typhimurium* or *Pseudomonas fluorescens* at 21° for

Table I

Effect of drying time on nutrient films in dry tubes

Test organism. *Esch. coli* (Type I) Inoculum 11,000 per rod, dried and incubated for 3 days at 21°

Drying time, min.	Total count after incubation, per rod	Multiplication
0	136,800,000	12,300
,,	62,400,000	5620
3	2,300,000	235
,,	3,900,000	351
4	<1000	<0·09
,,	<1000	<0·09
5	1000	0·09
,,	<1000	<0·09
6	<10	<0·001
,,	436,000	39·2
7	120	0·01
,,	50	0·004
8	110	0·01
,,	80	0·007
9	568,000	51·0
,,	10	0·001
10	<10	<0·001
,,	<10	<0·001

40 hours to atmospheres of 98% e.r.h., bacterial counts of the order of 25×10^6 to 500×10^6 could be attained from inocula of between 1000 and 10,000 per rod. This meant that air supply and nutrients were adequate for copious growth provided that a high e.r.h. was maintained.

To facilitate comparison, growth or decline of the organisms is recorded in terms of ' multiplication ', i.e. the number after exposure to the conditions being studied divided by the initial number. If the ' multiplication ' is less than unity, growth has either not occurred or has not occurred sufficiently rapidly to replace dying cells; if it is greater than unity, the number of sub-divisions, or ' generations ' that has occurred is the power to which 2 must be raised to correspond with the ' multiplication ' recorded. Log tables for this purpose have been published.[3] The mean generation time can be calculated by dividing the number of generations by the exposure period, but as the observations made in any one experiment were insufficient to establish the duration of the different growth phases, such calculations may not be of much significance.

Exposure media

(i) *Nutrient gelatin I* (for use at temperatures $\ngtr 25^\circ$). 1·0% peptone (Oxoid), 0·5% Lemco beef extract, 0·5% sodium chloride, 15·0% gelatin, autoclaved for 20 minutes at 10 lb.

(ii) *Nutrient gelatin II.* 1 ml. of sterile 10% glucose solution added aseptically to 9 ml. of (i).

(iii) *Nutrient gelatin III* (for use at temperatures $>25^\circ$). Similar to (i) but boiled for 3 min. instead of autoclaving.

(iv) *Nutrient broth.* 1% Lemco, 1% peptone (Oxoid), 0·5% sodium chloride.

(v) *Nutrient broth + farina.* Equal quantities of (iv) at double strength and 2% boiled farina paste.

(vi) *Bread pulp.* 100 g. crumb from centre of freshly baked loaf, mixed aseptically with 225 ml. of sterile water in top-drive macerator.

(vii) *Macerated hard-boiled egg.* Three hard-boiled eggs macerated aseptically in 200 ml. of sterile water.

The recovery procedures varied according to which of the following diluents and culture media were used. Unless otherwise stated, the plating medium used was standard agar (*a* below).

Diluents

(1) Sterile water.

(2) ¼ strength Ringer solution (prepared from Oxoid tablets).

(3) ¼ strength Ringer solution + 0·1% sodium citrate (a possible means of resuscitating moribund organisms).[4]

(4) Salt solutions (3% or more) in conjunction with culture media with added salt.

Culture media

(*a*) Standard agar. 0·5% peptone (Oxoid), 0·3% yeast extract (Marmite), 1·0% whole milk, 1·5% agar.

(*b*) Standard agar + 3% NaCl.

(*c*) Nutrient broth + 3% NaCl.

Results

Using the above technique, a study was made of various combinations of the following variables:

(A) Nature, strain and previous culture conditions of the organisms.
(B) Period of exposure of the organisms in the films during the test.
(C) Exposure temperature.
(D) Medium in which the organisms are exposed.
(E) Recovery procedure (including diluent and culture medium).
(F) E.r.h. during exposure.

The object of these experiments being to determine limiting conditions of growth, it was realized that the normal statistical methods of expressing the probability of growth under any given conditions are not applicable. In Table II a summary of all the rod tests made with *Esch. coli* and with Salmonella strains and with one strain of *Staphylococcus* is given. This Table shows the number of instances in which growth occurred (i.e. multiplication >1) and the number in which growth may have been significant (i.e. more than 1 division in 24 hours). For purposes of simplification, the conditions of growth of the inoculum, the nature of the exposure medium, and the media used for dispersing the film and culturing the surviving organisms have not been included, but this information is given in subsequent Tables for those instances where growth at comparatively low relative humidities was detected. The fact that (see Table II) no instances of significant growth were found in the 22 tests over 10·5% salt solution, whereas 24 out of 162 over 12% salt solution showed growth, suggests that some at least of these 24 could be attributed to failure of the technique, particularly as replicates of many of them did not give evidence of growth. However, the possibility that some of these anomalous results were due to genuine mutations or adaptations to the adverse conditions had to be considered.

Tables III and IV give details of the experimental conditions leading to multiplications of more than one generation per day in some or all replicates in the tests summarized in Table II. It will be seen that there was some doubt about the equilibration in some of these tests. In the earlier ones (Expt. 7) method (*a*) was used; the uncertainties of this method have already been considered. In experiments 41, 42 and 43 the doubt arose more from the hydrophilic nature of the medium used, i.e. bread pulp. The drying time required to remove the amount of water necessary to establish equilibrium was 2½ minutes and in these experiments only this drying treatment was given. It was realized, however, after experiment 42 that inequalities in particle size of the bread pulp might have caused the films to be thicker, and more difficult to dry, in some parts than in others. In experiment 43, therefore, the bread pulp, after preparation in a top-drive macerator, was triturated between two sterile test-tubes, one being of such a size as just to slide within the other. In experiment 43, the inocula consisted of sub-cultures from the isolated rods from experiment 42 that had supported growth of *Esch. coli* and *Salm. typhimurium* respectively and hence the organisms may have become trained to tolerate a higher osmotic pressure than usual. They did not, however, grow as vigorously over 12% salt as the parent cultures. Ten replicates in which *Esch. coli* was used produced less than two generations per day; seven out of ten replicates in which *Salm. typhimurium* was used did not grow and the remaining three produced less than two generations per day.

There is a possibility that, even with uniform films, air-drying will remove moisture mainly from the surface layers and that equilibrium throughout the film may not be established before growth occurs in the under layers. If this is so, one would expect growth to occur initially and then cease; as only one count per rod was possible with the technique employed, this point could not be verified directly. It has, however, been observed in some experiments that, over 9% salt solution, Salmonella cultures in the logarithmic phase produce more definite growth than

Table II

Summary of rod tests (with varying culture conditions for inoculating culture) and various recovery procedures

Organism	% NaCl regulating e.r.h.																	
	6·0			7·5			9·0			10·5			12·0			15·0		
	Total	Growth		Total	Growth		Total	Growth		Total	Growth		Total	Growth		Total	Growth	
		+	?		+	?		+	?		+	?		+	?		+	?
Esch. coli Type I	77	58	6	24	1	4	98	8	7				61	11	5	17	0	0
Salm. typhimurium NCTC 5714	50	48	1	60	51	0	80	34	11	4	0	0	60	9	2	20	0	0
Salm. typhimurium var. Binns NCTC 73	3	3	0				3	0	0				3	0	0			
Salm. typhimurium var. Copenhagen NCTC 5712	3	3	0				3	3	0				3	0	2			
Salm. typhimurium NCTC 5710	23	22	0	28	23	4	61	24	6	18	0	1	41	4	7	29	0	0
Salm. enteritidis var. Caco NCTC 5188	3	3	0				3	2	1				3	0	0			
Salm. enteritidis var. Jena. NCTC 5670	3	3	0				3	2	1				3	0	1			
Salm. enteritidis NCTC 5694	3	3	0				3	0	0				3	0	0			

Organism	% NaCl regulating e.r.h.																	
	9·0			12·0			15·0			18·0			21·0			24·0		
	Total	Growth		Total	Growth		Total	Growth		Total	Growth		Total	Growth		Total	Growth	
		+	?		+	?		+	?		+	?		+	?		+	?
Staph. pyogenes NCTC 6571	25	25	0	33	26	7	39	23	11	24	13	2	15	4	0	12	3	0

Total = Total number of rods used for each concentration of salt solution
Growth + = Number of rods giving viable counts showing more than 1 division/24 h.
Growth ? = Number of rods giving viable counts showing multiplication of more than 1, but less than 1 division/24 h.

those in the stationary growth phase. This may mean that growth of young cultures occurs below the surface of films before equilibrium is established, or it may mean that the preliminary stages of cell division have occurred in an appreciable proportion of the cells before they were incorporated in the films and that the remaining stages could proceed in an unfavourable environment.

One obvious way to overcome the uncertainties concerning equilibration was to increase the drying time. This was tried in experiment 44 using *Staphylococcus aureus* as the test organism and macerated hard-boiled egg as the exposure medium. In the first test in this experiment,

Table III

Experimental details of rod tests (selected from those summarized in Table II)

Test organism: Escherichia coli *Type I*

Expt. No.	A	B	C	D	E	F			
						over 9·0% NaCl		over 12·0% NaCl	
						Multiplication	Generations per day	Multiplication	Generations per day
7*	18 h., 37° (25,000)	23	21	i	1	4·88	2·3		
	18 h., 37° (25,000)	23	21	i	3	26·0	4·7	2·24	1·21
						12·0	3·6	0·36	—
	18 h., 37° (36,000)	23	21	i	3			3·38	1·8
								1·30	0·4
41†	18 h., 37° (19,700)	48	21	vi	3	4·36	1·1		
					3	0·68	—		
			30	vi	3	5·06	1·2	244·0	4·0
						30·4	2·5	0·05	—
						67·4	3·3	3·04	0·8
42†	18 h., 37° (9750)	48	37	vi	3			21·4	2·2
								4 replicates <1	
43†	Suspension of colonies from 42 (12·0%) (2800)	48	37	vi	3			4·28	1·0
								6·43	1·3
								4·64	1·1
								5·72	1·3
								6·43	1·3
								6·78	1·4
								5·0	1·2
								3·92	1·0
								3·57	0·9
								2·14	0·5
44	18 h., 37°	48	37	vii	3	8·85	1·6		
						0·07	—		
Test organism: Salmonella typhimurium, *NCTC* 5714									
7*	18 h., 37° (5900)	23	21	i	2	4·06	2·0	3·38	1·8
						7·8	3·0	3·72	1·9
				i	3	8·84	3·1	2·71	1·4
						18·8	4·2	6·1	2·6
41†	18 h., 37° (12,500)	48	30	vi	3	1840	5·4	6·4	1·3
						3730	5·9	57·5	2·9
						1350	5·2	6·09	1·3
42†	18 h., 37° (1850)	48	37	vi	3	22	2·3	68·0	3·0
						2·5	0·7	<0·007	—
						0·17	—	<0·007	—
43†	Suspension of colonies from 42 (12%) (6050)	48	37	vi	3			6·6	1·4
								1·15	0·1
								2·98	0·8
								7 replicates <1	

Columns A to F refer to the varying experimental conditions listed above
The numbers in brackets in column A are the initial counts per rod

Table III (*contd.*)

Expt. No.	A	B	C	D	E	F			
						over 9·0% NaCl		over 12·0% NaCl	
						Multiplication	Generations per day	Multiplication	Generations per day
	Test organism : Salmonella typhimurium *NCTC* 5710								
50	18 h., 37° (8750)	72	21	i	3	274	2·7	212·0	2·5
						145	2·4	0·34	—
						282	2·7	0·53	—
52	96 h., 21° (5% NaCl broth) (6250)	72	21	i	3	336	2·8	13·9	1·3
						1040	3·3	>480·0	3·0
						43	1·8	25·8	1·6
						138	2·4	0·77	—
						945	3·3	7·36	1·0
						2690	3·8	4·65	0·7
73	72 h., 30° (8% NaCl broth) (200)	72	21	i*a*	4, *b*	1·9	0·9	4 replicates	
						0·5	—		
						0·65	—		
						2·2	1·1		
75	24 h., 37° (1850)	72	25		4, *b*	0·062	—	3 replicates	—
						0·42	—		
						1·83	0·9		
	96 h., 30° (8% NaCl broth) (2550)	72	25	1*a*	4, *b*	12·3	3·6	3 replicates	—
						12·0	3·6		
						1·8	0·8		

* Equilibration doubtful: method (*a*)
† Equilibration doubtful due to hydrophilic nature of exposure medium

Columns A to F refer to the varying experimental conditions listed above
The numbers in brackets in column A are the initial counts per rod

Table IV

Experimental details of rod tests (*selected from those summarized in Table III*)

Test organism : Staphylococcus pyogenes (aureus) *NCTC* 6571

Expt. No.	A	B	C	D	E	F					
						over 18·0% NaCl		over 21·0% NaCl		over 24·0% NaCl	
						Multiplication	Generations per day	Multiplication	Generations per day	Multiplication	Generations per day
41	18 h., 37° (3250)	48	30	vi	3	12·0	1·8	0·03	—		
						8·65	1·6	0·37	—		
						2·46	0·6	0·04	—		
42	18 h., 37° (4400)	48	37	vi	3	105·0	3·4	10·9	1·7		
						60·7	3·0	0·33	—		
						151·5	3·6	0·11	—		
44	(1) 18 h., 37° (5150)	72	21	vii	3	1060·0	3·4				
						825·0	3·2				
	,,	72	37	vii	3	4500·0	4·1	794·0	3·2	925·0	3·3
						3340·0	3·9	1080·0	3·4	564·0	3·1
						4460·0	4·0	1390·0	3·5	1400·0	3·5
	(2) 18 h., 37° (6425)	72	37	vii	3	11·4	1·2	0·05	—	0·18	—
						60·5	2·0	0·03	—	0·10	—
						64·2	2·0	0·09	—	0·12	—

the films were dried for 2½ minutes. In these films limited growth occurred over 21% and 24% NaCl solution, but in the second part of the test, the drying time was increased to 10 minutes, and growth was not found to occur below the e.r.h. corresponding to 18% NaCl solution. With more thorough drying of films containing *Esch. coli* and *Salm. typhimurium*, however, it was found that some of the organisms in the inoculum became non-viable during the drying stage, and this suggested that the vitality of the remainder may have been affected. Table V gives the results of an experiment in which *Salm. typhimurium* NCTC 5710 was dried in films for varying times. The inoculum in one part of the experiment was grown in nutrient broth and exposed to drying in nutrient gelatin, and the survivors were recovered by dispersion in quarter-strength Ringer solution with 0·1% citrate and plated with standard agar. In the other part of the experiment, the culture of the inoculum and all the subsequent manipulations were conducted with similar media but for the addition of 3% NaCl. This addition was made because there were indications from other experiments, including that described in the next paragraph, that this organism (*Salm. typhimurium* 5710), after growing in comparatively high salt concentrations, tended to lyse if brought into contact with media containing less than 3% of salt. The results in Table V show that a 3-hour culture from a medium without added salt becomes severely diminished in numbers on drying even for 3 to 6 minutes, and that on some rods there were only a few survivors after 12 minutes. A 24-hour culture from a medium without added salt was less affected by drying. A 3-hour culture in broth containing 3% salt was also less affected, and a 24-hour culture in such a medium gave about 50% survivors even after drying for 18 minutes.

Table V

Effect of drying on viability of Salmonella typhimurium, *NCTC* 5710

Age of culture ..	.. 3 hours		3 hours		24 hours		3 hours		24 hours	
Culture medium	..		Nutrient broth				Nutrient broth + 3% NaCl			
Exposure medium	..		Nutrient gelatin				Nutrient gelatin + 3% NaCl			
Drying time (min.)	Count	σ	Count	σ	Count	σ	Count	σ	Count	σ
0	22,300	4900	3800	1400	8900	800	4200	700	5600	1600
3	12,200	1300	2900	800	7600	700	2500	500	6600	700
6	5600	3100	1800	200	7600	1000	3900	700	4500	800
9	5050	1900	1900	1300	7300	1900	2000	500	4600	1200
12	260	310	800	1100	5250	800	1700	400	4200	1000
15	95	89	190	190	2850	1200	1400	500	3500	800
18	890	750	210	220	3400	2200	1500	900	2900	400

σ = standard deviation (6 replicates)

The question of acclimatization to progressively higher salt concentrations was studied by means of liquid cultures. The inoculum consisted of one drop (approx. 0·03 ml.) of a culture that had produced definite growth at any earlier stage. Incubation throughout was at 20°. The results are shown in Table VI, expressed in terms of the number of days after inoculation required for the appearance of turbidity. In the first five stages it will be seen that there was apparent acclimatization to 6%, 7% and 8% of salt, but in none of the tests was it possible to detect visible growth in nutrient broth containing 9% or more of salt. Even the ability to tolerate 8% of salt was variable, although, as in Stage IX, the inoculum had been grown at such a concentration. No contaminants were detected, hence the effect could not be attributed to antagonistic effects from other organisms. It is noteworthy that growth could be detected by the rod technique at salt concentrations of 9% and above, but the rates of growth were such that, in a liquid medium, the appearance of visible turbidity would be extremely slow.

Discussion

In spite of many attempts to eliminate causes of variations between film cultures under ostensibly the same culture conditions, variations have remained and must be attributed to the cultures themselves. Powell[5] has recently conducted observations comprising measurement of generation times of individual bacteria within cultures. He confirmed the earlier observations of Kelly & Rahn[6] that individual generation times of freely growing cultures vary considerably. For instance, a species having a mass generation time of about 20 minutes was found to have individual generation times varying from 6 to 46 minutes. It is therefore not surprising that

Table VI

Acclimatization of Salmonella typhimurium *to increasing salt concentrations*
(Expressed as days before appearance of visible turbidity in inoculated nutrient broth + salt at 20°)

Stage	Inoculum grown in broth with			Added salt % in test medium					
	% Salt	At stage	Days	3	4	5	6	7	8
I	0		1	3	3	4	5	17	
II	6	I	12			1	1	2	5
III	7	II	2				3	5	6
IV	8	II	5					2	8
V	6	III	3				1	2	2
VI	8	II	7					9	22
VII	6	V	1				1	6	22
VIII	7	V	2				6	7	
IX	8	V	2				6		
X	8	II	9				4	4	20
XI	8	V	4				4	4	
XII	7	XI	11				1	8	

comparatively small inocula exposed to borderline conditions for growth do not give replicating results. Dean & Hinshelwood,[7] when considering the factors affecting the growth of bacterial colonies in the presence of drugs, point out that in any culture subject to lethal influences there is a wide distribution of survival times, and that it is those that survive longest that have a chance to become adapted to the unfavourable environment. Resistance to high osmotic pressure does not necessarily follow the same adaptation pattern as drug resistance. According to Robinson *et al.*[8] halophilism in *Micrococcus halodenitrificans* is associated with the presence of a mechanism that maintains a comparatively low osmotic pressure within the cell in spite of high salt concentration in the medium. As a consequence of this, enzyme systems, such as nitritase, which show maximum activity in presence of 2·2% NaCl when present in intact cells, have an optimum salt concentration of 0·9% in cell-free state. Mitchell & Hoyle[9] have recently reviewed the evidence that such an osmotic barrier exists in the plasma membrane under the cell wall of various bacterial types. It appears to be more robust in resting cells than in proliferating cells, hence osmotic conditions allowing survival will not necessarily permit cells to grow. The present evidence shows that *Esch. coli* Type I and *Salm. typhimurium* will not grow if osmotic pressures corresponding to more than 7·5 to 9% salt are being exerted. Other factors may modify this osmotic effect. For instance, Christian[10] has shown that *Salmonella oranienburg* is not retarded by glycerol as much as by sucrose, glucose or $NaCl$–KCl–Na_2SO_4 mixture when these substances are present in amounts producing the same lowering of water activity (a_w) down to the value 0·97 ; also that washed suspensions of this organism do not show a diminution in respiration rate (measured by the Warburg technique) when a_w is lowered to 0·96 by glycerol, whereas the other solutes depress respiration. This effect of glycerol may possibly be explained by the evidence of Morowitz[11] that the cellular water of certain bacteria can be replaced by glycerol solutions. Possibly glycerol may be produced by lipolysis of fat when present in sufficient quantity on badly washed implements or equipment in the food industry to allow the survival of Salmonella under air conditions that, with reasonable cleanliness, would have caused their destruction.

The technique developed shows clearly that staphylococci can grow at higher osmotic pressure than can *Salm. typhimurium* or *Esch. coli* Type I, but in the higher salt concentrations (18% or more) growth is very slow and it is doubtful whether a significant amount of toxin would form.

Application to the determination of vulnerability of foods to infection

By means of a slight modification of the method described above, it is possible to determine on what parts of a given article of food certain micro-organisms would grow, assuming that moisture content is the controlling factor. The principle of the modification is that the food sample is used to control the relative humidity inside the apparatus instead of using a salt solution, the test organisms being introduced as a dried film in nutrient media as before. There is thus no addition of liquid to the food with the inoculum, and the test organism is kept in the presence of a good medium for growth, provided that it can absorb the necessary amount of water vapour from the food sample, which must be large enough not to be affected by the small moisture loss involved.

In a typical example, slices 1 to 1·5 mm. thick were cut rapidly from the surface and from various depths below the surface of a freshly baked and cooled loaf. These were immediately placed in dry tubes and a rubber bung carrying an inoculated and dried nutrient gelatin film of the test organism (*Esch. coli*) inserted into each tube. It was found that the slices from 2 to 3 mm. and more below the surface would allow the test organism to grow. In other words, the protective zone preventing the spread of any surface contamination with this organism was 2 to 3 mm. thick initially. On further exposure to air of comparatively low relative humidity, the thickness of the zone would increase. The crust itself was found to cause an appreciable diminution in the count in the test rod, indicating that a volatile germicidal substance (e.g. furfural) may have been present.

In another application of the test it was possible to show that, in a freshly prepared veal and ham pie, a protective zone against *Esch. coli* Type I and against *Salm. typhimurium* NCTC 5710 grown in a medium containing 8% of sodium chloride, extends throughout the pastry that has a dry appearance, but ends abruptly where the pastry begins to have a soggy appearance. If such pies are chilled before wrapping and then exposed to a normal atmosphere during wrapping, they absorb up to 0·5 g. of water from the atmosphere per 180-g. pie. A moisture-proof wrapping would allow the surface zone so moistened to remain in a condition in which bacteria introduced during or before wrapping could grow. This would be deduced by an experienced food technologist and a wrapping not completely moisture-proof would be used, but it is a point which cannot readily be proved directly.

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SOME FACTORS AFFECTING THE PHYSICAL TOXICITY OF FUMIGANTS

By K. A. HASSALL

The fumigant toxicities to the grain weevil *Calandra granaria* of 39 alkyl chlorides, bromides and thiocyanates have been estimated with considerable accuracy. The difference between toxicities of isomers on the thermodynamic scale is small, except near the cut-off point, or when instability or chemical reactivity are evident. Near the cut-off point, the characteristic sudden rise in the ratio p_t/p_s (toxic vapour pressure/saturation vapour pressure) is more marked the lower the vapour pressure of the isomers, so that a secondary compound may be toxic when the normal one is non-toxic. Vapour pressure may also be a limiting factor for a whole series when the saturation vapour pressures of even the lower homologues are small. For chlorides and bromides, p_t/p_s values change slowly with carbon content below the cut-off member, but for unit saturation vapour pressure the bromides are the more toxic. When a molecular volume correction factor, V, is introduced, the points for chlorides and bromides form a single line, the slope of which approximates to unity. The equation, $\log p_t/V = \log p_s + k'$, gave better results than $\log p_t = b \log p_s + k$, when it was applied to various published data, and experimental evidence was therefore found justifying a mainly theoretically derived equation of McGowan.[4]

Experiments using different times of exposure, and with death as the criterion of toxicity, indicated no difference of behaviour between physical and chemical poisons. Doubling the exposure time approximately halved the LD_{50} and hence the p_t/p_s value. This provides further evidence that the arbitrary physical/chemical barrier of $p_t/p_s = 0{\cdot}05$ has little practical significance.

Introduction

The characteristics of substances causing physical toxicity have been discussed by Ferguson[1] and McGowan.[2] Ferguson[1] has shown that phase distribution effects can be eliminated by comparing the thermodynamic activities, ' a ', at which equitoxicity is evident, instead of comparing external concentrations directly. For a vapour, p_t/p_s approximates to ' a ', where p_t and p_s are the toxic vapour pressure for a given effect, and the saturation vapour pressure, respectively.

Equations of the type

$$\log p_t = b \log p_s + k$$

often fit experimental data satisfactorily for individual series, but contribute no information about the nature of physical toxicity. Moreover, accurate prediction of toxicity for compounds not in the series is impossible from these equations alone. For different homologous series, b often lies between 0·75 and 0·95 but in substitution series it may be higher.[3] When non- or slightly polar series have equations with similar values of b, small, but consistently occurring, differences are found between the constants k. In highly polar series the values of k are smaller than in non-polar.

Phase distribution effects having been removed, examination of isotoxic thermodynamic activities (p_t/p_s) becomes important for two reasons. First, variations in these between different substances may indicate what other properties are related to physical toxicity; accounting for any differences in p_t/p_s then becomes a necessary first step in the elucidation of the mechanism of physical action. Secondly, once a generalization is established concerning the isotoxic p_t/p_s values of different substances, a practical result would be the more accurate prediction of isotoxic concentrations of physical poisons. McGowan[2, 4] and Mullins[5] are among those who have suggested that such factors as molecular volume and density may be responsible for the range of isotoxic p_t/p_s values which has been observed, but experimental evidence is still meagre and often unreliable.

Experimental work in the past has been mainly designed to show that isotoxic physically-acting compounds act in a narrow p_t/p_s range. For this purpose it was more desirable to have numerous approximate results than fewer accurate ones. Safeguards to eliminate day-to-day changes in the sensitivity of the test organism were seldom taken, and often few dosages were used. Results have usually been obtained without statistical analysis or without making the probit lines parallel. Whether or not the observed, rather small differences in p_t/p_s are of importance in relation to the mechanism of action of physical poisons has, in consequence, not always been certain.

The main object of the present study was to assess the toxicities of some non-polar fumigants more accurately than has probably yet been done. This led to the conclusion that an equation similar to that of McGowan[4] can be applied to the results, and that some of the differences in isotoxic p_t/p_s values noted by other authors are probably due to molecular size.

Experimental

Procedure

Fumigation tests were carried out for 5 h. at 25° on grain weevils *Calandra granaria*, after which the weevils were kept in a moist atmosphere for 4 days before the percentage kill was found. The apparatus and technique were those described earlier.[6] Some of the less toxic halides were measured into the flasks from a semi-micro burette, but most were weighed out in small glass phials which were crushed within the fumigation flasks about an hour before the experiments were due to begin. When the two methods could be compared side by side using one substance, the two estimated LD_{50} were not significantly different.

In all tests, two or more substances were tested at a time, at seven or more concentration levels of each, spaced approximately equidistantly on a logarithmic scale. Since the experiments took a year to complete, tests were also carried out using the three substances chosen as group standards, namely, *n*-pentyl chloride, *n*-butyl bromide and *n*-butyl thiocyanate, to ensure that no change in relative weevil sensitivity had meanwhile occurred.

In other tests, the effect of time of exposure on LD_{50} was studied with a selection of alkyl halides, alcohols and a hydrocarbon at two times of exposure. The substances were tested singly, the 5-h. and 10-h. tests beginning at the same time. Where possible, probit lines were made parallel before the LD_{50} for 5-h. and 10-h. exposure were estimated.

Materials

Chlorides.—Several of the lower chlorides were purchased, but most of those with five or more carbon atoms were synthesized from the corresponding alcohols. Most of these were of commercial grade only and were fractionated before use. Several of the higher chlorides were prepared by the method described by Copenhaver & Whaley[7] or a modification of it. The reaction mixture consisted of zinc chloride, concentrated hydrochloric acid and the appropriate alcohol, maintained at a constant temperature in an oil bath ; the chloride was distilled off as it was formed, through a temperature-regulated upright condenser. For chlorides above C_5, the reaction is less vigorous and the products of higher boiling point, and it was found more convenient to allow the reaction to proceed under reflux and then fractionally to distil the separated upper layer. Good yields of *sec.*-butyl chloride and of 2-chloropentane (the only *sec.*-pentyl chloride studied) were obtained. The yield of 1-chloro-2-ethylbutane (‘*iso*’hexyl chloride) was poorer. This compound, which does not appear to have been previously reported, boiled at 123–126° (uncorr.) with decomposition. The method was not found satisfactory for several branched-chain chlorides, including active pentyl chloride, 4-chloro-2-methylpentane (‘*sec.*’-hexyl chloride) and 3-chloro-2 : 4-dimethyl-pentane, since low- and often also high-boiling fractions were formed in considerable quantity. For these three, and also for *sec.*-pentyl chloride, 4-chloroheptane (‘*sec.*’-heptyl chloride) and 2-chloro-octane, better results were obtained by omitting the zinc chloride and increasing the reflux time.

tert.-Pentyl and *tert.*-hexyl chloride (1-chloro-2 : 2-dimethylbutane) were readily prepared by shaking the alcohols with concentrated hydrochloric acid for a few minutes. They are, however, rapidly destroyed by cold water or alkali. Washing was therefore done very quickly with water only, the product dried with calcium chloride and purified by fractional distillation.

Bromides.—Several hexyl and heptyl bromides were prepared by the method described by Vogel[8] from red phosphorus, bromine and the alcohol. *n*-Hexyl and *n*-heptyl bromides were obtained in good yield. ‘*iso*’-Hexyl bromide (1-bromo-2-ethylbutane), b.p. 141·5–142·5° (uncorr.), and ‘*sec.*’-hexyl bromide (4-bromo-2-methylpentane), b.p. 134–136°, were obtained in moderate yield and do not appear to have been previously reported. It was not found possible to prepare 3-bromo-2 : 4-dimethylpentane by this method ; a fraction with a low boiling point and lighter than water was obtained, and a second fraction containing bromine had a very high boiling point and was highly lachrymatory.

tert.-Pentyl and *tert.*-hexyl bromides were prepared by shaking the alcohols with 50% hydrobromic acid, the product being rapidly washed and dried. The products were then further purified by vacuum distillation.

Results

The toxicities of 18 chlorides are presented in Tables I*a* and I*b* and of 21 bromides and thiocyanates in Table II. Log LD_{50} (m) were estimated by the method of probit analysis, after constraining the lines, for substances tested simultaneously, to parallelism whenever this was possible. It was found that for certain substances this constraint was not justifiable and true relative dosage values (M) could not be obtained. For this reason the five compounds which always gave lines with high probit slopes were tested separately against *n*-pentyl chloride, and their LD_{50} are given in Table I*b*. To bring these results as nearly into line with those recorded in Tables I*a* and II as the data permit, values in column 4 have been derived after taking into account differences between the average log LD_{50} (m) of *n*-pentyl chloride used each time as standard, and the average m value of 1·984 for this substance used as standard in Table I*a*.

The toxicities of the three substances used as standards, namely, *n*-pentyl chloride, *n*-butyl bromide and *n*-butyl thiocyanate, were average values obtained from 6, 4 and 2 tests respectively. In view of the importance attached to intra-series differences between the constants k in equations of the type

$$\log p_t = \mathrm{b} \log p_s + \mathrm{k} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

several tests were carried out with these three substances side by side. It was confirmed that the average values gave in fact fully representative relative toxicities, so that all the compounds in Tables I*a* and II have had toxicities determined under strictly comparable conditions.

The method of calculation of molar dosages and p_t/p_s values has been described more fully earlier.[9] Relative dosage values (M) were obtained by the method of Finney[10] and from them relative molar dosage values (M^*) were obtained by means of the relationship

$$M^*_{12} = M_{12} + \log W_1 - \log W_2$$

where W_1, W_2 are the molecular weights of the test substance and group standard respectively.

Table I

Toxicities of chlorides relative to n-*pentyl chloride*

1 Chloride	2 No. of tests	3 M, average (*n*-pentyl = 0·0) ± S.E.$_M$.	4 Log mol./l. × 10^4 *n*-pentyl = 0·956	5 p_t/p_s	6 Log p_s
a) Low probit slopes (b = 4–8)					
n-Butyl	1	− 0·423 ± 0·028	1·440	0·49	2·023
iso-Butyl	2	− 0·595 ± 0·024	1·612	0·52	2·167
sec.-Butyl	3	− 0·619 ± 0·028	1·636	0·51	2·195
n-Pentyl	6	0·0	0·956	0·51	1·518
iso-Pentyl	3	− 0·041 ± 0·020	0·997	0·42	1·641
active-Pentyl	2	− 0·070 ± 0·033	1·026	0·43	1·657
sec.-Pentyl	3	− 0·134 ± 0·018	1·090	0·46	1·693
n-Hexyl	2	+ 0·261 ± 0·024	0·641	0·80	1·009
' *iso*'-Hexyl	2	+ 0·201 ± 0·026	0·701	0·59	1·196
sec.-Hexyl	4	+ 0·188 ± 0·022	0·714	0·61	1·196
' *sec.*'-Hexyl	3	+ 0·079 ± 0·024	0·823	0·46	1·430
n-Heptyl	1	+ 0·294	0·560	> 1·00	0·565
' *sec.*'-Heptyl	2	+ 0·381	0·473	0·79	0·845
(*b*) High probit slopes (b = 10–24)					
n-Propyl	1	—	1·831	0·37	2·530
sec.-Propyl	4	—	2·042	0·40	2·713
tert.-Butyl	6	—	0·990	0·06	2·473
tert.-Pentyl	6	—	0·831	0·16	1·882
tert.-Hexyl	2	—	0·721	0·35	1·446

See text for derivation of molar dosages of compounds giving lines of high slope. No calculated regression lines were obtained for *n*-heptyl and ' *sec.*'-heptyl chlorides

Table II

Toxicities of bromides and thiocyanates

	No. of tests	*M*, average (*n*-butyl = 0·0) ± S.E$_M$.	log mol./l. × 10^4	p_t/p_s	Log p_s
(*a*) Bromides (*n*-butyl = 0·841)					
Ethyl	1	− 0·130 ± 0·031	1·072	0·047	2·668
n-Propyl	2	− 0·067 ± 0·013	0·955	0·125	2·127
sec.-Propyl	3	− 0·735 ± 0·014	1·623	0·37	2·324
n-Butyl	4	0·0	0·841	0·33	1·597
iso-Butyl	4	− 0·229 ± 0·012	1·070	0·36	1·780
sec.-Butyl	6	− 0·301 ± 0·010	1·142	0·40	1·814
tert.-Butyl	2	+ 0·243 ± 0·017	0·598	0·06	2·092
n-Pentyl	3	+ 0·271 ± 0·027	0·528	0·46	1·130
iso-Pentyl	3	+ 0·165 ± 0·018	0·634	0·41	1·286
active-Pentyl	1	+ 0·176 ± 0·046	0·623	0·40	1·294
sec.-Pentyl	4	+ 0·063 ± 0·017	0·736	0·49	1·312
tert.-Pentyl	2	+ 0·351 ± 0·018	0·448	0·15	1·532
n-Hexyl	1	+ 0·40	0·360	> 1·00	0·602
'*iso*'-Hexyl	1	+ 0·083 ± 0·039	0·678	> 1·00	0·883
'*sec.*'-Hexyl	1	+ 0·243 ± 0·041	0·518	0·59	1·013
tert.-Hexyl	2	+ 0·159 ± 0·017	0·602	0·62	1·076
(*b*) Thiocyanates (*n*-butyl = 0·486)					
n-Propyl	1	− 0·010 ± 0·040	0·552	0·21	0·493
sec.-Propyl	1	− 0·215 ± 0·042	0·757	0·19	0·753
n-Butyl	2	0·0	0·486	0·46	0·088
iso-Butyl	1	− 0·225 ± 0·038	0·711	0·51	0·268
sec.-Butyl	2	− 0·088 ± 0·028	0·574	0·37	0·280

Once a molar LD$_{50}$ had been assigned to the standard, the molar concentrations for the remaining substances were obtained from the equation

$$\log (\mathrm{LD}_{50})_1 = \log (\mathrm{LD}_{50})_2 - M^*_{12}$$

Vapour pressures are related to molar concentrations by the expression

$$\log p_t = \log [\mathrm{LD}_{50}(\mathrm{mol./l.}) \times 10^5] - 0{\cdot}731$$

Results of tests in which different exposure times were used appear in Table III. In most cases the probit lines had slopes which were not significantly different at different times, but a notable exception was *n*-propyl chloride. For this substance the high slope observed at 5 h. was not obtained at 10 h. Table III also shows that, in general, doubling the exposure time resulted in the corresponding LD$_{50}$ being rather less than halved, an effect which has been frequently noted for *chemically* toxic substances. It would thus appear that, at least when death is used as a criterion of physical toxicity, not only the concentration of the poison at the site of action, but also the time it stays there, determines the intensity of the response. This confirms a previous finding of McGowan.[10a]

Table III

Effect of time of exposure on m *for some physical and chemical fumigants*

Substance	m_5 ± S.E$_m$.	m_{10} ± S.E$_m$.	Common b ± S.E$_b$.
Chloroform	2·409 ± 0·007	2·178 ± 0·016	None (10, 6)
Heptane	2·273 ± 0·011	2·019 ± 0·010	7·14 ± 0·43
Methyl alcohol	1·956 ± 0·009	1·766 ± 0·007	8·91 ± 0·45
Ethyl alcohol	1·968 ± 0·007	1·822 ± 0·008	10·31 ± 0·76
n-Propyl chloride	2·713 ± 0·004	2·578 ± 0·007	None (18, 11)
n-Butyl chloride	2·357 ± 0·010	2·110 ± 0·011	7·53 ± 0·50
n-Pentyl chloride	1·971 ± 0·013	1·635 ± 0·012	8·56 ± 0·65
n-Hexyl chloride	1·643 ± 0·016	1·410 ± 0·010	None (6, 8)
n-Butyl bromide	1·954 ± 0·009	1·707 ± 0·009	9·74 ± 0·59
n-Pentyl bromide	1·718 ± 0·016	1·408 ± 0·019	7·52 ± 0·71
n-Butyl iodide	0·820 ± 0·014	0·531 ± 0·014	5·90 ± 0·35
n-Pentyl iodide	0·870 ± 0·017	0·577 ± 0·021	5·31 ± 0·44

m_5, m_{10} = log LD$_{50}$ (mg./l.) for 5- and 10-h. exposure respectively. b, probit slope ; where no common slope, individual values given in parenthesis

Discussion

An examination of the p_t/p_s values in Tables I and II corresponding to the LD_{50} for chlorides and bromides, shows that the *tertiary* halides, and also the first two bromides, are atypical. For these eight substances, the p_t/p_s values change rapidly with carbon content, whereas for the remainder they are fairly stable over a considerable range. The anomalous position of the *tertiary* compounds is presumably due to the ease with which they are hydrolysed. The ethyl and propyl bromides have higher chemical reactivities than any of the other bromides (e.g. Menschutkin[11]), and the low relative saturations at which these and some other lower members of homologous series exert a toxic action have been attributed by Ferguson & Pirie[3] to their chemical reactivity.

For the remaining halides, the most striking feature is the stability of the p_t/p_s value in a series up to a point near the cut-off point, when a sudden rise occurs which is in general the more pronounced the lower the vapour pressure. Thus the rise between *n*-pentyl and *n*-hexyl chloride is 0·29 units, but between *sec.*-pentyl and *sec.*-hexyl chloride only 0·15 units. For bromides the effect is similar. The reason for this rise is not certain, but factors governing the speed of attainment of equilibrium and of accessibility of the molecules of toxicant are probably involved. Burtt[12] pointed out that if degree of saturation is analogous to electrical potential, absolute solubility is analogous to capacity. The present results confirm that ' capacity ' effects assume greatest importance when the concentration in the external phase is lowest.

It has long been recognized that the toxicities of isomeric alcohols follow the order

$$n > iso > sec. > tert.$$

when molar external concentrations are the index of toxicity.[13] Ferguson[1, 14] has shown that this is largely a phase-distribution effect, and contributes no information on the exact relationship of toxicant molecules to their substrate at the site of action. He considered[14] that for highly polar narcotics, the effect of carbon chain branching was to increase the potency greatly (i.e. to decrease the isonarcotic p_t/p_s values). For less polar series, the effect was much less marked, and for the isomeric pentanes the toxicity decreased with chain branching. Except possibly for *n*-pentyl chloride and bromide which appear to be rather less toxic than their isomers, there is little indication in the present study that carbon chain branching has any appreciable effect on toxicity on the p_t/p_s scale when the standard errors of M are taken into account. Since cyclic compounds tend to be somewhat less toxic than aliphatic ones with similar vapour pressures (see for example the data of Ferguson & Pirie[3]) the simulation of a ring form by normal pentyl chloride and bromide might account for their lower toxicity.

Unlike the halides, the p_t/p_s value in the thiocyanates is not stable up to a point near the cut-off point (Table II*b*, and also Hassall[6]). The rise is unlikely to be due to chemical reactivity, for not only do thiocyanates have a typical anaesthetic effect on weevils, but also the p_t/p_s values at which *n*- and *sec.*- isomers operate are roughly the same. This contrasts with the situation found in the chemically toxic iodides (Hassall[9]) where *n*-iodides are often 3–10 times more toxic than their *sec.*-isomers on the p_t/p_s scale. The rise may be due to the very low vapour pressures of all the thiocyanates used.

Turning next to intra-series effects, Ferguson & Pirie[3] showed experimentally that toxic vapour pressure, p_t, of physical poisons in any one series was related to the saturation vapour pressure, p_s, by an equation of the type (1). Here b is a measure of the tendency of p_t/p_s to rise in the series, while k measures the intrinsic toxicity of the group as a whole. Fig. 1 shows that the equation holds well for chlorides and bromides if the lower *n*-bromides and all *tert.*-compounds are excluded, and it happens that for these two closely related series the k values are not greatly different. Nevertheless, whenever chlorides and bromides have been tested together (e.g. Ferguson & Pirie,[3] Hassall[6]), the points for chlorides on such a graph always lie to the left of those for bromides, and the difference thus appears to be real. For other series the differences between k values are larger, and in general the following order of k values can be observed: aromatic compounds > aliphatic hydrocarbons > chlorides > bromides > thiocyanates (> highly polar series)

If, therefore, for any set of data involving more than one series of non-polar physical poisons, the best straight line is drawn through the points, such differences will be reflected in the standard

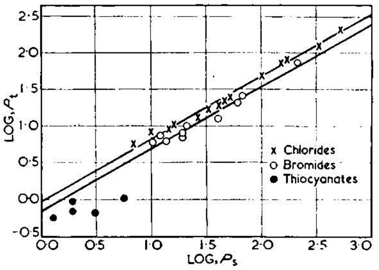

FIG. 1.—*Relation of log* p *to log* p_s *for alkyl chlorides, bromides and thiocyanates*

errors of the common slope, b, and in the size of χ^2, which measures the goodness of fit of all points to the line. These values have been calculated for ten sets of data, and appear in columns 4 and 5 of Table IV.

The chief objection to equations of the type (1) is that the empirical constants b and k give no further insight into the mechanism of narcosis and must be determined for every series of physical poison. It has long been recognized that molecular volume or surface area may affect narcotic potency. The evidence, however, has either been of a specialized nature such as that of Warburg (summarized by Winterstein[15]), and of Brink & Posternak,[16] or mainly theoretical in origin (Mullins,[5] McGowan[4]). Inspection of the data in this and earlier papers led to the conclusion that if p_t/V was substituted for p_t in equation (1), the differences between the k values of different series were diminished. A detailed statistical analysis was then undertaken of all data on non-polar and halogenated fumigants available in the literature, with the results shown in columns 6 and 7 of Table IV. The χ^2 value in column 7 is in nearly every case smaller than that in column 5 and is never appreciably larger. As would be expected, the volume correction factor has more effect when the substances used were not all closely related than when only single series were tested. Moreover when log p_t/V is plotted against log p_s, the line of best fit usually has a slope approximating to 1·0 (although inclusion of cut-off members lowers this somewhat). Use of log $p_t . C_1$ (where C_1 is the molar concentration of the toxicant in its own pure liquid ; see McGowan[4]), gave results as satisfactory as those in columns 6 and 7. Surface area,

Table IV

Comparison of slopes and χ^2 *for* $\log p_t = b \log p_s + k$ *and* $\log p_t/V = b \log p_s + k'$

1	2	3	4	5	6	7
Author	Materials[a]	n	y = log p_t		y = log p_t/V	
			b ± S.E$_b$.	χ^2	b ± S.E$_b$.	χ^2
Ferguson[14]	Aliphatic	17	0·832 ± 0·069	1·18	0·931 ± 0·055	0·75
Ferguson & Hawkins[17]	Inert gases	6	0·835 ± 0·149	0·23	0·977 ± 0·140	0·22
Ferguson & Pirie[3]	Aliphatic, mostly halides	33	1·007 ± 0·048	0·92	1·096 ± 0·024	0·22
Ferguson & Pirie[3]	Aromatic	5	0·947 ± 0·057	0·012	1·017 ± 0·042	0·007
Fühner[18]	Paraffins without octane	3	0·868 ± 0·026	0·000	0·991 ± 0·034	0·001
	with octane	4	0·772 ± 0·057	0·009	0·887 ± 0·061	0·010
Hassall[6]	Aliphatic	10	0·969 ± 0·081	0·30	1·011 ± 0·027	0·034
Hassall (this paper)	Halides only	23	0·884 ± 0·035	0·15	1·015 ± 0·033	0·13
	Halides plus thiocyanates	28	1·001 ± 0·039	0·49	1·070 ± 0·031	0·22
Joachimoglu[19]	Aliphatic halides[b]	8	0·894 ± 0·055	0·071	1·024 ± 0·067	0·11
Moore[20]	Mostly aliphatic	5	1·016 ± 0·043	0·006	1·041 ± 0·019	0·001
Tattersfield & Roberts[21]	Aromatic[c]	10	0·51 ± 0·063	0·11	0·649 ± 0·027	0·021

[a] in all cases possible chemical toxicants amongst test substances have been omitted

[b] Substances applied in aqueous solution ; for these mol./l. have been plotted against p_s

[c] Absorption of substances into rubber of apparatus may account for low slopes and some p_t values higher than saturation

measured by $V^{\frac{2}{3}}$, gave results which were rather less satisfactory than those using molecular volume, but the results were unfortunately not clear-cut enough to enable a decision to be made between the surface and bulk phase theories of narcosis.

Although the introduction of a volume correction makes it possible for non-polar aliphatic substances to be plotted on a single line, and similarly for non-polar aromatic compounds, these two lines do not coincide. It thus appears that the presence of a benzene ring necessitates a higher concentration at the site of action than would be expected from the toxicities of aliphatic substances. Alternatively, the degree of departure from ideality of solution at the site of action may be different for these two types of compound. The equation

$$\log p_t/V = \log p_s + k'$$

can be compared with the equation of McGowan[4]

$$\log p_t + \log C_l = \log p_s + \log C_b + 0{\cdot}0014[P]$$

where C_b = molar concentration at the site of action, and $[P]$ = parachor. This equation has never been tested experimentally. Excluding the rather dubious parachor factor, which was obtained by subtracting two much larger experimentally derived quantities, and remembering that C_l has the units of $1/V$, the two equations are similar. In particular a slope of 1·0 would be expected when $\log p_t/V$ was plotted against $\log p_s$, and k' would be expected to incorporate the concentration at the site of action, together with other constants not dependent on the series. Of the data in Table IV only two sets are greatly at variance with McGowan's equation, namely, that of Tattersfield & Roberts[21] and the aliphatic compounds studied by Ferguson & Pirie.[3] Of these, the b value for the former is unreliable in that absorption may well have occurred into rubber and this would have been most marked for the substances present in least amount. The high value of b calculated by Ferguson & Pirie's results[3] is due to the presence of substitution series among the compounds tested, suggesting that in addition to molecular volume, some other property may play a minor rôle in physical toxicity. For all ordinary ranges of p_s, however, the equation

$$\log p_t = \log p_s + \log V + k'$$

appears to allow an accurate prediction of physical toxicity, k' being somewhat larger for aromatic substances and smaller for polar substances than it is for the non-polar aliphatic compounds.

Physical poisons offer one of the few examples where it is possible, with reasonable certainty and accuracy, to predict the toxicity of one homologue if those of several others have been estimated. In Fig. 2*a*, for example, the extrapolated lines joining the points for the higher *n*- and *sec.*-chlorides pass close to the observed values for the log molar LD_{50} of *n*- and *iso*-propyl chloride. When it is remembered how different the slopes of the probit lines were for propyl (16 to 24) and other chlorides (5 to 8) this is somewhat surprising. This does not happen when LD values far from the 50% region are employed (Fig. 2*b*). It suggests that when probit slopes of closely related compounds are very different, a comparison can still be made of LD_{50}, but of

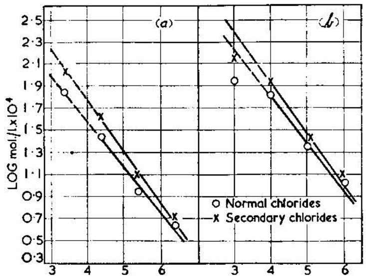

FIG. 2.—*Relationship of molar dosages for* (a) 50% *and* (b) 99% *kill, to carbon content*

no other levels of toxicity, i.e., that whatever the unknown factors are which affect the slopes of probit lines, the pivoting occurs somewhere near the centre, and that they do not greatly affect the position of this pivoting point.

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THE WATER-SOLUBLE CARBOHYDRATES OF GRASSES. III.*—First and Second Year Growth

By R. WAITE

The water-soluble carbohydrates, glucose and fructose (reported together as total free hexoses), sucrose and fructosan have been determined in eight strains of rye-grass of varying rates of growth and in two strains of timothy and one of cocksfoot in their first and second years of growth. In the rye-grasses, the extremely rapid growth and flowering of annual varieties resulted in much smaller accumulations of fructosan but more free hexoses than in biennial or perennial plants. Flower formation in the timothy grasses in the first year similarly delayed the storage of fructosan until after head emergence.

In second-year growth the fructosan content of all species increased rapidly early in the year but flower formation and early development caused a marked fall in the fructosan content of the timothy and cocksfoot strains but not of the rye-grasses. The reverse was later true during stem elongation after flower emergence, when the fructosan content in the rye-grasses fell sharply whilst those of the timothy grasses and cocksfoot rose to a second peak. The practical aspect of this difference in the soluble carbohydrate content of grasses at similar stages of growth is mentioned.

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Introduction

In a previous paper[1] in this series the changes in the water-soluble carbohydrates during the annual life cycle of four perennial grasses were reported. It was shown that although there was little difference between the contents of glucose, fructose and sucrose of the four grasses throughout the growing season, there was a marked difference in the way the fructosan content of the rye-grass (*Lolium perenne*) varied with stage of growth compared with that of the timothy (*Phleum pratense*), fescue (*Festuca pratensis*) and cocksfoot (*Dactylis glomerata*). The fructosan content of the rye-grass continued to rise steadily during that stage of growth when the flower initial was enlarging rapidly, whereas during the same stage of development in the other three species, the fructosan content, after an initial rise, fell abruptly for a period of 2–3 weeks.

This difference in behaviour of the fructosan content of various grasses is important because in temperate climates fructosan is at times a major component of the grass dry matter, rising to as much as 20–25%. The amount of fructosan in grasses has a considerable bearing on the success, or otherwise, of silage making[2] and probably plays a useful part in rumen metabolism. It was not clear whether the ability of the rye-grass to store carbohydrate uninterruptedly during the period of rapid flower growth was peculiar to the species, or whether the particular strain used, S23, was, by its slow general growth habit, partly responsible.

In these experiments all four grasses were well established and variability in the vigour of the root systems might have accounted for some of the differences observed in the sugar concentrations. It was decided, therefore, to grow a number of strains of rye-grass of varying rates of growth (' earliness ') including annuals and a biennial as well as perennials, and to follow the changes in the soluble carbohydrate fractions during both the first and second growing seasons. For comparison the strains of timothy, fescue and cocksfoot previously used, were sown again.

Experimental

A deficiency of both phosphorus and potash in the soil was corrected before applying 2 cwt./acre of a mixed fertilizer (N : P : K, 12 : 4 : 12) some weeks before sowing.

The following grasses were sown in April 1953 in small (60 sq. yd.) plots:

Rye-grass, annuals. Western Wolths and Wimmera
,, biennial. Irish Italian
,, perennials. Ayrshire, S24, New Zealand Mother, Danish Hunsballe and S23
Timothy, Scotch Commercial and S48
Cocksfoot, S143

There was excellent germination and the grasses grew well. One plot was sown with fescue S53, but the slow development of the seed allowed excessive weed growth and no results are recorded here for this grass.

The air temperature in 1953 from March to June was 2–3° F colder than in 1954 but from July until October these conditions were reversed. The average for the whole period from March to October was the same (51° F) in both years. In 1954 the rainfall in all months except June was exceptionally high, the monthly average from March to October being 102 mm. compared with 60 mm. for the same period in 1953.

Samples from all plots (except the fescue) were taken weekly during the growing season by the methods previously described[1] and, as before, were dried in a forced draught oven and ground within ¾–1 h. from the time of cutting. This technique was previously shown to prevent any appreciable enzymic breakdown of the fructosan or loss of the simpler sugars.

Chemical analysis of the dried grass

In the previous work[1] the hexoses and sucrose had been directly extracted from the dried grass with 80% ethanol, but when the fructosan content of the grass was high, small amounts of some fructose-containing material, other than free fructose or sucrose, appeared to be extracted by this strength of alcohol. By paper chromatography applied to the hydrolysates it was found that the extra fructose originated from fructose oligosaccharides. Wylam[3] has since dealt with this point in some detail and, while we agree that 90% ethanol is a more selective reagent, our earlier results did not suggest that the amount of oligosaccharides extracted by 80% ethanol was as great as in the example she quoted. To minimize the amount of oligosaccharide material extracted with the simpler sugars the powdered grass was shaken overnight (17 h.) at 20° in 90% ethanol. These are the only changes made in the methods previously detailed.

Physical measurements associated with growth

The length of the grass at each sampling was obtained from the measurement of 100 tillers of the cut material. The stage of development and length of the growing point and, after flower initiation, of the true stem were noted at each sampling.

The ratio of the weights of leaf and stem was determined on the dried material before grinding.

Results

Growth

The increase in height of all the grasses as the growing season advanced followed the usual sigmoid pattern. In the first year the two annual rye-grasses grew much more quickly than the other varieties of rye-grass, reaching a height of 28 in. in 12 weeks compared with heights of 14–20 in. in 20 weeks for the perennial varieties. Both these annuals flowered. The only other grasses to flower in the first year were the two strains of perennial timothy, which flowered 11 weeks after sowing and grew rapidly to a height of 25 in.

In the second year the annual strains were absent but all the remaining grasses flowered, the rye-grasses growing to heights of 22–26 in. and the timothy and cocksfoot to 35 in.

In those species which did not flower in their first year the primary growing point remained small (less than 1 mm.) and vegetative. In the annual rye-grasses and timothy grasses in the first year and in all grasses in the second year, flower initiation was followed by a rapid elongation of the primary growing point and of the true stem.

The relative amounts of leaf and stem were governed mainly by whether a grass flowered or not. Non-flowering grass still possessed 1½–2 times as much leaf as stem until September, whereas in a flowering grass the carbohydrate analysis of the whole plant, after a height of 12–14 in. had been reached, was progressively similar to that of the stem alone.

Soluble carbohydrate

From a preliminary consideration of the results for both years it was clear that the five strains of perennial rye-grasses fell into two groups. The three varieties, Ayrshire, S24 and New Zealand Mother had very similar soluble carbohydrate contents, whilst those of the Danish Hunsballe more closely resembled those of the slower-growing S23 strain. In order to clarify the graphical presentation of the results, only those from the S24 and S23 strains are given to represent the early and late varieties of perennial rye-grass.

The changes in the soluble carbohydrate fractions of the whole aerial parts of the grasses are shown in Figs. 1–3.

The free glucose and fructose, although determined separately, were similar in quantity and are presented as the total free hexoses. In the biennial and perennial strains these did not amount to more than 2–4% of the dry matter, but in the two annual rye-grasses there was a period of several weeks when they were more than 4%. This was mainly the result of a high concentration of hexoses in the stem, rising to as high as 8% during the period of rapid growth from 8 to 25 in.

The sucrose contents followed a seasonal pattern very similar to that previously reported,[1] increasing to a sharp maximum early in the season when the height of the grass was about 4–6 in. In the first year of growth the highest sucrose concentrations occurred about 40 days before the highest fructosan contents, but in the second year this interval was reduced to 14 to 20 days before the peak fructosan value in May.

Where plants are making new growth directly from seed, the amount of the reserve carbohydrate fructosan formed in the first year grasses was smaller than in growth of the same height in the following year. This was particularly so in the grasses which flowered in their first year,

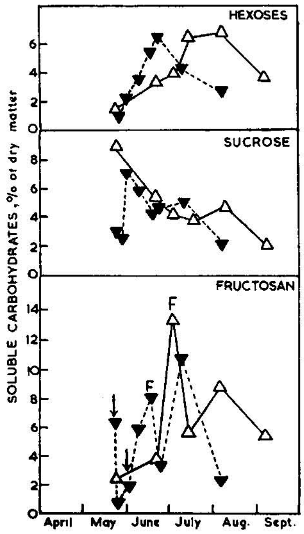

Fig. 1.—*Soluble carbohydrates in annual rye-grass*

△ Wimmera (1954)
▼ Western Wolths (1953)
↓ indicates change of the growing point from vegetative to floral
F indicates flower emergence

the two annual rye-grasses and the two perennial timothy grasses. In these plants the requirements for the rapid growth and flower formation already mentioned minimized or delayed the accumulation of fructosan (Figs. 1 and 3). Judged by fructosan content the annuals were better able to meet these demands up to the time of flower emergence than the perennial timothy grasses. In the second year the amount of fructosan in the timothy and cocksfoot grasses rose sharply from the beginning of April to about mid-May, with the fructosan content of the cocksfoot at a considerably lower level than that of the timothy grasses. At this time (indicated in Fig. 3 by an arrow) the primary growing points changed from a vegetative to a floral development and in the following 15 days rapidly increased in size from about 8 to 56 mm. Fig. 3 shows that during the same period the fructosan content fell abruptly in both these species. In the early and late varieties of rye-grass the fructosan content also increased in early and mid-May and to a much higher value in all strains than in the timothy grasses or cocksfoot. In these rye-grasses the flower initiation and development phase (the time indicated by an arrow in Fig. 2) was passed through without any check to the rapid increase in fructosan content, although the rate of elongation of the flower initial in the rye-grasses was quite comparable to that in the other species, increasing on average from 10 to 65 mm. in 17 days. After flower emergence the behaviour of the fructosan content in the two groups of grasses again differed considerably, rising to a second peak in the timothy grasses and cocksfoot but falling steadily in all the rye-grass varieties as the stem bearing the flower continued to lengthen.

Discussion

In the cotyledon and first leaves growing from the seed, carbohydrates are formed by photosynthesis and are used both as a source of energy and as material from which most of the other plant constituents are formed. It could therefore be some time before the young plant would have available sufficient excess of carbohydrate to create the temporary reserve which in the vegetative parts of grasses and cereals is in the form of fructosan. The present results indicate that even when the leaves were only 1–2 in. in length fructosan was already formed and continued to accumulate at a considerable rate in some grasses. The exception to this was when a grass flowered early in its first year of growth, when the need for simple metabolites rather than a convertible storage compound was shown by the presence of greater amounts of free

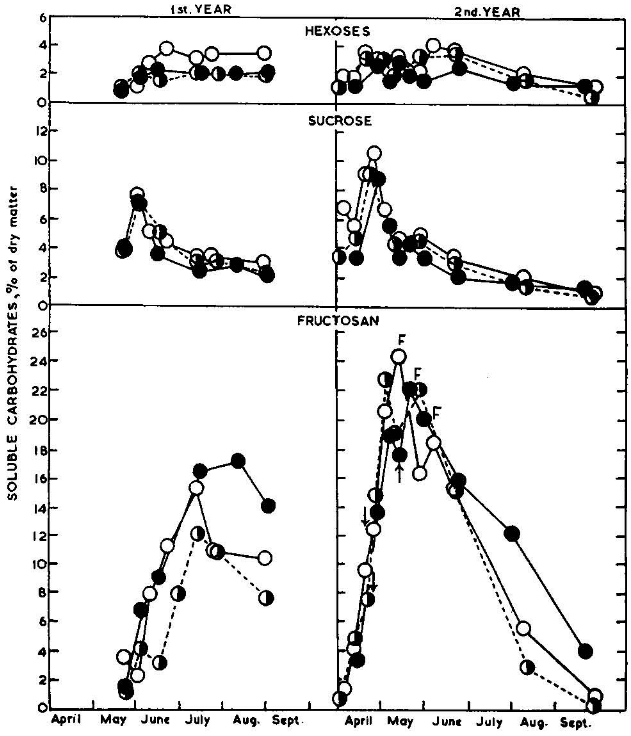

FIG. 2.—*Soluble carbohydrates in biennial and perennial rye-grass*

○ Italian ◑ S24 ● S23
↑ or ↓ indicates change of the growing point from vegetative to floral
F indicates flower emergence

hexoses than usual and smaller amounts of fructosan. This is probably typical of annual species whereas the high free hexose content was lacking in the perennial timothy grasses which also flowered in their first year. In general, the levels of fructosan and sucrose, from which it probably originates, were lower in all varieties in the first year of growth than in the second year. This was particularly so in cocksfoot where the fructosan content never rose above 3% (of the dry matter) in 1953 compared with 8% in 1954.

In both years the different rates of growth of the various strains of rye-grass appeared to have little effect on the maximum amount of fructosan formed. The ability of all the rye-grass strains investigated, other than the annuals, to accumulate large amounts of fructosan during the young leafy stage of growth (6–8 in.) at a time when the primary growing point, newly changed to the floral form, was rapidly enlarging, is a distinctive feature of this species. In the two timothy strains and in the cocksfoot this morphological development and growth was, as previously observed, attended by a marked fall in fructosan content. In contrast, the rapid growth of stem after flower emergence caused a sharp fall in the fructosan percentage in the rye-grasses whereas during the same period it rose in the other grasses, particularly in the timothy strains. This was not because the rye-grasses grew to a greater height than the timothy strains and cocksfoot ; the reverse was in fact true and the approximate yield of dry matter made at each sampling showed higher values for these latter species.

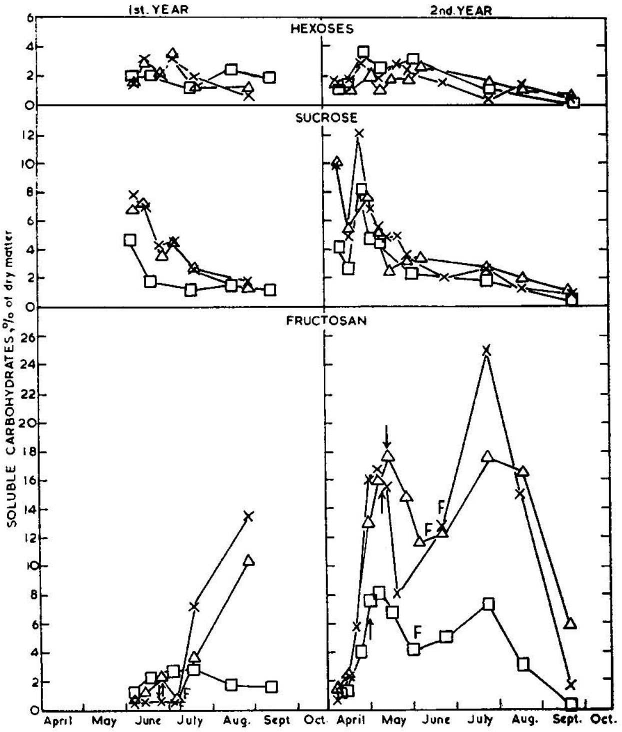

FIG. 3.—*Soluble carbohydrates in perennial timothy and cocksfoot*

□ Cocksfoot, S143 △ Timothy, S48 × Timothy, Scots
↑ or ↓ indicates change of growing point from vegetative to floral
F indicates flower emergence

The reason for this more rapid fall in fructosan content in the rye-grasses after heading is not known with certainty, but it is probably connected with the earlier withering of leaf material than in the other strains. Since the plant is still actively growing, the need for sugars for energy and tissue formation is apparently greater than the amount being currently synthesized and the reserve is mobilized to supply the deficit. Archbold[4] has reported much the same effect in barley, the natural decrease in stem fructosan content being accelerated when leaves were artificially removed. Conversely, the relatively high fructosan content of the first-year perennials at the end of the growing season was probably because the leaves were only beginning to wither at the end of August.

The fructosan contents of some American forage grasses, including cocksfoot and timothy, were reported recently by Phillips *et al.*[5] In cocksfoot the amounts varied between 0 and 2·3% (of the dry matter) and in timothy the maximum was 12%, values appreciably lower than those for the same species in Scotland. There are two probable reasons for this marked difference. Phillips *et al.*[5] used a mower with the cutter-bar set at a height of 2·5 inches and, since much of the fructosan in grasses is stored in the first internode of the stem, a high proportion would be left in the stubble. Secondly, the rate of growth of the American grasses was appreciably faster than ours, the annual growing cycle being completed in 44 days by the cocksfoot and 51 days by the timothy in comparison with 76 and 86 days, respectively, in Scotland. The present results suggest that this more rapid growth would tend to lower the amount of reserve carbohydrate and it seems likely that in warm climates fructosan may account for much less of the total soluble carbohydrates than in temperate or cold regions.

The sharp fluctuations in the amount of the chief soluble carbohydrate as the grass grows older differ markedly from the more predictable fall of such constituents as protein, ether extractives and ash or the rise of cellulose and lignin. The reason is, no doubt, that in addition to their utilization by the plant as a source of energy, the soluble carbohydrates are essentially intermediate compounds in the formation of a number of other products.

The considerable difference in the amount of fructosan in different species of grasses at the same stage of growth has an interesting practical implication. If a high fructosan content is desired, perennial rye-grasses are most likely to provide it when at a height suitable for grazing or ensiling, but for hay-making, timothy strains would be expected to be richest in this material. On the basis of results extending over several years, cocksfoot at any height is likely to contain only small quantities of fructosan.

Acknowledgments

The author wishes to thank Dr. J. Grainger of the West of Scotland Agricultural College for weather data and Misses McInnes, King & Wilson for assistance in the field and in the chemical analyses.

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THE FATTY ACID COMPOSITION OF LIPIDS IN THE MUSCLE AND FATTY TISSUES OF LOIN MUTTON CHOPS

By L. HARTMAN and F. B. SHORLAND

Dissection of loin mutton chops showed 13·4–17·0% bone, 42·2–48·5% muscle and 38·1–40·8% fatty tissue. In a sample showing fatty degeneration, the muscle tissue was not apparent and the dissection revealed 19·6% bone and 80·4% fatty tissue. In the bone-free tissues of normal chops 84·2–87·3% of the total fat was concentrated in the fatty tissues. The fatty acid composition of the lipids of the muscle and fatty tissues of the chops was similar to that observed by other investigators for sheep depot and muscle fats. The loin chop muscle lipids contained a somewhat higher proportion, approx. 3%, of C_{20} unsaturated acids as compared with 1% or less usually observed in sheep fats by other investigators. The fat in the fatty tissues contained more stearic acid and *trans*-acids, but less di- and polyethenoid acids and particularly tetraenoic (arachidonic) acid than the muscle fat.

Introduction

In most studies on the fatty acid composition of sheep fat, the perinephric, external tissue, or some other readily accessible fat, has been used for the determination of the composition of the depot fat. In the present investigation the selection of fat was made from the nutritional point of view, the loin mutton chop being one of the more important edible parts of the animal. Interest was initiated from an investigation of a sample of loin chops showing fatty degeneration obtained by the courtesy of Mr. W. Williams, Chief Chemist to Messrs. R. & W. Hellaby Ltd., Auckland, New Zealand.

Experimental

The loin mutton chops showing fatty degeneration were selected from a line of sheep 18–24 months old (E33). The chops showed no obvious muscle tissue, but appeared to consist of bone and fatty tissue only. To compare the fatty acid composition of the fat from the above chops with that of normal loin chops, samples from 18–24-months-old sheep were purchased locally (K51 and K53). The bone-free tissues were dissected into muscle and fatty tissue, except in the chops showing fatty degeneration, where such separation was impracticable. The fat from the muscle and fatty tissues was extracted as previously described.[1] The results of the dissection were as follows:

		Average wt. of chop, g.	Bone %	Muscle %	Fat %
K51	12 chops	137·3	13·4	48·5	38·1
K53	2 chops	191·9	17·0	42·2	40·8
E33	4 chops	282·4	19·6	80·4	

The composition of the tissues is shown in Table I.

Table I

Composition of muscle and fatty tissues of loin mutton chops

(Figures in brackets indicate % of phospholipid in fat calculated by multiplying the % of P by 25)

Sample		Fat-free dry matter, %	Fat %	Water %	M.p. of fatty acids, ° C
E33	Adipose and muscle tissues combined (fatty degeneration)	7·8	64·7 (1·6)	27·5	47–48
K51	Adipose tissue	4·4	86·1 (0·8)	9·5	47–48
	Muscle (normal sample)	25·2	9·8 (8·8)	65·0	43–44
K53	Adipose tissue	3·5	85·9 (2·0)	10·6	46–47
	Muscle (normal sample)	19·0	15·6 (5·2)	65·4	43–44

The ester fractionation analyses were made as earlier outlined,[2] while the composition of the di- and poly-unsaturated acids was determined on the fat samples by spectrophotometric procedures.[3] The results are collected in Tables II and III, together with data from the depot fats of sheep obtained by other workers using modern ester fractionation techniques.

Discussion

The dissection data show that the muscle tissues contain 10–15% of fat, and that the proportions of adipose and muscle tissue in the normal chop are about equal. This means that trimming the fat can remove up to nearly 90% of the total fat in the chop, and that the practice of grilling a chop effects a substantial reduction in fat intake, which could be significant in the formulation of a high protein-low fat content diets.

Table II shows that the fatty acid composition of the lipids from the adipose and muscle tissues of the chop is generally similar to that found by other workers for depot and muscle fats. Comparative data in Table II illustrate that sheep fats contain somewhat less than the 27–30% palmitic acid normally found in the depot fats of the larger land animals. The fat from the chop showing fatty degeneration, apart from its somewhat higher content of stearic acid, is similar to

Table II

Fatty acid composition of sheep fats. Fatty acids % (w/w)

(*a*) Adipose tissues of mutton chops

Sample	Saturated				Unsaturated				Total *trans*-acids
	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}	
E33	—	24·3	29·0	0·2	—	2·7 (2·0H)	42·9 (2·2H)*	0·9 (as 6·0H)	16·4
K51	2·9	21·8	26·5	0·4	0·3 (2·0H)	3·2 (2·0H)	43·7 (2·2H)	1·2 (as 6·0H)	15·6
K53	2·9	22·5	22·6	1·6	0·4 (2·0H)	3·8 (2·0H)	45·4 (2·2H)	0·8 (as 6·0H)	15·5

(*b*) Muscle tissues of mutton chops

Sample	Saturated				Unsaturated				Total *trans*-acids
	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}	
K51	1·4	24·3	21·6	0·3	0·2 (2·0H)	0·6 (2·0H)	48·4 (2·4H)	3·2 (as 6·0H)	10·7
K53	0·6	26·1	19·1	0·8	tr.	0·8 (2·0H)	49·3 (2·3H)	3·3 (as 6·0H)	11·1

(*c*) Depot fats of sheep

Sample	C_{14}**	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}
	Controlled diets[4]							
Perinephric								
Super-maintenance	2·3– 2·9	24·0– 26·2	24·9– 28·3	—	0·3– 0·7	0·9– 2·4	42·0– 44·4 (3·3– 5·7)***	0·6– 1·1
Sub-maintenance	1·8– 3·0	23·0– 26·8	30·1– 37·8	—	0·2– 0·2	0·9– 1·3	34·3– 38·3 (2·2 4·3)	0·8– 1·1
External tissue								
Super-maintenance	3·6– 4·0	27·8– 28·3	13·5– 16·2	—	0·2– 0·4	0·6– 1·6	50·5– 52·7 (1·9–4·8)	0·6– 0·7
Sub-maintenance	2·6– 4·0	24·3– 33·9	15·3 24·6		0·3– 0·3	0·7– 1·2	44·2– 45·1 (1·5–4·9)	0·5– 0·9
Total fatty tissues New Zealand[5]	3·8	25·0	22·2	0·7	0·5	1·7	44·2 (2·2)	0·9
Muscular tissues New Zealand[5]	4·3	24·6	15·4	0·7	0·5	2·6	50·1 (4·0)	1·8
Indian[6]	2·9	27·8	27·7	1·5	0·4	2·7	36·4 (3·4)	0·6
Somali[7]	2·2	23·0	14·9	—	0·3	2·5	56·5 (0·8)	0·6

* Mean unsaturation expressed in terms of hydrogen

** Includes traces of C_{12}

*** Figures in brackets indicate % linoleic acid based on total fatty acids

the fat from the fatty tissues of the normal chop. Comparison of the fat of fatty tissues and muscle of normal chops shows that the muscle fat contains over 3% of C_{20} unsaturated acids or about 3 times as much as is present in the fat of the fatty tissues (see Table II). The fatty tissue fat, as compared with muscle fat, contains more (3·5–6%) stearic acid but less (2·5–3·6%) palmitic acid, suggesting that it would be less digestible than the muscle fat, as is also indicated by the difference in the melting points of fatty acids shown in Table I.

The spectrophotometric analysis shows that only a portion of the C_{20} unsaturated acids can be present as tetraenoic (arachidonic) acid (compare Tables II*a* and II*b* with Table III) which is concentrated to a very much greater extent in the muscle lipids than in the fat from the fatty tissues. The proportions of non-conjugated di- and trienoic acids are also greater in the muscle

Table III

Composition of the di- and polyethenoid acids as a % (w/w) of the total fatty acids

(*a*) Adipose tissues

Sample	Conjugated			Non-conjugated			Total
	Diene	Triene	Tetraene	Diene	Triene	Tetraene	Di- + polyethenoid
E33	1·97	0·05	—	1·66	0·75	0·21	4·64
K51	2·89	0·06	—	0·13	0·88	0·12	4·08
K53	2·30	0·05	—	0·98	0·48	0·07	3·88

(*b*) Muscle tissues

Sample	Conjugated			Non-conjugated			Total
	Diene	Triene	Tetraene	Diene	Triene	Tetraene	Di- + polyethenoid
K51	2·18	0·08	0·10	1·99	1·01	0·63	5·99
K53	2·10	0·06	—	1·45	0·53	0·58	4·72

fat than in the adipose fat. This shows that muscle tissues are an important source of essential fatty acids, their content of arachidonic acid being almost equal to that of the fatty tissues.

In the previous analyses of sheep fats it has been customary (cf. Hilditch[8]) to attribute the excess of unsaturation above that required for a monoenoic acid to linoleic acid. This practice is subject to the criticism that triene as well as diene constituents are present as indicated by Table III. It seems therefore preferable either to record the mean unsaturation of the C_{18} unsaturated acids in terms of hydrogen or to measure the actual content of diene and triene constituents by the modern spectrophotometric techniques.

As could be expected the fats of the muscle tissues are richer in phospholipids than those of the fatty tissue (see Table I). Usually phospholipids as compared with glyceride fractions contain more stearic acid.[8] In mutton chops, however, the increased phospholipid content of the muscle fat as compared with fat from the fatty tissue is associated with a diminution of stearic acid content.

It has previously been shown by Hartman *et al.*[9] that the fats of pasture-fed ruminants contain *trans*-acids which are presumably formed by the action of hydrogenases in the rumen on the dietary unsaturated fatty acids. Such acids are apparently absent from the fats of non-ruminants unless included in the diet. The *trans*-acid contents of the mutton chop fats determined by the infra-red technique described in a previous communication[9] show a *trans*-acid content of 10·7–16·4% (see Table II) as compared with 11·2% in sheep perinephric fats.[9] The distribution of the *trans*-acids follows the same pattern as that of stearic acid, but is the reverse of that found for total C_{18} unsaturated acids.

According to Callow's views[10] the proportion of dietary fat as compared with endogenous fat will diminish with the rate of growth. Thus it would be anticipated that the *trans*-acid contents of the muscle fat would be higher than that of the adipose tissue, but the opposite has been found. *trans*-Octadec-9-enoic (elaidic) acid when fed to rats has been shown by Sinclair[11] to be preferentially distributed in the phospholipids as compared with the glycerides. As with stearic acid, the distribution of the *trans*-acids between the adipose and the phospholipid-containing muscle fat of the chops was contrary to expectation. The pattern of the distribution of fatty acids between different tissues, therefore, is not predictable in the light of present knowledge.

Acknowledgments

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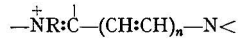
STUDIES IN FUNGITOXICITY I.—Fungitoxicity of Certain Carbocyanines

By M. PIANKA and J. C. HALL

Thirty-five carbocyanines were examined for fungitoxicity and some were found to be active. This newly found biological property was limited to carbocyanines of a fairly low molecular weight, and was dependent on the heterocyclic base or bases from which the carbocyanine was derived. Alkyl substituents enhanced the fungitoxicity of the dyes irrespective of their position. Tests with some dyes showed that these penetrated to the protoplasts of the spores, staining them irreversibly.

Introduction

Paul Ehrlich's early work lay in the field of dyes, which he tested for their potential chemotherapeutic properties. Sulphonamide drugs originated from dyestuffs chemistry, Prontosil (2 : 4-diaminoazobenzene-4'-sulphonamide) having been found active against streptococci in 1935.[1] In a series of papers published between 1924 and 1934, Browning and his collaborators[2, 3, 4] described the antiseptic properties of the dyes anil- and styryl-quinolines and cyanines, all of which contain the conjugated system



$$-\overset{+}{N}R{:}\overset{|}{C}-(CH{:}CH)_n-N<$$

This also occurs in the well-known antiseptic, acriflavine (in acriflavine $n = 1$, in carbocyanines $n = 2$). Some cyanines, e.g. 1 : 1'-dimethyl-6 : 6'-dimethoxycarbocyanine iodide[2] and some thiacarbocyanines[5] were found to inhibit greatly the growth of staphylococci.

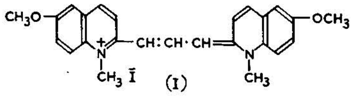
Ogata[6] found that neocyanines—trinuclear cyanines with two acidic radicals—had antileprotic properties. Other workers found that certain cyanine dyes possessed antifilarial activity.[7]

The above reported findings prompted us to submit certain carbocyanines to testing for fungitoxicity, with the hope of establishing some correlation between chemical structure and effect on fungal spores.

Experimental

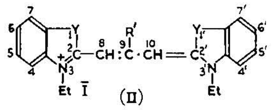
Nomenclature of carbocyanine dyes

Carbocyanines are cyanine dyes linked by a chain consisting of three methine groups (methine chain). Thus the previously mentioned 1 : 1'-dimethyl-6 : 6'-dimethoxycarbocyanine iodide has the formula **I**.



(I)

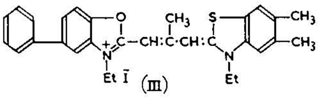
With other nuclei a suitable prefix is used, thus thia- denotes a benzthiazole, oxa- a benzoxazole, selena- a selenazole and thiazolo- a thiazole nucleus. The numbering used in this paper is based on the general scheme shown in **II**.



(II)

The symmetrical carbocyanine, in which Y = O, S or Se and R' = Et is termed 3 : 9 : 3'-triethyloxa- or thia- or selena-carbocyanine iodide.

The unsymmetrical carbocyanine of the formula **III**



(III)

is termed 3 : 3'-diethyl-9 : 5' : 6'-trimethyl-5-phenyloxathiacarbocyanine iodide.

Preparation of the dyes

The preparation of dye No. 2 (see Results) was by the method of Kendall & Fry ;[8] the preparation of the dyes Nos. 3–6, 11–16, 22–24, 28–29, 31–35 was described by Barany & Pianka.[9] Dye No. 7 was prepared by the method of Mills,[10] dye No. 25 by that of Clark ;[11] dyes Nos. 17–19 were synthesized by the methods of Brooker & White,[12] whose method[13] was also used for dye No. 16.

All the dyes were recrystallized twice from methanol.

3 : 3'-Dimethyloxacarbocyanine iodide (Dye No. 1)

2-Methylbenzoxazole (1·3 g.) and methyl toluene-*p*-sulphonate (1·9 g.) were kept at 140–150° for 4 h. Pyridine (6 c.c.) and ethyl orthoformate (6 c.c.) were added to the cooled reaction mixture, which was then kept for 6 h. at 120–140°. The hot solution was added, with vigorous stirring, to a solution of potassium iodide (6·5 g.) in water (32·5 c.c.) and alcohol (32·5 c.c.). The dye separated instantly. After 4 h. it was filtered and recrystallized. Purple-violet needles were obtained, melting at 274–275° (Found : N, 6·39. $C_{19}H_{17}O_2N_2I$ requires N, 6·48%).

3 : 3'-Diethyl-8-methylthiacarbocyanine iodide (Dye No. 8)

Preparation of the intermediate 2-2'-acetanilidovinyl-3-ethylbenzthiazolium ethyl sulphate.—2-Methylbenzthiazole (7·4 g.) and diethyl sulphate (8 g.) were heated at 140–150° for 30 min. The quaternary salt thus obtained was heated with *NN'*-diphenylformamidine (10 g.), acetic anhydride (5 c.c.) and glacial acetic acid (5 c.c.) for 3 h. in an oil-bath kept at 100°. The cooled reaction mixture was repeatedly triturated with methanol and ether (1 : 2). The yellow solid that separated was filtered, washed with ether and recrystallized from methanol. Yellow crystals, m.p. 200°, were obtained (Found : N, 6·20. $C_{21}H_{24}O_5N_2S_2$ requires N, 6·25%).

Preparation of the dye.—2-Ethylbenzthiazole (3·2 g.) and diethyl sulphate (2·3 c.c.) were heated at 150° for 20 min. To the cooled quaternary salt were added the above 2-2'-acetanilidovinyl-3-ethylbenzthiazolium ethyl sulphate (2·8 g.) and pyridine (10 c.c.), and the mixture was heated at 120–140° for 1 h. The dye was then converted to its iodide, as with the previous dye. On recrystallization, dark green crystals, m.p. 254°, were obtained (Found : N, 5·71. $C_{22}H_{23}N_2S_2I$ requires N, 5·53%).

3 : 3'-Diethyl-8 : 10-dimethylthiacarbocyanine iodide (Dye No. 9)

2-Ethylbenzthiazole (3·26 g.) and diethyl sulphate (2·3 c.c.) were heated at 150° for 20 min. Pyridine (7 c.c.) was added and then ethyl orthoformate (2·7 c.c.), and the mixture was heated at 140–150° for 1 h. On pouring into excess of 10% aqueous potassium iodide, an oil separated.

This was well washed with water, dissolved in hot methanol and poured again into excess of 10% aqueous potassium iodide. After 24 h. at 0° a very small amount of solid separated, which was filtered and recrystallized. A black microcrystalline powder, m.p. 241–242°, was obtained (Found: N, 5·30. $C_{23}H_{25}N_2S_2I$ requires N, 5·38%).

3 : 3′-*Diethyl*-8 : 9 : 10-*trimethylthiacarbocyanine iodide* (*Dye No.* 10)

The conditions were as described above, except that ethyl orthoacetate (3·4 c.c.) was used instead of ethyl orthoformate. The condensation to the dye took 3 h. A minute amount of solid separated after 48 h. at 0°; it was filtered and recrystallized from methanol and a little ether. Dark purple crystals, m.p. 233–234°, were obtained (Found: N, 5·21. $C_{24}H_{27}N_2S_2I$ requires N, 5·24%).

When ethyl orthopropionate (3·2 c.c.) was used instead of ethyl orthoacetate, no 3 : 9 : 3′-triethyl-8 : 10-dimethylthiacarbocyanine iodide could be recovered.

3 : 3′-*Diethyl*-4 : 5 : 4′ : 5′-*dibenzthiacarbocyanine iodide* (*Dye No.* 20)

This dye was prepared by the method of Brooker & White,[12] but the dye was converted to the iodide instead of the bromide. On recrystallization, a dark blue powder, m.p. 238°, was obtained.

3 : 3′-*Diethyl*-9-*methyl*-4 : 5 : 4′ : 5′-*dibenzthiacarbocyanine bromide* (*Dye No.* 21)

2-Methyl-β-naphthathiazole (16·5 g.) and ethyl toluene-*p*-sulphonate (16·5 g.) were heated at 180° for 5 h. A solid was obtained. The 3-ethyl-2-methyl-β-naphthathiazolium toluene-*p*-sulphonate (6 g.), pyridine (25 c.c.) and ethyl orthoacetate (5·5 c.c.) were heated at 120–140° for 3 h. The dye was converted to the bromide.[12] On recrystallization a dark purple powder was obtained, m.p. 236–238°.

3 : 9 : 3′-*Triethylselenacarbocyanine iodide* (*Dye No.* 27)

2-Methylbenzselenazole (4·5 g.) and diethyl sulphate (3·1 c.c.) were heated at 160° for 15 min. Pyridine (16 c.c.) and ethyl orthopropionate (2·8 c.c.) were added and the mixture was heated at 120–140° for 2 h. The dye was converted to the iodide. On recrystallization an olive-green powder was obtained, m.p. 234–237° (softens at 140°) (Brooker & White[12] gave m.p. 146–148°; U.S.P. 2,378,783 reported m.p. 210–211°).

3 : 9 : 3′-*Triethyl*-5′ : 6′-*dimethyl*-5-*phenyloxathiacarbocyanine iodide* (*Dye No.* 30)

Preparation of the intermediate 2-(2-*anilinobut*-1-*enyl*)-3-*ethyl*-5-*phenylbenzoxazolium ethyl sulphate.*—2-Methyl-5-phenylbenzoxazole (6 g.) and diethyl sulphate (4·5 g.) were heated at 160° for 20 min. Ethyl *N*-phenylthiolpropionimidate (6·1 c.c.) was then added and the mixture was heated in an oil-bath at 170° for 30 min. The mixture was repeatedly triturated with methanol and ether (1 : 1). The solid that separated was filtered off, washed with ether until the washings were colourless, and recrystallized twice from methanol. Yellow crystals, m.p. 197°, were obtained. On keeping, the solid became gummy and turned to a mass of glass-like appearance.

Preparation of the intermediate 3-*ethyl*-2 : 6-*dimethylbenzthiazolium iodide*

2 : 6-Dimethylbenzthiazole (11·1 g.) and ethyl iodide (6·3 c.c.) were heated at 100° in a closed tube for 48 h. The contents of the tube were ground with excess of acetone, filtered, washed with acetone and then with ether and recrystallized from ethanol. Crystals, m.p. 157–158°, were obtained.

Preparation of the dye.—The above-mentioned freshly prepared anilinobutenyl compound (1·85 g.), 3-ethyl-2 : 6-dimethylbenzthiazolium iodide (1·7 g.) and pyridine (15 c.c.) were heated at 130° for 2 h. The dye was converted to the iodide and recrystallized. Plum-red crystals, m.p. 360°, were obtained (Found: N, 4·87. $C_{31}H_{33}ON_2SI$ requires N, 4·61%).

Fungitoxicity tests

All the tests were carried out by the well-established Montgomery-Moore[14] slide germination technique: 0·015 c.c. of a solution of a dye in methanol at the required concentration was spread uniformly over circular areas, 15 mm. across, delimited on 3 × 1 in. microscope slides. The methanol was allowed to evaporate. To the dry slides 0·04 c.c. of a suspension of spores, diluted to contain 15–20 spores per low-power field of the microscope ($\frac{2}{3}$ in. objective, × 10 eye-piece), was applied to the treated circular areas. The slides were then placed in moist Petri dishes and kept in an incubator at 21° for 20 h. Counts for relative percentage germination of the spores were then carried out. The concentrations of the dyes required to kill 95% of the spores (LD_{95}) were obtained by plotting probits of percentage mortality against the appropriate log concentration values. Goodness of fit was checked by means of the χ^2 test. Tests were repeated a sufficient number of times until reproducible results were obtained. The average LD_{95} values are reported for the following fungi: *Venturia inaequalis* (Cooke) Wint.; *Botrytis cinerea* Pers.; and *Fusarium bulbigenum*, Cooke and Massee, var. *lycopersici* (Brushi) Wollenw. (LD_{95} values were chosen as they give greater critical difference in activity levels than LD_{50} when no slope figures are appended.)

Results

Table I shows the results of fungitoxicity tests on 35 cyanine dyes prepared by methods described above or in the literature.

Imbibition of the dyes by spores from aqueous solutions

Two simple dyes, Nos. 1 and 7, were dissolved in distilled water and to the coloured solution a suspension of spores of *Botrytis* was added. After setting aside for 20 h., the spores were washed repeatedly by centrifuging with distilled water. Under the microscope, the protoplasts of the spores were found to be deeply stained, whereas spores devoid of cell contents were either colourless or only very faintly coloured. The protoplasts of the germ tubes of those spores that had germinated in sub-lethal concentrations of the dyes were also found to be stained.

Conclusions

The following points on the relationship between fungitoxicity and chemical structure of carbocyanines emerge from a study of the results reported above:

(*a*) Only changes in the cationic part of the carbocyanine have an effect on the activity; as far as one can judge from the limited results available, the anion does not appear to influence the activity.

(*b*) Alkyl substituents appear to enhance the activity of the carbocyanines irrespective of their position in the methine chain or the nucleus. Thus 3 : 3′-diethylthiacarbocyanine iodide (No. 7) is inactive, whereas the 8-methyl-, 8 : 10-dimethyl-, 8 : 9 : 10-trimethyl-, 6 : 6′-dimethyl-, 6 : 9 : 6′-trimethyl- (Nos. 8–12), 5 : 6 : 5′ : 6′-tetramethyl- and 5 : 6 : 9 : 5′ : 6′-pentamethyl-derivatives (Nos. 14 and 15) are all active. Among the oxacarbocyanines the 5 : 6 : 5′ : 6′-tetramethyl- and 5 : 6 : 9 : 5′ : 6′-pentamethyl-derivatives (Nos. 5 and 6) are highly active. Also in the unsymmetrical oxathia- (Dyes Nos. 28 and 29), oxaselena- (Dyes Nos. 31 and 32) and selenathia-carbocyanines (Dyes Nos. 33–35), alkyl substitution enhances the activity of the parent compounds. Sen & Joshi[15] also observed that alkyl substitution generally increased toxicity.

(*c*) Oxacarbocyanines are more active than the corresponding thia- and selena-carbocyanines. Katz[16] also found that derivatives of 2-hydrazinobenzoxazole possessed marked, whereas the benzthiazole analogues had little, antifungal activity.

(*d*) Dibenzthiacarbocyanines and tetraphenylthiazolocarbocyanine (Dye No. 24) are inactive. In general, increase in molecular weight and in the bulk of the molecule causes a decrease in biological activity. Thus even alkylated 3 : 3′-diethyl-4 : 5-4′ : 5′-dibenz- and -6 : 7-6′ : 7′-dibenzthiacarbocyanines (Dyes Nos. 18, 19 and 21) are inactive, though alkylation rendered the inactive 3 : 3′-diethylthiacarbocyanine iodide very active. Also 3 : 3′-diethyl-5 : 5′-diphenyloxacarbocyanine iodide is much less active than its parent compound. Edgerton & Burckhalter,[17] who studied the amoebicidal activity of derivatives of 8-hydroxyquinoline, itself fungicidal, also observed a decrease in the activity with increasing molecular weight; and Sen & Joshi[15] observed that higher ether-esters of cresotinic acid were much less fungicidal than the lower ones.

(*e*) Selenacarbocyanines are fairly fungitoxic.

Table I

Results of fungitoxicity tests

No. of dye	Name of dye	M.p.	*Venturia*	*Botrytis*	*Fusarium*
			LD_{95} values, p.p.m.		
	Oxacarbocyanine iodide				
1	3 : 3'-Dimethyl-	274-275°	50	100	30
2	3 : 3'-Dimethyl-9-(*p*-toluidino)-	251-253°	50	55	35
3	3 : 3'-Diethyl-5 : 5'-diphenyl-	240°	260	325	90
4	3 : 3'-Diethyl-9-methyl-5 : 5'-diphenyl-	273-274°	150	160	100
5	3 : 3'-Diethyl-5 : 6 : 5' : 6'-tetramethyl-	280-281°	25	30	25
6	3 : 3'-Diethyl-5 : 6 : 9 : 5' : 6'-pentamethyl-	253-256°	20	25	20
	Thiacarbocyanine iodide				
7	3 : 3'-Diethyl-	269°(d.)	>1000	>1000	>1000
8	3 : 3'-Diethyl-8-methyl-	254°	60	75	65
9	3 : 3'-Diethyl-8 : 10-dimethyl-	241-242°	75	70	75
10	3 : 3'-Diethyl-8 : 9 : 10-trimethyl-	233-234°	80	80	70
11	3 : 3'-Diethyl-6 : 6'-dimethyl-	275-276°	35	85	45
12	3 : 3'-Diethyl-6 : 9 : 6'-trimethyl-	274°	185	110	90
13	3 : 9 : 3'-Triethyl-6 : 6'-dimethyl-	245-247°	25	30	40
14	3 : 3'-Diethyl-5 : 6 : 5' : 6'-tetramethyl-	265-267°	70	65	45
15	3 : 3'-Diethyl-5 : 6 : 9 : 5' : 6'-pentamethyl-	275-276°	65	75	65
16	3 : 3' : 9-Triethyl-5 : 6: 5 ' : 6'-tetramethyl-	254-255°	190	110	775
	Thiacarbocyanine bromide				
17	3 : 3'-Diethyl-6 : 7-6' : 7'-dibenz-	[278-280°(d.)	>1000	>1000	>1000
18	3 : 3'-Diethyl-9-methyl-6 : 7-6' : 7'-dibenz-	267-268°*	>1000	>1000	>1000
19	3 : 3' : 9-Triethyl-6 : 7-6' : 7'-dibenz-	290-292° †	>1000	>1000	>1000
20	3 : 3'-Diethyl-4 : 5-4' : 5'-dibenz-(iodide)	238°	>1000	>1000	>1000
21	3 : 3'-Diethyl-9-methyl-4 : 5-4' : 5'-dibenz-	236-238°	>1000	>1000	>1000
	Thiacarbocyanide iodide				
22	3 : 3'-Diethyl-6-methyl-4' : 5'-benz-	216°	460	150	770
23	3 : 3'-Diethyl-6 : 9-dimethyl-4' : 5'-benz-	252-254°	250	140	140
24	3 : 3'-Diethyl-4 : 5 : 4' : 5'-tetraphenyl- thiazolocarbocyanine iodide	247-250°	>1000	>1000	>1000
	Selenacarbocyanine iodide				
25	3 : 3'-Diethyl-	276-277°‡	115	105	100
26	3 : 3'-Diethyl-9-methyl-	292-293°	460	>1000	>1000
27	3 : 9 : 3'-Triethyl-	234-237°	130	145	150
	Oxathiacarbocyanine iodide				
28	3 : 3'-Diethyl-5' : 6'-dimethyl-5-phenyl-	270°	110	125	80
29	3 : 3'-Diethyl-9 : 5' : 6'-trimethyl-5-phenyl- (perchlorate)	250-253°	90	95	70
30	3 : 9 : 3'-Triethyl-5' : 6'-dimethyl-5-phenyl-	360°	105	105	160
	Oxaselenacarbocyanine iodide				
31	3 : 3'-Diethyl-5'-methoxy-5-phenyl-	262°	425	170	500
32	3 : 3'-Diethyl-9-methyl-5'-methoxy-5-phenyl-	247°	235	150	180
	Selenathiacarbocyanine iodide				
33	3 : 3'-Diethyl-5-methoxyselena- 4' : 5'-benzthiacarbocyanine	237-238°	>1000	>1000	>1000
34	3 : 3'-Diethyl-9-methyl-5-methoxyselena- 4' : 5'-benzthiacarbocyanine	227-228°(d.)	>1000	100	150
35	3 : 9- 3' :Triethyl-5-methoxyselena- 4' : 5'-benzthiacarbocyanine	210-213°(d.)	135	90	125

* Brooker & White[12] reported m.p. 261°(d.)
† *idem, ibid.*, reported m.p. 296°(d.)
‡ Clark[11] reported m.p. 270-271°(d.)
(d.) = melting with decomposition

(*f*) In general, the activity of the unsymmetrical dyes lies midway between those of the symmetrical parent dyes. It follows, therefore, that the activity is a function of the properties of the terminal heterocycles from which the dye is derived rather than of the shape of the dye.

As has been seen, the dyes were irreversibly adsorbed by the spores, irrespective of their activity. In consequence, the differences in activity of the dyes cannot be ascribed to their powers of penetration. Thus the explanation for their mode of action has to be sought in terms of interference with the vital processes connected with the metabolism of the protoplast.

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A RAPID METHOD FOR THE DETERMINATION OF LACTOSE IN MILK AND CHEESE

By A. JOHN G. BARNETT and G. ABDEL TAWAB

A colorimetric method is described for the determination of lactose in milk and cheese. It appears to be both speedy and accurate, as is evidenced by the facts that a lactose determination in milk takes about 15 min. to complete, while suitably designed experiments indicate a satisfactory degree of recovery of lactose. As illustrations of the potential uses of the method, figures are given for the lactose content of different types of cheese at different stages of maturity and for milk undergoing the process of souring.

Introduction

During the course of a study of the ripening of some locally-made type cheeses, it became necessary to measure the lactose contents of the different samples. Official methods[1] are somewhat laborious and, as far as the authors are aware, there has been only one attempt in recent years to evolve a reliable method capable of giving results at both routine and research levels. This is the method suggested by Fagan, Sibbach & Hussong,[2] in which anthrone, a reagent specific for carbohydrates, is used in sulphuric acid solution. Use has been made of the anthrone method in these laboratories for the determination of soluble carbohydrates in grass and silage,[3] but as the reagent does not keep for any length of time and as the technique involves heating and cooling procedures, attention was turned to the method of Dubois *et al.*[4] which has been used satisfactorily here for similar purposes.[5] The phenol–sulphuric acid reagent appears to be quite specific for carbohydrates, and equivalent amounts of different carbohydrates, under the same experimental conditions, give very similar colorimetric readings. As the purpose of the present work was concerned with the carbohydrates in milk and cheese, the results described below were obtained with the use of lactose as a standard. It has been possible to show that the other main components of milk and cheese have no interfering action, at the concentrations used, on the determination of lactose by this method.

Experimental

Reagents

(*a*) An 80% solution of phenol is prepared by warming together, until the crystals are dissolved, 80 g. of phenol (AnalaR) and 20 ml. of distilled water. The reagent will remain unchanged in appearance for several weeks if stored in a brown bottle.

(*b*) Sulphuric acid of AnalaR quality should be used. Ordinary grades of sulphuric acid serve the purpose but when the stock bottle is replenished new standard curves have to be prepared.

Procedure

Standard curve

(*a*) A standard lactose curve is obtained as follows. Pure lactose (0·05 g.) is dissolved in 1000 ml. of distilled water and aliquot portions of 0 to 2 ml. are placed in matched colorimeter tubes (EEL Electroselenium Co.) and diluted to 2 ml. To each tube are added 6 drops of 80% phenol from a 1-ml. graduated pipette, the pipette being filled to the mark for each addition. The actual amount of phenol added does not matter, within limits, as long as the same quantity is always used: the drop-method is easier to employ than a measurement procedure designed to add an exact amount, in the neighbourhood of 0·15 ml., to the tubes. One pipette only should be kept for the purpose of this addition. To each tube are now added 5 ml. of sulphuric acid using a bulb pipette. (There is no need to cool the tubes during this addition.) The colour, which is yellow/brown in nature, is developed at its maximum intensity immediately and appears to be stable for at least 1 hour. For convenience, the tubes may be set aside for 15 min. before reading the colours obtained on an EEL colorimeter using a blue filter. The instrument is set at zero with a blank prepared from 2 ml. of water, 6 drops of phenol solution and 5 ml. of sulphuric acid.

The line obtained by plotting the EEL readings against the volumes of standard solution employed is a very shallow smooth curve between zero and 100 µg./2 ml. concentrations (Fig. 1).

(*b*) *Determination of lactose in milk and cheese.*—The milk sample (1 g.) is accurately weighed in a small basin and transferred quantitatively with distilled water to a 1-litre flask, and made up to volume with distilled water. A filtration through two thicknesses of Whatman No. 42 filter paper gives a water-clear filtrate but, as mentioned below, filtration is not in fact necessary. For cheese, while a Waring Blendor may be used for some soft varieties, the best way of preparing the extract is to grind 1 g. (accurately weighed) of the cheese in a mortar with 10 ml. of water and to transfer the supernatant liquid to a 1-litre flask. The grinding and decantation procedure should be performed six times in all. The total contents of the mortar are then washed into the flask, shaken for a few moments by hand and the volume made up to the mark. The mixture is

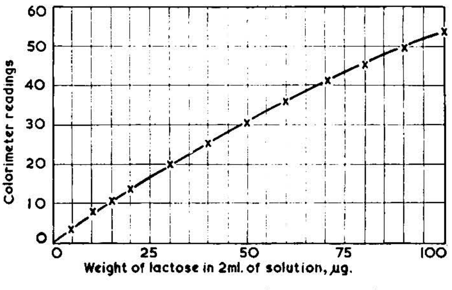

FIG. 1.—*Lactose concentrations from 100 µg./2 ml. to 5 µg./2 ml. plotted against EEL colorimeter readings*

then filtered through two thicknesses of Whatman No. 42 filter paper. This is a slow process but only 10 ml. are required to wash through the paper, funnel and receiving vessel and only a few ml. are subsequently required.

Using 2-ml. aliquots of the above milk and cheese preparation and a blank prepared as above, the lactose may be determined by the method described for the preparation of a standard curve. From the EEL readings, the lactose content in 2 ml. of the extract may be determined by reference to the standard curve. If the above quantities are adhered to, the lactose in the cheese or milk sample is given by the simple expression :

$$\% \text{ lactose} = \frac{\mu\text{g. lactose in 2 ml. of extract}}{20}$$

The maximum readings obtained on the EEL colorimeter for milk and cheese samples were between 50 and 54 while the minimum readings were as low as 4. These figures involve a range of 46 to 49 scale units which, by reference to Fig. 1, means that the average lactose equivalent per scale unit is about 2μg. A reasonable degree of accuracy from the instrument readings is thus obtained.

(*c*) *Interfering materials.*—The possibility of interfering substances has been investigated in the following way. Casein (0·3 g.), a mixture of fourteen amino-acids (0·05 g.), lactic acid (0·02 g.) and citric acid (0·005 g.) were separately placed in four 1-litre flasks and the contents made up to the mark with distilled water. In addition, 0·3 g. of butter fat was dissolved in about 2 ml. of alcohol and this was poured, a drop at a time with shaking, into 500 ml. of water in a 1-litre flask, the contents of which were then made up to volume with distilled water. These amounts correspond to the maximum quantities of the different components likely to be found in 1 g. of cheese, although as far as milk is concerned, they are vastly in excess. The contents of the flasks were then filtered as described above and 2-ml. portions, with a corresponding blank, used for lactose determination. Only with casein was a reading on the colorimeter obtained and that had a numerical value of about 2. The reading obtained by the examination of whole fresh milk is about 53 with our apparatus and standards. Actually it was shown that the casein samples used here contained traces of lactose.

(*d*) *Recovery experiments.*—In the recovery experiments with cheese and milk, the highest maximum and lowest minimum lactose recoveries obtained were 102% and 95% respectively. As seen from Tables I and II the recovery is excellent. Linear curves are obtained when the weights of lactose present are plotted against the volumes of the different mixtures used.

(*e*) *Speed of operation.*—Assuming the necessary apparatus was in place, the time for the determination of lactose in a single milk sample from the moment of starting to weigh the sample to the time of presentation of the result is very short, in this laboratory, 15 min. 7 sec. for an unfiltered milk sample and 15 min. 25 sec. for a filtered sample.

Applications of the method

(1) Using the procedure described above, it has been possible to estimate with ease the lactose content of a number of different locally-made type cheeses. These were prepared from the same bulk milk supply at the same time and each cheese was ripened under conditions approximating as closely as possible to those appropriate to each variety. The types used were Gervais, Coulommier, Pont l'Évêque, Dutch and Small-Holder. The lactose measurements were merely a part of a general investigation into the ripening changes over the period of manufacture of the cheese from the time of preparation to the time it would normally be marketed. This ranged from a few days to eight weeks : the results obtained are summarized in Table III. Although the main interest here is speed and accuracy in obtaining results, it may be noted in passing that the high values at the start of the ripening periods of the soft cheese are what might be expected from unpressed material, while the much lower values for the first hard cheese samples reflect the effects of the pressing process intrinsic to their mode of manufacture.

(2) Four samples of milk, one pasteurized, one certified, one raw and one pasteurized and inoculated with a culture of *Esch. coli*, were incubated at 37°. The samples were obtained on the same day from different market sources. Lactose content was measured at intervals with the results shown in Fig. 2.

Table I

Recoveries of total lactose from mixtures of standard lactose solution (100 µg./2 ml.) and milk

Solution A*					Solution B					Solution C				
Standard lactose, ml.	Solution A ml.	µg. sugar/2 ml.		% Recovery	Standard lactose, ml.	Solution B, ml.	µg. sugar/2 ml.		% Recovery	Standard lactose, ml.	Solution C, ml.	µg. sugar/2 ml.		% Recovery
		Found	Calc.				Found,	Calc.				Found	Calc.	
0	2·0	100	100	100	0	2·0	47·5	50	95	0	2·0	24·0	25·0	96·0
0·2	1·8	99	100	99	0·2	1·8	55·0	55	100	0·2	1·8	31·5	32·5	96·9
0·4	1·6	100	100	100	0·4	1·6	60·0	60	100	0·4	1·6	40·0	40·0	100
0·6	1·4	99	100	99	0·6	1·4	62·0	65	95·4	0·6	1·4	47·5	47·5	100
0·8	1·2	100	100	100	0·8	1·2	70·0	70	100	0·8	1·2	54·0	55·0	98·2
1·0	1·0	100	100	100	1·0	1·0	73·5	75	98	1·0	1·0	62·0	62·5	99·2
1·2	0·8	99	100	99	1·2	0·8	80·0	80	100	1·2	0·8	70·0	70·0	100
1·4	0·6	100	100	100	1·4	0·6	85·0	85	100	1·4	0·6	76·5	77·5	98·7
1·6	0·4	98·5	100	98·5	1·6	0·4	88·5	90	98·4	1·6	0·4	87·0	85·0	102·4
1·8	0·2	98·5	100	98·5	1·8	0·2	92·5	95	97·4	1·8	0·2	91·0	92·5	98·4
2·0	0	100	100	100	2·0	0	100	100	100	2·0	0	100	100	—

* *Solution A* = 1 ml. milk diluted to 1000 ml. *Solution B* = Solution A diluted with an equal volume of water. *Solution C* = Solution B diluted with an equal volume of water

Table II

Recoveries of total lactose from mixtures of standard lactose solution (100 µg./2 ml.) and extracts from Pont l'Évêque cheese

Extract 1					Extract 2					Extract 3				
Standard lactose, ml.	Extract ml.	µg. sugar/2 ml.		% Recovery	Standard lactose, ml.	Extract ml.	µg. sugar/2 ml.		% Recovery	Standard lactose, ml.	Extract, ml.	µg. sugar/2 ml.		% Recovery
		Found	Calc.				Found	Calc.				Found	Calc.	
0	2·0	81·5	81·5	100	0	2·0	26·5	26·5	100	0	2·0	5·5	5·5	100
0·1	1·9	82·5	82·3	99·7	0·1	1·9	30·5	30·2	99·0	0·1	1·9	11·0	10·3	93·7
0·3	1·7	84·0	84·1	100·1	0·3	1·7	37·0	37·6	101·6	0·3	1·7	20·0	19·7	98·5
0·5	1·5	86·0	85·9	99·9	0·5	1·5	45·0	44·6	99·1	0·5	1·5	28·5	29·2	102·5
0·8	1·2	88·5	88·9	100·4	0·8	1·2	55·5	55·8	100·5	0·8	1·2	43·5	43·3	99·5
1·0	1·0	89·5	90·7	101·3	1·0	1·0	63·5	63·2	99·5	1·0	1·0	55·5	52·8	95·1
1·5	0·5	94·5	95·2	100·7	1·5	0·5	81·0	81·4	100·5	1·5	0·5	76·0	76·4	100·5
1·8	0·2	98·0	98·2	100·2	1·8	0·2	92·5	92·0	99·3	1·8	0·2	90·5	90·5	100
2·0	0	100	100	100	2·0	0	100	0	100	2·0	0	100	100	100

* Extract 1 was made from cheese 1 day old, Extract 2 from cheese 1 week old and Extract 3 from cheese 4 weeks old

Table III

The lactose content of five locally made ' type ' cheeses measured over the period from their time of manufacture to that stage at which each would normally be marketed

	% Lactose in fresh cheese				
	Coulommier	Gervais	Dutch	Pont l'Évêque	Small-Holder
Start	4·52	4·28	0·82	4·06	1·05
After 1 day	3·62	2·87	—	—	—
,, 2 days	3·44	1·97	—	—	—
,, 3 ,,	3·37	1·67	—	—	—
,, 1 week	—	—	0·18	1·32	0·85
,, 2 weeks	—	—	0·18	0·62	0·85
,, 3 ,,	—	—	—	0·42	0·35
,, 4 ,,	—	—	—	0·28	0·27
,, 5 ,,	—	—	—	—	0·26
,, 6 ,,	—	—	—	—	0·25
,, 7 ,,	—	—	—	—	0·25

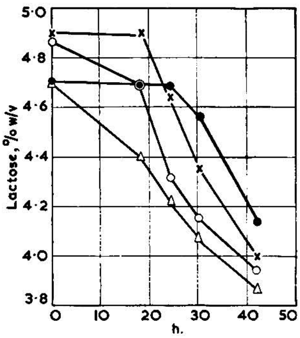

FIG. 2.—*The variation in lactose content of four milk samples allowed to sour at 37°*

×——× Certified ●——● Pasteurized
○——○ Raw △——△ Pasteurized + culture of *Esch. coli*

Summary

(1) A very rapid method is described for the determination of lactose in milk and cheese, utilizing the reaction which occurs between a phenol–sulphuric acid mixture and a carbohydrate.

(2) The validity of the technique is supported by recovery experiments with both milk and cheese extracts and a lactose standard, while lactose may be estimated in any concentration from zero to 100 μg./2 ml. of extract containing this component.

(3) As examples of applications of the method, studies have been made on the decline in lactose content during the souring of four grades of milk and in the ripening of five different varieties of cheese.

Acknowledgment

The authors are indebted to the members of the Dairying Department (Craibstone) of the North of Scotland College of Agriculture for their preparation of the cheeses used in this and associated work.

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A RAPID PROCEDURE FOR THE CHROMATOGRAPHIC SEPARATION AND SPECTROPHOTOMETRIC ESTIMATION OF CERTAIN PASTURE LIPOIDS. I.—Carotene, Xanthophyll and Chlorophyll

By N. A. WORKER

A method is described for the extraction and chromatographic separation of carotene, xanthophyll and chlorophyll from pasture plants.* The pigments are extracted with acetone, transferred to light petroleum, chromatographed on alumina into three fractions and then estimated spectrophotometrically. The method is rapid and accurate and is suitable as a routine assay procedure.

Introduction

During a recent investigation in this laboratory a rapid and accurate chromatographic technique suitable for routine assay was required for the estimation of carotene, xanthophyll and chlorophyll in pasture plants. A review of the literature indicated that although some excellent methods had been devised none was entirely satisfactory. Many, for example, were not suitable for routine work involving large numbers of samples. Some involved saponification, a tedious and time-consuming step, and others required the use of adsorbents which were known to allow only extremely slow passage of solvents. Also speed and simplicity had sometimes been sacrificed to obtain an absolute analytical precision which for comparative purposes is seldom required. A technique has therefore been developed which allows a quantitative separation of the pigments on one column of adsorbent, and which is simple, accurate and rapid, and thus ideally suited to the routine assay of large numbers of samples. No specialized apparatus is needed and the adsorbent used is a readily available standard brand which requires no preparation.

Experimental

Reagents

Alumina:	Aluminium oxide for chromatography (May & Baker, Ltd), Brockmann grade III
Solvents:	Light petroleum, boiling range 40–60° A.R. Acetone, A.R.
Eluants:	1. 2% acetone (v/v) in light petroleum 2. 1% ethanol (v/v) in light petroleum 3. 8% ethanol (v/v) in light petroleum

Extraction procedure

For accurate results some general precautions must be observed during extraction.[1]

Convenient quantities are 5 g. of fresh grass or 1 g. of dried grass ground to pass through a 0·5-mm. circular mesh sieve and thoroughly moistened with a few drops of water. The sample is extracted under nitrogen in a macerator of the Waring Blendor type with 60 ml. of acetone in the cold for 5 minutes. The mixture is filtered through a Buchner funnel and the residue re-extracted under nitrogen for 5 minutes with an additional 60 ml. of acetone. This mixture is filtered as before and the residue washed with 25 ml. of acetone. The combined filtrates are made up to 150 ml. An aliquot of 3 ml. is dried on a water-bath at 60° and taken up in 2–3 ml. of light petroleum for chromatography.

Preparation of column

The column is prepared by a method essentially similar to that used for the separation of carotene and vitamin A alcohol by Thompson, Ganguly & Kon,[2] with minor modifications. The alumina is weakened by suspending in eluant 3 to an approximate Brockmann value of IV.

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* Species examined in the present investigations included ryegrass, cocksfoot, red and white clovers, but there is no reason why the method should not be used for examining other pasture or crop plants

The alumina suspension is poured into a tube to form, after settling, a column approximately 3 cm. high. When all the eluant has percolated through, the top is strengthened with 1 ml. of light petroleum and it is then ready for immediate use. (More than 1 ml. will cause undue destruction of chlorophyll.) Six columns have been found a convenient number to handle at one time.

Separation of pigments

The pigment solution is poured on to the column which adsorbs the chlorophyll and xanthophyll in a narrow band at the top and allows the carotene to move through. (Overloading of the column will cause undue destruction of all the pigments.) The chromatogram is then developed with 10 ml. of eluant 1 which quantitatively strips the carotene fraction. Further development with 25 ml. of eluant 2 removes chlorophyll with less than 3% loss. Xanthophyll is quantitatively recovered by final development with 25 ml. of eluant 3. During chromatography the tops of the columns must not be allowed to become dry but must be topped up just as the last drop of the previous batch of eluant is about to percolate through. In addition the general precautions mentioned by Davidson[1] must be observed. The eluates when suitably diluted (carotene to 15 ml., xanthophyll to 30 ml., and chlorophyll to 30 ml.) are ready for spectrophotometry.

Estimation

The concentrations of carotene and xanthophyll in the eluates are determined from the absorption maxima at 451 mμ and 441 mμ, respectively (Beckman model DU photoelectric spectrophotometer) and the value of E at these wave-lengths. For carotene, $E^{1\%}_{1\,\text{cm.}}$ is 2500. In the case of xanthophyll, it has been customary in this laboratory to use the same value for $E^{1\%}_{1\,\text{cm.}}$ as for carotene, viz., 2500 as the xanthophyll fraction resulting from the above separation is a mixture of non-carotene pigments of uncertain composition. For pure xanthophyll, Davidson, however, has reported a figure of 3980 computed from earlier work by Karrer & Würgler.[3]

In the case of chlorophyll it is necessary to make an adjustment for the variations in $\lambda_{\text{max.}}$ with variations in the ratio of chlorophyll-*a* and -*b*. Absorption curves for the pure isomers were prepared from pure solutions obtained by chromatography on sucrose of the mixed chlorophyll fraction obtained by chromatography on alumina. The maxima in the curves occurred at 664 and 645 mμ for chlorophyll-*a* and -*b* respectively, but each had appreciable absorption at the $\lambda_{\text{max.}}$ of the other. Corrections (found by experiment) were therefore applied to the optical densities of 1-cm. layers of solutions (D_a and D_b, respectively) by deducting $0{\cdot}2065D_b$ in the case of chlorophyll-*a* and $0{\cdot}2028D_a$ in the case of chlorophyll-*b*. That these corrections were valid was shown by tests on known mixtures of solutions of the two chlorophylls when the difference between the absorption found and that calculated was not more than 1%. The E values at the $\lambda_{\text{max.}}$ used in the calculations were 1020 for chlorophyll-*a* and 568 for chlorophyll-*b* (results of Zscheile & Comar[4]).

Recovery experiments

The results of recovery experiments in which known amounts of the different pigments were blended with acetone, transferred to light petroleum, chromatographed on alumina and

Table I

Recovery of pigments after blending with acetone and chromatographing on alumina

Carotene			Xanthophyll†			Total chlorophyll		
Added, μg.	Recovered, μg.	Recovery, %	Added, μg.	Recovered, μg.	Recovery, %	Added, μg.	Recovered, μg.	Recovery, %
5·0	4·9	98	10·0	9·8	98	50·0	49·0	98·0
10·0	10·0	100	20·0	19·8	99	100·0	97·4	97·4
15·0*	14·8	99	30·0*	29·6	98·7	150·0*	145·5	97·0
20·0	19·7	98·5	40·0	39·5	98·8	200·0	193·8	96·9
50	48·6	97·2	100·0	96·9	96·9	500·0	468·0	93·6

* Approximate amounts present on column during a normal separation

† Crude xanthophyll obtained by chromatography of grass extract on alumina and estimated using $E^{1\%}_{1\,\text{cm.}}$ 2500

re-estimated are shown in Table I. From these results it is apparent that over the usual range of concentrations obtained with pasture plants, recoveries were well within the limits of normal experimental error.

Discussion

The procedure described for the extraction and separation of carotene, xanthophyll and chlorophyll from pasture plants offers several advantages over previous ones. Firstly, saponification is not necessary; secondly, the method is simple and direct requiring a minimum number of steps and no special apparatus; and thirdly, the pigments can be separated on a chromatographic column of adsorbent which is an easily obtainable proprietary brand of alumina, allowing a rapid passage of solvents and requiring no pre-treatment. Recovery experiments with pure pigments over a wide range of concentrations indicate results to be both accurate and reproducible. The procedure is thus ideally suited for routine assay and comparative work with large numbers of samples. It is essential, however, to observe all the precautions noted in the experimental section.

A routine method for the chromatographic separation of tocopherol from pasture plants and its assay by the Emmeric–Engel reaction will be described in a later paper.

Acknowledgments

The results of this investigation form part of a thesis submitted for the degree of Ph.D. of the University of New Zealand. The author wishes to express sincere thanks to Dr. W. A. McGillivray of the Biochemistry Department, Massey College, for advice and helpful criticism, and to the New Zealand Department of Scientific and Industrial Research for a grant towards the investigation. The technical assistance of Miss Fay Frecklington is gratefully acknowledged.

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ERRATUM

In the paper by R. Coles *et al.* entitled ' Influence of dietary carotene on the mortality pattern of fowl ', appearing in *J. Sci. Fd Agric.*, 1956, **7**, 692–699, the carotene content of the grass meal used in the experimental ration (Tables II and V) should read 250 mg. per kg.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 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

JULY, 1957

I.—AGRICULTURE AND HORTICULTURE

General : Soils and Fertilizers

Characteristics of some Tanganyika soils. A. Muir, B. Anderson and I. Stephen (*J. Soil Sci.*, 1957, **8**, 1—18).—Physical, chemical and mineralogical characteristics of a number of profiles are presented. A. H. Cornfield.

Genesis and pedo-chemical characteristics of " karail " black clayey soils in the lower Gangetic basin of Uttar Pradesh. R. R. Agarwal, C. L. Mehrotra and R. N. Gupta (*J. Indian Soc. Soil Sci.*, 1956, **4**, 65—71).—Characteristics of the soils are presented and discussed. A. H. Cornfield.

Mineral studies of some reddish-brown and black soils of Rajasthan. R. V. Tamhane and N. Rao (*J. Indian Soc. Soil Sci.*, 1956, **4**, 105—109).—The nature of the heavy minerals present in the two types of soil is described. A. H. Cornfield.

Deduction of Schofield's capillary potential equation from Kelvin's formula. C. L. Dhawan, B. Verma and B. K. Handa (*J. Indian Soc. Soil Sci.*, 1956, **4**, 61—63).—The method of deducing the equation is described. A. H. Cornfield.

Hygrophotographic method for depicting soil moisture. J. Sivadjian (*Soil Sci.*, 1957, **83**, 109—112).—A method is described for obtaining a pictorial record of the moisture distribution in soils. Bromide photographic plates are immersed in developer in the dark, brought into open daylight, developed fully, fixed in hypo, washed, bleached with 3% aq. $HgCl_2$, washed and immersed in 0·5% aq. KI, again washed and dried. The plate is now sensitive to light in which it assumes a violet black colour. Moisture restores the initial yellow colour. The blackened plates are buried in soil to obtain a picture of the distribution of the moisture present. Bromide paper prints are made in the usual way from the exposed plates. T. G. Morris.

Field experience with the neutron-scattering method of measuring soil moisture. G. L. Stewart and S. A. Taylor (*Soil Sci.*, 1957, **83**, 151—158).—The method was calibrated by removing cores of soil, at intervals of 10 cm. to a depth of 160 cm. for moisture determination, and then lining the hole with an Al tube, the neutron source and probe being lowered into the hole. Readings were taken at 10-cm. intervals as it was removed. The standard source of counts was a 1-litre graduated cylinder in a container full of water. The correlation coefficient relating neutron count ratio (soil/standard) with water ratio (vol. of water in the sample/bulk vol.) was practically constant (0·95) from 30 cm. down to 160 cm. For depths <30 cm. the value was 0·76. In two soils studied the gravimetric and neutron count results agreed well below 30 cm. depth. Org. matter and clay content appeared to have no effect. T. G. Morris.

Evaporation of water from heated soils. P. T. Ramacharlu (*J. Indian Soc. Soil Sci.*, 1956, **4**, 129—134).—Rates of loss of moisture from a Gangetic alluvial soil heated to 60—1000° are reported. Results are compared with moisture losses from a sand and are discussed in relation to aggregation. A. H. Cornfield.

Firebreaks and soil erosion. L. C. Lightfoot (*J. Agric. W. Aust.*, 1956, **5**, 353—354).—Cultural methods used to prevent erosion on soils from which the plant cover has been removed to act as firebreaks are described. A. H. Cornfield.

Pastures in a soil conservation programme. A. L. Clarke (*J. Agric. S. Aust.*, 1956, **59**, 486—492).—The importance of pastures in soil conservation programmes in areas where water erosion is a problem is discussed. A. H. Cornfield.

Hydrogen and calcium relationships of calcareous soils. C. V. Cole (*Soil Sci.*, 1957, **83**, 141—150).—The effects of CO_2 and sol. salts on the activities of H^+ and Ca^{++} ions in the soil solution of calcareous soils are examined. $CaCO_3$ and soil suspensions were equilibrated with air–CO_2 mixtures of definite composition. Using $CaCO_3$ of different sources and particle size, good agreement was obtained with other workers on the effect of P_{CO_2} on the solubility of the $CaCO_3$. With aq. $CaCl_2$ or $NaHCO_3$ instead of water as the medium for suspension and a fixed P_{CO_2}, values obtained for the $Ca(OH)_2$ activity were constant. Similarly, using a calcareous loam in suspension in different solutions a constant value was also obtained, indicating that the P_{CO_2} was a controlling factor in the system. T. G. Morris.

The rôle of soil clay minerals in phosphorus fixation. J. B. Hemwall (*Soil Sci.*, 1957, **83**, 101—108).—An hypothesis is advanced which assumes that P is fixed in clay minerals by its reaction with sol. Al, originating from the clay, to form insoluble Al phosphates. The p*K* ($= pAl^{3+} + pH_2PO'_4 + 2pOH'$) values for several clay–phosphate systems were determined at pH 4 over an equilibration period of up to 7 days at 30°. Clays which fixed appreciable amounts of P (e.g., Na and Al kaolinite and Al montmorillonite) had constant p*K* values and the Al in solution decreased on the addition of the P. With Na montmorillonite very little P was fixed, the p*K* value was high and the Al content did not change on the addition of P. The rate of fixation was dependent finally upon the rate of decomposition of the clay. T. G. Morris.

Influence of certain adsorbed cations on radish seedling development. H. A. Schreiber, L. E. Davis and R. Overstreet (*Soil Sci.*, 1957, **83**, 91—99).—In mixtures of Ca- and Na-homionic soils the max. elongation of radish seedlings occurred when the Na content was 10—20%, and the min. at 80%. Salinity, pH or exchange capacity were not correlated with these findings but chemical analyses indicated that as the adsorbed Na increased the Ca content fell and with it the elongation. In Ca–H systems there was no added elongation at low ($<60\%$) levels of adsorbed H ; at higher levels the elongation was less than that of the controls. As in the Ca–Na system the elongation decreased with Ca content. With Ca-Mg systems there was no stimulation of growth due to the Mg at low levels and above 60% a decrease occurred. Elongation fell sharply at levels of Ca$<40\%$ as in the other systems. T. G. Morris.

Relation between dithizone-extractable zinc in the soil and zinc uptake by maize plants. H. F. Massey (*Soil Sci.*, 1957, **83**, 123—129).—Samples of 34 Kentucky soils representing 16 series were heavily cropped with clover and millet to lower the available Zn content. Maize was then grown until the plants were 12 to 16 in. high. Dithizone-extractable Zn was estimated in the soil and in the plants after wet ashing. The soil pH was negatively correlated with the uptake of Zn by the plant and the amount of dithizone-extractable Zn in the soil was positively correlated with the uptake. No correlation was found between Zn uptake and the total Zn content of the soil. From the results an equation was derived connecting the Zn content (in μg.) in the plants (Y), the soil pH (X_1) and the dithizone-extractable Zn (p.p.m.) in the soil (X_2), viz., $Y = 99{\cdot}2 - 12{\cdot}2X_1 + 10{\cdot}9X_2$. Using this equation it was found that a Y value of 40 divided the Zn-deficient and non-deficient soils in the field. T. G. Morris.

Boron requirements of some Bihar soils. S. C. Mandal, M. A. Ali and H. N. Mukherjee (*J. Indian Soc. Soil Sci.*, 1956, **4**, 79—85).—Crop yields on an acid sandy soil were generally increased, whilst yields on soils of heavier texture were usually reduced, by application of borax (20 lb. per acre). A further application of B in the following year resulted in reduced yields even on the sandy soil. Available B (hot water-sol. B), before treatment was relatively low in the sandy soil as compared with the other soils. B toxicity symptoms occurred in many of the crops. Wheat and rice were relatively resistant to B injury as compared with the other crops. A. H. Cornfield.

Effect of continuous applications of manures and fertilizers on the carbon and nitrogen levels of soils. C. N. Acharya and K. Rajagopalan (*J. Indian Soc. Soil Sci.*, 1956, **4**, 111—123).—Application of farmyard manure annually to three soil types (8·8—19·2% clay) over 25 to 45 years increased the org. C content of the soils by 20—40%. Application of inorg. fertilizers, mustard cake or green manure had no significant effect on org. C content. None of the treatments had any significant effect on the N content of surface soils, although in one soil the N content of the subsoil was increased. The C/N ratio of the soils were increased somewhat by farmyard manure, slightly by green manure, but was not affected by inorg. fertilizers. A. H. Cornfield.

Aminopolysaccharides in soils. I. Colorimetric determination of hexosamines in soil hydrolysates. F. J. Stevenson (*Soil Sci.*, 1957, **83**, 113—122).—Soil (2 g.) is hydrolysed with 50% HCl in a closed vessel at 100° for nine hours, and after separation from the solid matter the hydrolysate is evaporated to dryness at 40°. The residue is suspended in water and passed through a column of Amberlite IRA 400, in the carbonate form, to remove humin, acids, Fe and Al. The effluent is then used for the hexosamine determination by the acetylacetone-*p*-dimethylaminobenzaldehyde method.

Any sugars present in the effluent are separated by Dowex-50 in the H form, the hexosamines and amino-acids being adsorbed and the sugars passing through. In seven soils examined the N present as amino-sugars was 7·6—12·9% of the total N. Cropping increased the hexosamine content of org. matter, and the org. matter in the B horizon was richer in N than that in the A. T. G. MORRIS.

Root nodule bacteria of *Prosopis stephaniana*. A. K. Khudairi (*Science*, 1957, **125,** 399).—*Prosopis* is the dominant legume in Iraq in the wild state. The nodules contain much NO_3' and it is suggested that the prolonged fertility of Mesopotamia is due to the NO_3' from the abundant growth of *Prosopis*. T. G. MORRIS.

Effect of specific enzyme substrate (saccharose) on the production of soil saccharase. St. Kiss (*Z. PflErnähr. Düng.*, 1957, **76,** 119—122).—Saccharase activity (Hofmann and Seegerer) determinations indicate the stability of soil enzymes, no increase being observed on moistening air-dry soils or on air-drying soils. Raw sugar increases the formation of saccharase as also, to a slight extent, does glucose. Peptone has little or no effect. M. LONG.

New fields for the application of the Mitscherlich equation. II. Contribution of soil nutrient forms to plant nutrient uptake. A. M. Balba and R. H. Bray (*Soil Sci.*, 1957, **83,** 131—139).—The Mitscherlich equation as expanded by the authors previously (cf. J.S.F.A. Abstr., 1957, i, 164) has been further modified to permit calculation of the contribution of each form of a nutrient present in the root zone, to the uptake of nutrients by the plant. Thus $v = A(1 - e^{-c_aB_a - c_bB_b - cx})$ where B_a and B_b are the amounts of total adsorbed and easily sol. P respectively and c_a and c_b are the corresponding efficiency factors and y and A are the total P uptake by the crop and the max. amount of P in the crop under the conditions of the experiment respectively. Using the equation, values from field experiments show that the adsorbed P is the most important source of the nutrient. The adsorption of added P was greater in soils with lower P fertility. T. G. MORRIS.

Effect of fertilizers and organic materials on the cation-exchange capacity of an irrigated soil. P. F. Pratt (*Soil Sci.*, 1957, **83,** 85—89).—The effect of long-term fertilizer treatment on the cation-exchange capacity of a sandy loam is reported. The cation-exchange capacity (C.E.C.) was unaffected by urea, K or P applied either alone or together. The C.E.C. of the top 6 in. of soil was raised significantly by manure in combination with the other three fertilizers. Of the K added in fertilizers 77% accumulated in the top 36 in. of soil. There was a highly significant correlation between C.E.C. and the C content of the top 6 in. Extrapolation of the line to zero C content showed that 2·4 mequiv. per 100 g. was the C.E.C. of the mineral portion of the soil; that of the org. C was estimated as 490 mequiv. per 100 g. irrespective of the type of org. matter added. T. G. MORRIS.

Economic analysis applied to nitrogen fertilizer rate experiments on irrigated maize. J. L. Paschal and B. L. French (*U.S. Dep. Agric.*, 1956, Tech. Bull. 1141, 73 pp.).—The application is reported of a method of economic analysis using the function $Y_j = M - AR,^{x_j}$ where Y_j is the yield of maize at the jth rate of fertilizer application, M = max. yield, A = increase in yield attributable to N fertilizer, R = ratio between successive increments in yield for uniform increments in fertilizer applied, and x_j is the quantity of fertilizer applied at the jth rate of application, for three plots of irrigated maize in Oregon, Washington and Nebraska. E. G. BRICKELL.

Comparison of the fertilizer action of nitrogen and magnesium as "Stickstoffmagnesia" and in other forms. E. Pfeil and V. Loeschcke (*Z. PflErnähr. Düng.*, 1957, **76,** 110—119).—In pot trials with oats, mustard, grass and potatoes, "Stickstoffmagnesia" $(NH_4),SO_4,MgSO_4,6H_2O)$ and $(NH_4)_2SO_4 + MgSO_4$ were superior to $(NH_4)_2SO_4$ alone, especially at high levels on N. Dry matter, starch and glucose contents are higher if N is applied as nitrate; the NH_4^+ ion produces tubers of abnormally high N content. Mg reduces this as well as the ratio % N : % carbohydrate formation to normal levels. High NH_4^+ ion application leads to a decline in free tryptophan and low-mol. tryptophan compounds in the expressed sap from the tuber. M. LONG.

Comparison of different forms of potassium. C. Gillern (*Bodenkultur*, 1956, **9,** 175—177).—The effects of "40% Kali" (refined kainit), "Patentkali" (50% K_2SO_4, 34% $MgSO_4$) and "Reformkali" (K_2SO_4, KCl and $MgSO_4$) on the growth of oats and tobacco are compared. With oats the last-named gave the best result, a second application giving no significant difference. With tobacco Patentkali was the more effective. M. LONG.

Effect of superphosphate on crop and soil relationships under alkaline conditions. B. Das (*J. Indian Soc.Soil Sci.*, 1956, **4,** 87—93).—Yields of berseem (*Trifolium alexandrinum*) and the uptake of P on an alkali soil (8·5% clay, pH 9·1) were increased by application of superphosphate (100 lb. P_2O_5 per acre). The treatment increased the C/N ratio of the soil, particularly of the surface layer. Microbiological activity (CO_2 production) in the soil was increased by P application. A. H. CORNFIELD.

Fertilizer phosphates on calcareous soils. P. Boishot and G. Sylvestre (*Agric. Chemic.*, 1956, **11,** No. 11, 45—46, 119).—A general account. A. H. CORNFIELD.

Phosphate fertilizers. S. I. Vol'fkovich (*Usp. Khim.*, 1956, **25,** 1309—1335).—World literature dealing with the production of phosphate fertilizers is reviewed. Methods of prep. are classified and information given on the following: decomposition by use of sulphuric and nitric acids; preparation of phosphoric acid and concentrates for use as fertilizer bases; production of liquid fertilizer bases and electro- and hydro-thermal treatment of native phosphates. (52 references.) A. L. B.

Enrichment of Duplex slag in P_2O_5 by Sylvester process. R. S. Mathur, H. W. Bhaskar Rao and Rabindar Singh (*J. sci. industr. Res.*, 1956, **15A,** 463—465).—The application of the Sylvester process (*Steel*, 1951, **129,** No. 22, 84) for the enrichment of Duplex slag in P_2O_5 for use as a fertilizer is investigated. The citrate-sol. P content is increased from 3·87 to 5·70% by correcting the basicity of the slag to ~ 2 by the addition of 3% SiO_2 and fixing the mixture at 1350°. The minute crystal size and intimate interlocking of the constituent minerals hinder the magnetic separation of the phosphate-rich phase on the treated slag. I. JONES.

Phosphate fertilizer trials. C. Gillern and T. Reichard (*Bodenkultur*, 1956, **9**, 155—161).—Comparison is made of basic slag, superphosphate and various hard and soft rock phosphates with a variety of crops and 3 soils of differing pH. Basic slag gave best results. Soft rock phosphate "Hyperphosphate" was effective on grassland especially in acid soil; its efficiency on oats was increased by simultaneous use of org. manures, possibly by restricting P fixation. M. LONG.

Effect of rock phosphate in field trials. E. Hofmann and A. Amberger (*Z. PflErnähr. Düng.*, 1957, **76,** 102—110).—Field trials comparing rock phosphate (Hyperphos) and basic slag are reported. In a very acid loam, highly deficient in P, basic slag was superior to Hyperphos for spring cereals, whilst there was no difference in the case of autumn-sown cereals. Liming to pH 6·5 did not affect the action of Hyperphos. In slightly acid and neutral soils there was no difference between the two fertilizers in the case of cereals, beet and potatoes. M. LONG.

Colorimetric determination of phosphates in raw materials and fertilizers. K. Lasiewicz and H. Zawadzka (*Chem. Anal.*, 1956, **1,** No. 1, 53—63).—The material under test was treated with either H_2SO_4–HNO_3, water, 2% citric acid or ammonium citrate solution. The solution was then treated with a reductor solution (metol, Na_2SO_3, and $NaHSO_3$ in water), and ammonium molybdate solution and left in a dark place for 10 min. The mixture was then treated with 2N-Na acetate, diluted to a known vol. and the blue colour measured with a photometer. The method gives straight-line calibration curves with 0·1—5 mg. of P_2O_5. More than 0·6 ml. of 50% ammonium citrate solution and H_2S interfere. Results are in good agreement with those from the conventional gravimetric method. K. F. SPOREK.

Determination of urea in fertilizers and cattle-foods. G. H. Jongen and H. W. Berkhout (*Chem. Weekbl.*, 1956, **52,** 909).—In a method for determining urea in mixed fertilizers, Watt and Chrisp (*Analyt. Chem.*, 1054, **26,** 142) obtained a greenish-yellow solution by mixing urea with *p*-dimethylaminobenzaldehyde, extinction being measurable at 420 mμ. NH_4, K, Ca, phosphate, sulphate and nitrate ions, in amounts even exceeding those normally present, did not interfere. But with cattle-foods, the aq. solutions were filterable with difficulty and were often coloured. It was possible to clarify them with Carrez solutions, but decolorization could be achieved only by shaking with norite, and particles of this disturbed clarity. The difficulties may be overcome by clarifying with Carrez solutions, and decolorizing with norite, in one operation. The urea content is found from a table which relates urea concn. to extinctometer readings at 20°. The relationship between urea concn. and extinctometer readings is practically rectilinear, both between 100 and 500 μg., and 500 and 1000 μg. urea per 5 ml. of filtrate. Some results for shrimp meal, fish meal, linseed meal and grass mea show accuracies of 98—101%. The method is applicable to materials containing 1—10% of urea. W. J. WRIGHT.

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.

Effect of soil management practices upon soil and leaf analysis in a peach orchard. C. W. Hitz, G. N. Gilligan and H. Amling (*Delaware agric. Exp. Sta.*, 1956, Tech. Bull. 316, 20 pp.).—In a peach orchard receiving different types of soil management and fertilizers over 10 years, soil org. C content decreased with time with clean cultivation and inorg. fertilizers, but remained constant or increased where poultry manure was applied or cover crops were grown. The most beneficial cover in this respect was rye–vetch in winter and soya-beans in summer. With similar K fertilization, the exchangeable K content of the soil after 10 years was higher with cover crops than with clean cultivation. Extractable soil P was more closely related to amount of P applied than to type of management. Values for trunk growth and leaf N, P and K contents are presented. The best growth occurred where all three nutrients were applied and vigour was not related to leaf content of any one nutrient. Poor growth under clean cultivation where NO_3' only was applied resulted from depletion of the other nutrients. A. H. Cornfield.

Apparatus for hydroponics research. F. H. Emmert (*Conn. agric. Exp. Sta.*, 1956, Bull. 322, 7 pp.).—The construction and operation of small sub-irrigated gravel culture units are described. A. H. Cornfield.

Plant Physiology, Nutrition and Biochemistry

Absorption of water from atmospheres of different humidity and its transport through plants. R. O. Slatyer (*Aust. J. Biol. Sci.*, 1956, **9**, 552—558).—The top and the roots of an intact plant were sealed in separate flasks. In each flask the vapour pressure was maintained at a particular level to establish a diffusion gradient favouring water movement from root to top or top to roots. With 1-year-old seedlings of short-leaf pine the absorption of moisture from one flask and its transfer through the plant to the other was demonstrated regardless of the direction. Absorption occurred from both saturated and unsaturated atm. The bearing of the results on the absorption of dew and of moisture from unsaturated atm. is considered. R. H. Hurst.

Basis of drought resistance in soya-bean. J. A. Clark and J. Levitt (*Physiol. Plant.*, 1956, **9**, 598—606).—In droughted plants the cell walls were less elastically extensible, respiration rates were lowered and the amount of lipins (light petroleum extract) per unit leaf area was higher than in adequately watered plants. Drought resistance results solely from restricted transpiration due to increased deposition of lipins in leaves. A. G. Pollard.

Protoplasmic streaming in plants sensitive and insensitive to chilling temperatures. D. A. Lewis (*Science*, 1956, **124**, 75—76).—Protoplasmic streaming virtually ceases at 10° and completely so at 5° or 0° in cold-sensitive plants such as the tomato, water melon, tobacco and sweet potato but in cold-resistant plants such as the radish and carrot streaming continues at 0° or 2·5°. Tomatoes chilled for more than 24 hr. and then exposed to 20° did not start streaming, but cold-resistant plants treated similarly showed rapid streaming when warmed. T. G. Morris.

Inhibition of photosynthesis by oxygen. I. Comparative physiology of the effect. J. S. Turner, M. Todd and E. G. Brittain (*Aust. J. biol. Sci.*, 1956, **9**, 494—510).—The O_2 inhibition of photosynthesis is demonstrated for 25 species, representing: Angiospermæ, Filicales, Bryophta, Chlorophyceæ, Phæophyceæ and Rhodophyceæ. The inhibition is rapidly produced and rapidly reversible. Under conditions of high light intensity and high CO_2 concn., the inhibition is not significant until the O_2 concn. exceeds 20%, and is large and variable between 20 and 100%. The inhibitory effect cannot be accounted for by a high rate of dark respiration accompanying photosynthesis at the high O_2 concn. In two angiosperms, a reduced rate of photosynthesis resulted on transferring the tissue from 20 to 5% of O_2, possibly due to stomatal control of the process. (15 references.) R. H. Hurst.

Stimulating effect of light upon growth and carbon dioxide fixation in *Blastocladiella*. I. The S.K.I. cycle. E. C. Cantino and E. A. Horenstein (*Mycologia*, 1956, **48**, 777—799).—The aquatic fungus *B. emersonii* and a mutant containing carotene both grew more rapidly in the light than in the dark. Experiments with radioactive bicarbonate showed that the light increased CO_2 absorption by the fungus and caused an increase in the concn. of succinate and a decrease in the concn. of ketoglutarate in the fungus. Glucose was utilized more rapidly by the fungus in the light than in the dark. L. G. G. Warne.

Enzymic oxidation of glucose to glucosone in red Alga. R. C. Bean and W. Z. Hassid (*Science*, 1956, **124**, 171).—Glucosone and galactosone occur as transient compounds in the enzymic oxidation of glucose and galactose respectively by the red alga *Iridophycus flaccidum*. T. G. Morris.

Aseptic studies on metabolism of nitrogen compounds in plants. L. G. Nickell (*Bull. Torrey Bot. Club*, 1956, **83**, 421—427).—*Lemna minor* was grown in a nutrient containing all essential inorg. nutrients plus thiamine, pyridoxine and nicotinamide. Further additions of arginine, ornithine, citrulline, *iso*leucine and lysine to this medium all increased growth but addition of canavanine depressed it. Ornithine, and to a greater extent arginine and citrulline, overcame the inhibition due to canavanine. 2 : 6-Diaminopurine inhibited growth in the absence but not in the presence of adenine, whilst adenine alone and penicillin *G* both stimulated growth. L. G. G. Warne.

Effect of mineral nutrition on content of free amino-acids and amides in tomato plants. I. Comparison of effects of deficiencies of copper, zinc, manganese, iron and molybdenum. J. V. Possingham (*Aust. J. biol. Sci.*, 1956, **9**, 539—551).—The quant. composition of free amino-acids in tomato plants grown in full nutrient and in cultures deficient in Zn, Cu, Mn, Fe and Mo are determined. All deficiencies except that of Mo increased the free amino-acid fraction. Fe and Zn deficiencies caused large increases in asparagine and glutamine, while Cu and Mn deficiencies increased many individual amino-acids but not the two amides. Qual. differences between deficient and full-culture plants included the accumulation of β-alanine with deficiency of Zn, Cu or Mo, and of pipecolinic acid with deficiency of Mn or Fe. Histidine was absent with Cu, Mn, Fe and Mo deficiency, phenylalanine was absent with Cu deficiency, and lysine absent with Cu, Mn and Mo deficiencies. (35 references.) R. H. Hurst.

Effect of neutron irradiation on the respiration of barley seed. I. Effects on growth and oxygen uptake on barley seedlings of different varieties. K. Mikaelsen, I.-P. Bjørnseth and H. Halvorsen (*Physiol. Plant.*, 1956, **9**, 697—711).—Exposure of barley seeds to neutron irradiation did not affect their germinative capacity but adversely affected seedling growth. Treated seedlings showed lowered rates of O_2 uptake after the first two days and contained subnormal proportions of water. Varietal differences in these effects were apparent. A. G. Pollard.

Mobility of ^{45}Ca after injection into Western White pine. W. K. Ferrell and F. D. Johnson (*Science*, 1956, **124**, 364—365).—^{45}Ca was injected into the trunks of the trees. The isotope was found in the foliage during the same year and one year later was present in appreciable amounts in the newly developed buds. T. G. Morris.

Uptake of silicon and phosphorus by wheat plants studied with radiochemical methods. L. Rothbuhr and F. Scott (*Biochem. J.*, 1957, **65**, 241—245).—A method, which involves use of ^{31}Si, is described for determination of $^{31}SiO_2$ with an accuracy of 0·5 μg. The material is ashed at as low a temp. as possible and the residue is then fused with Na_2CO_3. The resulting mass is dissolved in dil. HCl and the solution is placed in a liquid counter. The amount of $^{31}SiO_2$ is determined by comparing the counting rates with those of standard solutions. Young wheat plants absorb considerable amounts of SiO_2 as silicate. Added PO_4''' slightly decreases the uptake of silicate, whilst added silicate increases absorption of PO_4'''. J. N. Ashley.

Importance of chelating in agricultural chemistry and soil science. F. Scheffer, B. Ulrich and P. Hiestermann (*Z. PflErnähr. Düng.*, 1957, **76**, 146—155).—A review with 63 references. M. Long.

Uptake of iron as ferric sequestrene by *Vicia faba* and *Phaseolus vulgaris*. S. Heinonen and H. Waris (*Physiol. Plant.*, 1956, **9**, 618—623).—Exudates from cut stems of the two species supplied with Fe in inorg. forms contained less Fe than when the element was supplied as an EDTA chelate, largely because of pptn. of inorg. Fe in the nutrient solution. Some Fe probably passes into the ascending sap stream in the chelate form. A. G. Pollard.

The rôle of chelates in iron supply and in the metabolism of plants. G. Scholz (*Z. PflErnähr. Düng.*, 1957, **76**, 133—145).—A review with 120 references. M. Long.

Loss of nutrients from plant foliage by leaching as indicated by radio-isotopes. W. G. Long, D. V. Sweet and H. B. Tukey (*Science*, 1956, **123**, 1039—1040).—Rapidly growing plants were allowed to to absorb ^{32}P or ^{42}K either through the roots or the cut stems. The foliage was then leached with water, and the amounts of radio-isotopes removed were estimated. Beans and sweet potato plants lost no ^{32}P if it had been absorbed through the roots, but bean and rose cuttings lost 1·5 and 12·8% respectively of ^{32}P absorbed through cut stems. Bean plants after absorbing either ^{42}K or ^{86}Rb from nutrient solutions at high or low concn. in daylight or darkness were leached as before. Plants held in the dark lost 71 and 42% of the ^{42}K and 14 and 7% of the ^{86}Rb at the high and low salt levels respectively. For plants in the light losses were 5 and 12% of ^{42}K at the high and low levels respectively and 5% of the ^{86}Rb at both levels. T. G. Morris.

Cabinet for plant trials using artificial light exclusively. K. Ehrendorfer (*Bodenkultur*, 1956, **9**, 168—174).—Details of a cabinet for growing plants in artificial light are given. Fluorescent tubes giving an intensity of ~ 23 w./sq. m. of plant surface (3600 lumen) whose yellow-green fraction is less than that of sunlight, are used. M. Long.

Evaluation of nutrient media by plant analysis. A. Finck and E. Schlicting (*Z. PflErnähr. Düng.*, 1957, **76**, 97—102).—In a water culture experiment with oats, the total uptake of Na and K is more sensitive to the supply of nutrient than is the % content and is preferable for use in evaluating nutrient media. M. Long.

Absorption spectra of the anthocyanin pigment of red cabbage: a natural wide-range pH indicator. F. T. Wolf (*Physiol. Plant.*, 1956, **9**, 559—566).—The isolation and examination of the absorption spectra of the pigment from *Brassica oleracea*, L. var. *capitata*, L. f. *rubra*, L. is described. The colour change of the pigment over the pH range 1—10 was in the order, red, purple, blue, green, yellow with absorption peaks at 530, 615 and 420 mμ. A. G. Pollard.

Temperature and root-cell elongation. H. Burström (*Physiol. Plant.*, 1956, **9**, 682—692).—Cell elongation and cell-wall properties in roots of wheat grown at different temp. are examined. The final cell length decreased with rise in temp. due to shortening of the period of elongation. Simultaneously the cell wall lost plasticity, the Ca requirement was raised and sensitivity to inhibition by coumarin diminished. These effects of increasing temp. resemble those of increasing concn. of indolylacetic acid in the tissue. At 10° coumarin inhibited only the initial stages of the elongation process. A. G. Pollard.

Initiation of mitosis and cell division by kinetin and indolylacetic acid in excised tobacco pith tissue. N. K. Das, K. Patau and F. Skoog (*Physiol. Plant.*, 1956, **9**, 640—651).—In *in vitro* cultures of tobacco pith cells addition of kinetin (**I**) + indolylacetic acid (**II**) stimulated mitosis and cell division. Use of **I** alone did not affect mitosis and **II** alone caused some mitosis which did not result in appreciable cell division. A. G. Pollard.

Growth-substances in ether extracts of potato tuber. W. C. Burton (*Physiol. Plant.*, 1956, **9**, 567—587).—Ether extracts of potato peel contain both inhibitors and stimulators of elongation of *Avena* coleoptiles. No evidence was obtained of relationships between the concn. of these substances and the occurrence of sprouting in the tubers. A. G. Pollard.

Differences in auxin content of wheat roots caused by changes in pH of the nutrient solution. H. Rufelt and P. Fransson (*Physiol. Plant.*, 1956, **9**, 693—696).—Transference of roots of wheat plants from nutrient solutions at pH 5 to those at pH 7·5 increased the easily-extractable indolylacetic acid (**I**) concn. in the roots 1 hr. later but at the end of 24 hr. the value had diminished again. The temporary increase was probably due to the stimulation, at the higher pH, of the system within plant cells by which tryptophan is converted into **I**. A. G. Pollard.

Auxin and sexuality in *Cannabis sativa*. J. Heslop-Harrison (*Physiol. Plant.*, 1956, **9**, 588—597).—Application of α-naphthylacetic acid in lanolin to the underside of hemp leaves following appropriate photoperiodic induction caused the formation of female flowers in " male " plants at sites at which male flowers would occur normally. The overall flowering response and also the modification of leaf form associated with flowering were reduced. Relationships between flower sexuality and the concn. of auxin around the meristems during the differentiation of flower primordia are discussed. A. G. Pollard.

Effect of gravity on the flowering of soya-beans. J. E. Fisher (*Science*, 1957, **125**, 396).—Soya-bean plants were made to grow downwards, lead weights being attached to the growing tip. In some downward-growing plants vegetative suckers were removed as soon as they were 1 cm. long. Inverted plants flowered earlier and at lower nodes than did the control. Removal of the suckers stimulated even earlier flowering. As the inverted plants aged the stems showed symptoms typical of the injury by external applications of auxin. T. G. Morris.

Enzymically catalysed oxidation of indolylacetic acid. E. R. Waygood, A. Oaks and G. A. Maclachlan (*Canad. J. Bot.*, 1956, **34**, 905—926).—Wheat leaf extracts, catalase and horseradish peroxidase catalyse the decarboxylation and oxidation of indolylacetic acid at pH 5·0—6·0 in the presence of critical concn. of Mn and monohydric phenols or resorcinol. One mole of CO_2 is liberated and 1 mole of O_2 consumed per mole of substrate. Manganic ions formed by a phenol–peroxidase–peroxide system initiate the decarboxylation and oxidation. An ether-sol. factor from wheat leaves and maleic hydrazide can substitute for the active phenols. Catechol, quinol, pyrogallol, scopoletin and riboflavin, etc., competitively inhibit the oxidation. A reaction sequence involving an org. peroxide or radical rather than H_2O_2 is suggested. (28 references.) R. H. Hurst.

Effect of catalase, riboflavin and light on the oxidation of indolylacetic acid. E. R. Waygood and G. A. Maclachlan (*Physiol. Plant.*, 1956, **9**, 607—617).—The mechanism of the decarboxylation and oxydation of indolylacetic acid (**I**) by catalase consists of initiation by $Mn^{\cdot\cdot\cdot}$, the formation of peroxide and the catalysed peroxidation of org. H-donors followed by oxidation of $Mn^{\cdot\cdot}$. The action of catalase systems is unaffected by light unless certain inhibitors are present and is retarded by riboflavin phosphate (**II**), the latter effect being counteracted by blue light. The growth-promoting action of **II** on plant growth and the restriction of growth by light may be explained by the **II**–**I**-light interactions. A. G. Pollard.

Selective inhibition of the geotropic response by *N*-1-naphthylphthalamic acid. T-M. Tsou Ching, R. H. Hamilton and R. S. Bandurski (*Physiol. Plant.*, 1956, **9**, 546—558).—In the period during which the geotropic bending of *Avena* coleoptiles reached max. the rates of straight growth and of geotropic curvature were directly related. The same applied to young roots and shoots of peas and maize. Treatment with *N*-1-naphthylphthalamic acid inhibited straight growth and curvature to extents which were disproportional in pea and maize roots and shoots but not in *Avena* coleoptiles. Treatment of maize roots with 2 : 4-dichlorophenoxy-*iso*butyric acid caused proportional inhibition of straight growth and geotropic curvature. A. G. Pollard.

Chemistry of arylboric acids. VI. Effects of arylboric acids on wheat roots and the rôle of boron in plants. K. Torssell (*Physiol. Plant.*, 1956, **9**, 652—664).—The action of 24 of these compounds on wheat roots and oat coleoptiles is examined. Growth of wheat roots was strongly stimulated, the mechanism being one of cell elongation. Phenylboric and 4-methoxyphenylboric acids were particularly active. No effects on oat coleoptiles were apparent. A. G. Pollard.

Effect of gibberellic acid on growth of maize roots. W. G. Whaley and J. Kephart (*Science*, 1957, **125**, 234).—Gibberellic acid was added to nutrient solutions in which excised, apical, 10-mm. segments of maize roots were grown for 24 hours. Segments of both primary and adventitious seminal roots of two inbred lines of maize and their hybrid were used. No dwarfism was present in the lines. The primary roots of one line were unaffected by the gibberellic acid at the concn. used (0—20 μg.). Those of the other line and the hybrid were significantly stimulated by the acid in 10 μg. per ml., and further stimulated by 20 μg. per ml. The effect on the seminal roots was similar to that of the primary. T. G. Morris.

Chlorosis produced by fluorine on citrus in Florida. I. W. Wander and J. J. McBride, jun. (*Science*, 1956, **123**, 933—934).—A chlorotic condition of citrus leaves unlike normal chlorosis was observed on trees near a superphosphate factory. High concn. of F were present, but high F contents were not always associated with chlorosis. T. G. Morris.

Crops and Cropping

Effect of potassium and potassium–phosphorus applications on the yield and quality of winter wheat. E. Primost (*Bodenkultur*, 1956, **9**, 162—167).—The grain yield is dependent only on the level of N supplied, whilst the baking quality, estimated by gluten content, is improved by the application of K at high N levels, the timing of the top-dressing being immaterial. The germinating power is not significantly affected by K–P fertilizer application. M. Long.

Quality of wheat as affected by manures and fertilizers. II. Thiamine, riboflavin and niacin contents. Y. P. Gupta and N. B. Das (*J. Indian Soc. Soil Sci.*, 1956, **4**, 125—128).—The thiamine content of wheat grain was increased by all treatments (org. manures and inorg. N, P, K alone and in various combinations) and in particular where P had been applied. The riboflavin content was generally unaffected by org. manures but was usually increased by N, P and K. The niacin content was increased only by PK and green manure treatments. There were no significant correlations between protein content and content of any of the vitamins or between content of the vitamins themselves. A. H. Cornfield.

Effects of ammonium sulphate and lime on paddy. S. Digar (*J Indian Soc. Soil Sci.*, 1956, **4**, 73—77).—Application of $(NH_4)_2SO_4$ (20—40 lb. of N per acre) yearly over 10 years to a clay soil increased yields of rice grain and straw in all but one of the years. Application of lime (4—8 cwt. per acre) three times over the 10-year period increased slightly grain yields but had no effect on yields of straw. Neither treatment, alone or in combination, had any significant effect on soil pH or exchangeable Ca content. A. H. Cornfield.

Effects of bulky organic manures on yields of rice. M. N. Basak (*J. Indian Soc. Soil Sci.*, 1956, **4**, 95—103).—When applied on the basis of equal amounts of N, town, village, and water hyacinth composts and sewage sludge were as effective as was $(NH_4)_2SO_4$ (**I**)

(40—60 lb. N per acre) in increasing yields of rice grain over three years. Application of superphosphate (20 lb. P_2O_5 per acre) had no effect on yields. The org. manures showed no better residual effect than did **I**. The rate of mineralization of org. N in flooded rice soils was directly related to temp. A. H. Cornfield.

Wet padi manurial experiments in Malaya. E. F. Allen and R. Henderson (*Malayan agric. J.*, 1956, **39**, 2—39).—Results of 223 replicated and 337 unreplicated trials are presented. NH_3 generally gave better results than did an equiv. amount of NO_3. "Ammophos" fertilizers gave results comparable with mixtures of $(NH_4)_2SO_4$ and double superphosphate. No yield increases occurred through applying Ca of Mg. Yields were sometimes reduced by Mg. P_2O_5 50 lb. per acre as superphosphate usually gave yield increases equiv. to 100 lb. of water-insol. P_2O_5 (fossil bat guano or rock phosphate). In the early stages of growth padi made excellent response to even small dressings of fertilizer. Fertilizer requirements in various districts are described. A. H. Cornfield.

Zinc content of potato leaves. H. J. Klosterman and C. O. Clagett (*N. Dakota agric. Exp. Sta., Bimo. Bull.*, 1956, **19**, 24—27).—The Zn content of potato leaves from a variety of locations ranged from 13 to 87 p.p.m. (dry matter basis). Zn content was not related to variety of potato and wide variations occurred in every area sample. No visible evidence of Zn deficiency occurred in any of the plants analysed. A. H. Cornfield.

Storage stability of vitamin C in potato granules at various moisture levels. E. Kissmeyer-Nielsen (*Amer. Potato J.*, 1956, **33**, 353—354).—Loss of vitamin C from potato granules stored at 25° for two months increased with moisture content of the granules (11% to 15% water). A. H. Cornfield.

Potassium for subterranean clover. E. N. Fitzpatrick and T. C. Dunne (*J. Agric. W. Aust.*, 1956, **5**, 321—326).—Symptoms of K deficiency in subterranean clover are described and illustrated. Application of K to K-deficient soils increased the yields of forage, clover and protein. Max. yields were obtained with 112—224 lb. of KCl per acre. Yields of clover were very low where no K was applied, even on areas which had been re-seeded with the legume. A. H. Cornfield.

Effect of fertilizers on protein content of guinea grass. M. B. Jamil (*Malayan agric. J.*, 1956, **39**, 121—132).—Protein (%) in guinea grass increased almost linearly with applications of $(NH_4)_2SO_4$ (2—10 cwt. per annum). Protein yields per acre increased to the greatest extent with the first 2-cwt. dressing but to lesser extents with further dressings. The % of protein was not significantly different whether an NPK fertilizer was split into 2, 3, 6 or 12 applications over the year. A. H. Cornfield.

Molybdenum content of pasture after top-dressing with molybdates. I. J. Cunningham and K. G. Hogan (*N.Z. J. Sci. Tech.*, 1956, **38 A**, 248—267).—The effect of top-dressing on the Mo content of pasture varied markedly with soil type. When MoO_4'' (2 oz. per acre) was applied to pastures which had normal Mo contents, seven of 64 North Island soils gave abnormally high response to the Mo, whereas none of 50 South Island soils showed a similar effect. The amount of Mo in the pasture was directly related to the amount applied, but on responsive soil the increase was much higher and more persistent. Perennial ryegrass took up slightly more Mo than did white clover. The response of the soil must be considered before Mo is applied, so as to avoid Mo levels that might be toxic to grazing stock. Responsive soil types are identified. $CoSO_4$ top-dressings (5 and 10 oz. per acre) did not affect the Mo content of pasture. Appreciable transfer of Mo from top-dressed to adjacent plots occurred in the excreta of grazing sheep. (10 references.) R. H. Hurst.

Copper and molybdenum contents of pastures grown on the different soils of New Zealand. I. J. Cunningham, K. G. Hogan and J. N. Breen (*N. Z. J. Sci. Tech.*, 1956, **38, A,** 225—238).—Analyses of 900 pasture samples collected in spring from 280 soils in the North and South Islands indicated that relatively small areas in both Islands are deficient in Cu (<5 p.p.m.) and that in North Island large areas (approx. 5 million acres) and smaller areas in the South Island have above-normal contents of Mo ($\nless 3$ p.p.m.). R. H. Hurst.

Pasture responses to potassium, molybdenum and phosphorus on Cressy shaley clay loam. D. F. Paton (*Tasmanian J. Agric.*, 1956, **27**, 329—338).—On soil which had previously been cropped for many years marked increases in yields of both herbage and clover were obtained over two years by applying superphosphate (1—3 cwt.), NH_4 molybdate (4 oz.) and KCl (1 cwt. per acre). $Ca(OH)_2$ (1 ton per acre) had no effect on yields. On a previously virgin area yield responses were obtained only by application of superphosphate. A. H. Cornfield.

Grazing capacity of wiregrass-pine ranges in Georgia. L. K. Halls, O. M. Hale and B. L. Southwell (*Georgia agric. Exp. Sta.*, 1956, Tech. Bull. N.S. 2, 38 pp.).—A study was made of the relationships between the kinds and amounts of herbage, shrubs, pine tree overstory and grazing capacity of burned wiregrass range over five years. A. H. Cornfield.

Effect of steam-sterilized soil on growth of re-plant apple trees. E. G. Bollard (*N.Z. J. Sci. Tech.*, 1956, **38A**, 412—415).—Steam-sterilization of soil permitted as much growth of Northern Spy stock as in fresh soil. In certain cases, soil sterilization appeared to improve growth. No parasitic attack on tree roots in either unsterilized or sterilized soil was observed. J. S. C.

Soils and manures for fruit. T. Wallace (*Minist. Agric. Lond.*, 1956, Bull. 107, 41 pp.).—Natural soil conditions of texture, depth, degree of weathering, drainage and mineral content are important in determining successful growth of fruit crops. Suitable manurial dressings are suggested for particular crops (apples, pears, plums, cherries, blackcurrant, redcurrant, gooseberries, raspberries and strawberries). R. H. Hurst.

Excess soluble salts in glasshouse tomato soils. K. J. McNaught and B. J. Houson (*N.Z. J. Sci. Tech.*, 1956, **38A**, 449—465).—Osmotic pressures of saturation extracts of glasshouse tomato soils were correlated with wt. of salts and conductivities. The effects of soil/water ratios on the amounts of dissolved ions were studied over a wide range. Conductivity determinations are a more reliable index of osmotic effects than salt-wt. figures. Results are discussed in relation to current glasshouse practices in this respect. (23 references.) J. S. C.

Self-incompatibility of flax (*Linum usitatissimum*, **L.**). H. Börner and B. Rademacher (*Z. PflErnähr. Düng.*, 1957, **76**, 123—132).—Flax grown in nutrient solution in which flax has previously been grown exhibits at 26 days a decrease in shoot dry wt. and root and shoot length whilst root dry wt. is not significantly different from the control. After 76 days dry shoot wt. and shoot length are reduced 28·5% and 13% respectively. No pathogenic microorganisms appear which could explain the results. M. Long.

Use of lime on flue-cured tobacco. R. Thomson, J. Watson and R. Monk (*N.Z. J. Sci. Tech.*, 1956, **38A**, 299—308).—In a trial on clean soil where black root-rot is not a factor, the application of ½ ton of ground limestone per acre every second year increased the yield of flue-cured tobacco without delay in maturity or reduction in quality. The pH of the soil was raised to a degree undesirable in soils where the fungus is present; above pH 5·6 the disease becomes more active. Exchangeable Ca was increased but exchangeable K and Mg were little affected. Sol. ash and CaO contents were the only mineral constituents of the cured leaves to be affected significantly. Total reducing sugars and N were not affected by liming. Seasonal variations in the chemical composition of the leaves were marked. R. H. Hurst.

Boron and flue-cured tobacco. R. Thomson (*N.Z. J. Sci. Tech.*, 1956, **38A**, 326—331).—The addition of borax (5 lb. per acre) to flue-cured tobacco fertilizer caused some increase in yield and marked increase in leaf quality; 10 lb. per acre delayed early growth and subsequent maturity of the crop. The effect of B on the crop was apparently correlated with rainfall at planting time. The use of borax had little or no influence on reducing sugars and N contents of the leaves. The amount of borax used increased proportionally the B content of the leaves. Seasonal effects on N and B levels in leaf were noted. R. H. Hurst.

Sugar content of maple syrup. F. H. Taylor (*Vermont agric. Exp. Sta.*, 1956, Bull. 587, 39 pp.).—The % of sugar in maple sap varied widely from tree to tree even in the same bush. Sap sugar (%) in a given tree varied with season, although tree-to-tree variation between neighbouring trees was maintained throughout the season. Sap sugar (%) was low early in the season, rose to a max., and then decreased towards the end of the season. A. H. Cornfield.

Pest Control

Colorimetric determination of lindane [γ-benzene hexachloride] in soils and crops. E. P. Lichtenstein, S. D. Beck and K. R. Schulz (*J. agric. Food Chem.*, 1956, **4**, 936).—A method, based on that of Schechter and Hornstein (*Analyt. Chem.*, 1952, **24**, 544), is described for the determination of γ-benzene hexachloride directly in crops and in soils with both high and low org. contents. S. C. Jolly.

Fate of aldrin and dieldrin in the animal body. J. M. Bann, T. J. DeCino, N. W. Earle and Yun-Pei Sun (*J. agric. Food Chem.*, 1956, **4**, 937—941).—Aldrin administered orally or parenterally is converted largely and rapidly (within 24 hr.) to dieldrin in beef and dairy cattle, pigs, sheep, rats and poultry, and the dieldrin is stored unchanged in the animal body. (28 references.) S. C. Jolly.

Soil fumigation. IV. Sorption of ethylene dibromide on soils at field capacity. V. Diffusion of ethylene dibromide through soils. F. Call (*J. Sci. Food Agric.*, 1957, **8**, 137—142, 143—150).—IV. Sorption of ethylene dibromide on 20 soils of varied texture, at moisture contents corresponding to field capacity, and in presence of air, was studied. Isotherms were linear and the sorption coeff. (slope of the isotherm) could be correlated with surface area, with org. matter-, with moisture-, and less closely with clay content; the sorption coeff. could be predicted with reasonable accuracy from the moisture content alone. Most of the ethylene dibromide within the soil is in the sorbed state; for a typical field soil horizon A_2 at a porosity of 0·250 only 1·1% is in the vapour phase. (17 references.)

V. In the diffusion of ethylene dibromide vapour through soils of different types, both unsteady- and steady-state coeff. agree with calculated values from known soil properties. Blocked pores are an important factor in the steady-state and sorption of vapour on the soil has great influence on the unsteady-state coeff. (17 references).

E. M. J.

Use of heptachlor in soil treatment. J. Lhoste, J. d'Aguilar and J.-L. Gérard (*C. R. Acad. Agric. Fr.*, 1956, **42**, 784—788).—Field trials show that heptachlor applied to soil is very active against larvæ of *Agriotes* spp. The extent to which the insecticide penetrates the plant to form toxic residues was also investigated and it is concluded that only rapidly-growing roots are seriously affected.

J. S. C.

Physicochemical studies of pesticides. I. Aggregation phenomena in DDT suspensions. G. T. Barnes and A. E. Alexander. **II. Effect of salts and clays on stability of DDT suspensions.** D. K. O'Neill and A. E. Alexander (*Proc. 2nd int. Congr. Surface Activity, Lond.*, 1957, Prepr. 1, BJ 334—340; BK 462—468).—I. When suspensions of DDT stabilized by sulphated butyl oleate (dips to control cattle tick) are shaken there is a slow deposition of about $\frac{3}{4}$ of the DDT. The rate of deposition is greatly increased by shaking in presence of fibres. By measurements with hair, wool, glass and glass made hydrophobic, it is shown that only hydrophobic fibres are effective. The effect is attributed to an increase in the rate of aggregation of micro-crystals on the surface of the fibre.

II. Experiments with $CaCl_2$, $MgCl_2$ and "Calgon" show that deposition is retarded by electrolytes, there being a different optimum concn. for each salt, above which DDT is salted out. The stabilizing effect is attributed to increased surface activity of the stabilizer. Most clays also increase the stability: about 0.2 to 0.5% of bentonite is effective. Soils behave similarly to clays, but are less active.

A. B. Densham.

Structure and insecticidal activity of some diethyl-substituted phenyl phosphates. T. R. Fukuto and R. L. Metcalf (*J. agric. Food Chem.*, 1956, **4**, 930—935).—Excellent correlation was found between inhibition of insect choline-esterase (*C*) by 24 diethyl-substituted phenyl phosphates and lability of the P–O–aryl bond as measured by hydrolytic stability, i.r. stretching frequencies and Hammett's σ values, although steric factors apparently operate in some cases; there was some correlation between *C* and insect toxicity. Insect metabolism, steric factors and ionic charges appear to influence insecticidal activity. Several new compounds, including diethyl *p*-cyanophenyl phosphate and diethyl *p*-methylthiophenyl phosphate and its sulphoxide and sulphone oxidation products, which have high σ values, have high insecticidal activity. (27 references.)

S. C. Jolly.

Fungicidal activities of some aryloxyacethydroxamic acids. Z. Eckstein and T. Urbański (*Bull. Acad. polon. Sci.*, 1956, III, **4**, 627—630).—A number of aryloxyacethydroxamic acids (**I**) were prepared by action of excess of NH_2OH and NaOMe on a methanolic solution of the Et ester of the aryloxyacetic acid, and their properties are presented. **I** are colourless cryst. substances giving in alcoholic solutions a colour reaction with $FeCl_3$ typical for hydroxamic acids. They form green chelate compounds with Cu salts. Results are given for the fungicide activity of 22 deriv. of **I** against *Fusarium culmorum*, *Rhizoctonia solani* and *Alternaria solani*. The activity is also compared with that of the fungicides Spergon and *p*-chloro-*m*-cresol. Relations between the activity of the compounds and their chemical structure are discussed.

R. J. Magee.

Active principles of Brazilian liana. III. J. R. Pucci and P. Philipp (*An. Ass. brasil. Quím.*, 1952 [1956], **11**, 181—185).—The determination of rotenone in Brazilian liana was carried out by three methods, namely, the A.O.A.C. "Official and Tentative Methods of Analysis", 6th Edn., 1945, p. 74, the volumetric method, and a rapid method. Four samples were used and results were compared. Reasonable concordance was obtained between the various methods; any major difference was due to the presence of appreciable quantities of resin. The presence of resin partially influenced the rapid method and it was found necessary to prolong the time of extraction.

H. Pritchard.

Effects of pea root exudates on physiologic races of *Fusarium oxysporum* Fr. f. *pisi*, (Linf.) Snyder & Hansen. E. W. Buxton (*Trans. Brit. mycol. Soc.*, 1957, **40**, 145—154).—Three pea varieties showed differential resistance to three physiologic races of *F. oxysporum* f. *pisi* and the exudates from their roots affect spore germination of the three races by depressing the germination of the races to which they are resistant more than that of the races to which they are susceptible. In roots inoculated with a race they resist, mycelium developed only in the outer cortex. Simultaneous infection with *F. solani* and *F. oxysporum* reduced the wilting due to the *F. oxysporum* whilst extracts of pea stems infected with *F. solani* inhibited germination of *F. oxysporum* spores.

L. G. G. Warne.

***In vitro* effects of mycothricin on plant pathogenic bacteria and fungi.** G. Rangaswami (*Mycologia*, 1956, **48**, 800—804).—This antibiotic inhibited the growth of seven bacteria and six fungi tested. Inhibition of growth requires a greater concn. of the antibiotic than does inhibition of spore germination.

L. G. G. Warne.

Interpretation of results from a soil fumigation trial. J. G. Bald and R. N. Jefferson (*Plant. Dis. Reptr*, 1956, **40**, 840—846).—Methods of interpreting results obtained in studies of the control of gladiolus diseases and pests with Vapam (Na methyldithiocarbamate) and fungicides are discussed.

A. H. Cornfield.

Source of controlled vacuum for pipetting nematodes. H. W. Ford (*Plant. Dis. Reptr*, 1957, **41**, 89—90).—An apparatus is described for applying constant controlled suction to a micro-pipette for selection of desired nematodes from a dish of mixed specimens.

A. H. Cornfield.

Determination of *OO*-diethyl *O*-*p*-nitrophenyl thiophosphate [parathion] by column chromatography. W. Paulus and H. J. Mallach (*Arzneimittel-Forsch.*, 1955, **6**, 766—767).—The adsorption of parathion (E605) (**I**) on 18 substances was investigated with a variety of solvents. Suitable adsorbents include alumina, active carbon, Florisil and silica gel. **I** is most strongly adsorbed from benzene or light petroleum solutions and most readily eluted by ether or $CHCl_3$. Column chromatography is recommended for application in the purification and analysis of **I**.

A. R. Rogers.

2 : 3 : 5-Triphenyltetrazolium chloride for obtaining pathogenic types from stock cultures of halo blight, *Pseudomonas phaseolicola*, and common blight, *Xanthomonas phaseoli*, organisms. B. C. Smale and J. F. Worley (*Plant Dis. Reptr*, 1956, **40**, 628).—Red colonies of *P. phaseolicola*, from 12-month-old stock cultures, which developed in potato–dextrose agar containing 0·005% of tetrazolium salt were highly pathogenic whilst white colonies were weakly pathogenic. Although individual colonies of *X. phaseoli*, developed in the same way, displayed colour variations, the degree of pathogenity was not related to colour. Organisms of both species freshly isolated from infected plants nearly all produced red colonies.

A. H. Cornfield.

Hyperparasitic chain of a fungus, a bacterium and its phage on wheat. Z. Klement and Z. Király (*Nature, Lond.*, 1957, **179**, 157—158).—The bacterial hyperparasite of the rust fungi, *Puccinia* spp., reported in America by D. S. Pon *et al.* (*Phytopath.*, 1954, **44**, 707) has now been found in Hungarian rust populations. The suggested name for this organism is *Xanthomonas uredovorus*. A bacterial virus which is parasitic on this organism has also been isolated. The host–parasite complex represents a hyperparasitic chain, beginning with the wheat as a tertiary host and terminating in the phage as tertiary parasite.

J. S. C.

Regional seed-treatment tests for control of common smut of winter wheat, 1955. L. H. Purdy (*Plant Dis. Reptr*, 1956, **40**, 996—1000).—Results of tests with a large variety of materials are reported.

A. H. Cornfield.

Insecticide–fungicide seed treatments for control of seed-maize maggot, *Hylemya cilcrura*, Rondani, and seed decay organisms. R. N. Hofmaster and T. J. Nugent (*Virginia Truck Exp. Sta.*, 1956, Bull. 116, 31 pp.).—Satisfactory control of the maggot and of seed decay organisms was obtained by treating each bushel of seed with Arasan SF-X (2 oz.) or Orthocide 75 (0·66 oz.) plus aldrin, dieldrin, heptachlor or γ-$C_6H_6Cl_6$ (0·25 oz.) in 3% aq. Methocel (sticker).

A. H. Cornfield.

Control of parasitic nematodes of maize by soil fumigation. J. Y. Oakes, C. N. Bollich, D. R. Melville, M. J. Fielding and J. P. Hollis (*Plant Dis. Reptr*, 1956, **40**, 853—854).—Good control of nematodes was obtained by application of Dowfume MC2 (98% methyl bromide + 2% chloropicrin; 2 lb. per 100 sq. ft.) or DD (25 gal. per acre) but not by Dowfume W-85 (ethylene dibromide; 7·5 gal. per acre). Yields of maize sown six weeks later were increased by all treatments, DD being the most effective.

A. H. Cornfield.

Control of bacterial wilt of sweet maize with antibiotics and Tween 20. J. L. Dockwood and L. E. Williams (*Plant Dis. Reptr*, 1956, **40**, 622—625).—Application of streptomycin (100 p.p.m.) or Terramycin (50 p.p.m.) foliage sprays reduced the incidence and severity of bacterial wilt of sweet maize at two locations but had no effect on yields. Tween-20 (a wetting agent) at 1000 p.p.m. (concn. normally used) decreased disease incidence at one location only. At 10,000 p.p.m. Tween-20 as a foliage spray or at 3000 p.p.m. as a spray directed into the whorls reduced disease incidence but was severely phytotoxic. A. H. Cornfield.

Control of the autumn armyworm, *Laphygma frugiperda*, (S.), in maize in Colombia. R. F. Ruppel, M. Benavides and A. Saldarriaga (*Plant Prot. Bull.*, 1957, **5**, 69—74).—The budworm form of the autumn armyworm in young maize was controlled by sprays of toxaphene (2 kg.), aldrin (0·5 kg.), Isodrin (0·5 kg.), endrin (0·25 kg.) or parathion (0·25 kg. per hectare). Heptachlor (0·5 kg. per hectare) and a 5% toxaphene bait failed to give complete mortality, but final maize yields were as high as with the other treatments. Aldrin and Isodrin were slightly phytotoxic. A. H. Cornfield.

Control of root-parasitic nematodes of rice in Texas and Louisiana. J. G. Atkins, M. J. Fielding and J. P. Hollis (*Plant Prot. Bull.*, 1957, **5**, 53—56).—Of a no. of materials tested as soil fumigants, Dowfume MC-2 (98% methyl bromide, 3 lb. per 100 sq. ft., applied one month prior to sowing) gave the best control of nematodes and increased the yields of rice to the greatest extent. A. H. Cornfield.

Control of straighthead of rice. N. S. Evatt and J. G. Atkins (*Plant Dis. Reptr*, 1957 ,**41**, 103—104).—Soil applications of Fe or Zn Versenates or foliage applications of Fe, Zn or Mn Versenates did not reduce the incidence of straighthead (probably a physiological disease) in rice. Application of " Feralum " (mixed Fe and Al sulphates, 1000—2000 lb. per acre) to the soil prior to flooding reduced the incidence of straighthead and doubled the yield of grain. A. H. Cornfield.

Control of the " dicky rice " weevil, *Maleuterpes spinipes*. E. H. Zeck (*Agric. Gaz. N.S.W.*, 1956, **67**, 430—431).—The pest was controlled with 0·05% DDT sprays in the spring or with cryolite (1·25 lb. + 1 quart white oil emulsion in 40 gal. per acre) when the weevils were active. A. H. Cornfield.

Potato haulm destruction with chemicals. Anon. (*Tasmanian J. Agric.*, 1956, **27**, 431).—Of a no. of materials tested pentachlorophenol (3·0—7·5 lb. in 30—100 gal. per acre) was the most effective and economical for killing potato haulms. A. H. Cornfield.

Organic phosphorus compounds as soil insecticides for control of sugar-beet root maggot. W. L. Gojmeras (*Plant Prot. Bull.*, 1957, **5**, 59—60).—When mixed with sugar-beet seed prior to sowing a number of org. P insecticides were ineffective in controlling insect attacks. Most of the materials reduced seed germination. A. H. Cornfield.

Control of lucerne flea and red-legged earth mite with malathion. A. J. K. Walker (*J. Agric. S. Aust.*, 1956, **59**, 386—387).—The lucerne flea was controlled by application of malathion (0·5 oz. per acre) 3—4 weeks after rains had opened the eggs. The red-legged earth mite was controlled by DDT (1 oz.), malathion (0·5oz.) + DDT (1 oz.), or γ-$C_6H_6Cl_6$ (2 oz. per acre) as soon as serious damage was apparent. A. H. Cornfield.

Effect of MCPB (sodium 2-methyl-4-chlorophenoxybutyrate) on young red clover (*Trifolium pratense*) and weeds. D. B. Edmond (*N.Z. J. Sci. Tech.*, 1956, **38A**, 397—402).—The effects of heavy rates (1—6 lb. acid equiv./acre) of MCPB on an association of young red clover and weeds was investigated in comparison with MCPA (1 lb. acid equiv./acre). MCPB did not significantly affect red clover yield but the heavier applications caused leaf scorch and petiole distortions. MCPA reduced clover yield: both chemicals reduced weeds. MCPB was slower and less effective than the equiv. MCPA, 1 lb. of the latter being about equal to 2 lb. of the former in affecting weeds. A list of susceptible and non-susceptible weeds is given. J. S. C.

Bacterial-induced chlorosis in soya-beans. P. R. Miller (*Plant Prot. Bull.*, 1956, **5**, 27).—Symptoms of the chlorosis, which appears to be associated with certain rhizobial inoculants, are described. A. H. Cornfield.

Bean, subterranean clover and lupin diseases caused by the bean yellow mosaic virus in Western Australia. H. L. Harvey (*J. Agric. W. Aust.*, 1956, **5**, 329—336).—Symptoms of the diseases in the various species are described and illustrated. A. H. Cornfield.

Control of fruit insects and diseases. L. A. Stearns, J. W. Heuberger and R. F. Stevens (*Delaware agric. Exp. Sta.*, 1956, Ext. F.44, 8 pp.).—The chemical control of insects and diseases of fruit trees and bushes is described. A. H. Cornfield.

Control of black spot of apple and pear. Anon. (*Agric. Gaz. N.S.W.*, 1956, **67**, 425—427).—Application of 0·1% HgPh chloride immediately following harvest in two successive years, with protective sprays in the intervening season, virtually eliminated black spot. A. H. Cornfield.

Control of the apple curculio (weevil), *Otiorrhynchus cribricollis*, Gylh. D. G. Shedley (*J. Agric. W. Aust.*, 1956, **5**, 599).—Of a number of materials tested, butt sprays of 0·5% dieldrin or γ-$C_6H_6Cl_6$ or foliage sprays of 0·05% dieldrin gave the best control. A. H. Cornfield.

Spray injury on apples in Tasmania. L. W. Miller (*Tasmanian J. Agric.*, 1956, **27**, 347—354).—Injury due to certain pesticides and solvents and as related to weather conditions, stage of growth, and health and variety of tree are described. A. H. Cornfield.

Control of brown rot of apricots. G. C. Wade (*Tasmanian J. Agric.*, 1956, **27**, 317—328).—Thiram (1·5 lb. of 80% concn. per 100 gal.) and Manam (Mn ethylene bisdithiocarbamate, 1 pint of 42% slurry per 100 gal.) controlled brown rot of apricots. Bordeaux mixture, $CuOCl_2$, lime-S, S, chloranil, dichlone, nabam + $ZnSO_4$ and ziram caused foliage or fruit injury, left undesirable residues, or were ineffective. Spraying between full bloom and shuck fall stages was necessary for good control. A. H. Cornfield.

Control of bull's-eye rot, *Neofabræa malicorticis* or *N. perennans*, on apple and pear fruit. J. R. Kienholz (*Plant Dis. Reptr*, 1956, **40**, 872—877).—The disease was controlled by spraying with ziram (1·5 lb. per 100 gal.) in the first cover spray for codling moth control followed by another application before autumn rains. Additional sprays were necessary when rainfall was excessive. A. H. Cornfield.

Systemic control of cherry leaf spot fungus by foliar sprays of actidione derivatives. J. M. Hamilton, M. Szkolnik and E. Sondheimer (*Science*, 1956, **123**, 1175—1176).—The oxime derivative of actidione at a concn. of 60 p.p.m. is effective as a systemic agent for the control of the fungus. The isomer, acetate and semicarbazone derivatives were effective systemically at 10 p.p.m. and conferred some degree of protection at 0·5 p.p.m. T. G. Morris.

Colorado peach mosaic suppression programme. G. M. List, N. Landblom and M. A. Sisson (*Colorado agric. Exp. Sta.*, Tech. Bull. 59, 28 pp.).—Suppression of the disease by examination of orchards and removal of diseased trees over 20 years is described. A. H. Cornfield.

Fungus diseases of citrus. J. R. Botham (*J. Agric. S. Aust.*, 1956, **59**, 501—506).—Diseases affecting the fruit, twigs and branches, and trunk and main roots and methods of control are described. A. H. Cornfield.

Control of anthracnose, *Colletotrichum glœsporioides*, Penz., on rough lemon seedlings. F. E. Fisher (*Plant Dis. Reptr*, 1957, **41**, 77—78).—Ferbam (2·5 lb. per 100 gal. applied every 7—10 days from Sept. 23 to Dec. 2) gave better control of anthracnose and resulted in better growth of lemon seedlings than did captan (2·5 lb.) or " Tribasic Cu " (1 lb. 50% per 100 gal.). In another year ferbam (1·5—2·0 lb.) and zineb (1·5 lb.) gave better control of both anthracnose and scab, *Elsinoe fawcetti*, than did captan (2 lb.) or $CuSO_4,5H_2O$ (2 lb. per 100 gal.). A. H. Cornfield.

Control of the Oriental red mite, *Anychus orientalis*, Zach., on citrus seedlings in Jordan. B. J. Klapperich (*Plant Prot. Bull.*, 1957, **5**, 57—58).—Watering the rooting zone of citrus seedlings with 0·1—0·2% Systox prevented attack of the leaves by the red mite for 26—41 days after treatment. A. H. Cornfield.

Chemicals screened for systemic effects against spreading decline disease of citrus. H. W. Ford (*Plant Dis. Reptr*, 1956, **40**, 861—865).—None of the 133 chemicals tested and listed for systemic effects against spreading decline disease [due to the burrowing nematode, *Radopholus smilis*, (Cobb.) Thorne] of citrus showed any promise of control, based on stimulation of new shoot growth by affected trees. A. H. Cornfield.

Control of tree-hoppers. E. H. Zeck (*Agric. Gaz. N.S.W.*, 1956, **67**, 428—430).—On citrus trees tree-hoppers were controlled by spraying in the spring with 0·02% parathion, 0·02% γ-$C_6H_6Cl_6$, or 0·05% DDT. On peaches leaf-hoppers were controlled with 0·1% DDT in the latter half of Oct. A. H. Cornfield.

Control of the Mediterranean fruit fly. C. F. H. Jenkins and D. G. Shedley (*J. Agric. W. Aust.*, 1956, **5**, 341—351).—The pest is controlled by spraying the trees with Na_2SiF_6 bait (Na_2SiF_6 1 oz. + white sugar 2·5 lb. per 4 gal. sprayed on 40 normal-sized trees or 100 vines) every six days commencing at least six weeks before the crop is ready for picking until two weeks after the trees have been stripped. The bait has given better control than have the org. insecticides. A. H. Cornfield.

Cercospora leaf spot (*Cercospora musæ*) of bananas. Anon. (*Agric. Gaz. N.S.W.*, 1956, **67**, 645—646).—Cercospora leaf spot of bananas was controlled by spraying monthly from Jan. to May with $CuOCl_2$ (1·5 lb.) + colloidal S (1 lb. per 40 gal.). A. H. CORNFIELD.

Fusarium wilt of bananas. I. Behaviour of *F. oxysporum* f. *cubense* in different soils. R. H. Stover (*Canad. J. Bot.*, 1956, **34**, 927—942).—Techniques used to study the behaviour of *F. oxysporum* in soil included : soil dilution plates, soil plates and buried slides. Differences between population counts in loam and sandy loam were attributed to a defect in the soil dilution plate method. Sporulation was greater in soils dried before plating than in soils maintained at 15—35% saturation ; it was greater in acid sandy loam than in alkaline loam, and was abundant below pH 7·0 in all soils. The optimum pH for sporulation in acidified loam was about 5·0, and in sandy loam from 5·0—7·0. In general, the rate of spread of disease in newly planted soils was correlated with soil texture, pH, and amount of growth and sporulation in soil plates and buried slides. The soil rather than the host probably exerts the primary influence on the fungus. (16 references.) R. H. HURST.

Control of pecan scab, *Cladosporium effusum*, (Wint.) Demaree. R. H. Converse (*Plant Dis. Reptr*, 1956, **40**, 870—871).—A fair degree of control was obtained with Puratized Agricultural Spray [7·5% tris (2-hydroxyethyl)(phenylmercuri)ammonium lactate ; 2·5 pints per 100 gal.] and Santobrite (75% Na pentachlorophenoxide 4·7 lb.) + Dormant Spray Oil No. 1 (1 gal. per 100 gal.). A. H. CORNFIELD.

Control of walnut blight, *Xanthomonas juglandis*, with agrimycin and copper compounds. P. W. Miller (*Plant Dis. Reptr*, 1956, **40**, 626—627).—Agrimycin (streptomycin 15 + Terramycin 1·5%) at concn. 100 p.p.m., Yellow Cuprocide (2 lb.) and Copper A Compound (4 lb. per 100 gal.) sprays were all equally effective in controlling walnut blight. Yellow Cuprocide caused a moderate amount of foliage damage. A. H. CORNFIELD.

Insect and disease control on vegetables. D. MacCreary, J. W. Heuberger and R. F. Stevens (*Delaware agric. Exp. Sta.*, 1956, Ext. F. 45, 8 pp.).—Methods of controlling insects and diseases on a large range of vegetables are described. A. H. CORNFIELD.

Fungicides and glycerin for control of cucumber scab, *Cladosporium cucumerinum*. S. Rich (*Plant Dis. Reptr*, 1956, **40**, 620—621).—Captan and zineb (2500 p.p.m.) sprays reduced the incidence of cucumber scab, whilst streptomycin (200 p.p.m.) had no effect. The addition of 1% of glycerin to the sprays increased the chemotherapeutic effect of zineb but had no effect on that of captan or streptomycin. Glycerin alone was ineffective. A. H. CORNFIELD.

Fungicide screening for control of tomato leaf-mould. H. Jacks and A. J. Webb (*N.Z. J. Sci. Tech.*, 1956, **38A**, 342—344).—Of 17 fungicides tested in the glasshouse for control of *Cladosporium fulvum*, Cke, the most effective were : dichlone, zineb, captan, salicylanilide and chloranil. R. H. HURST.

Effect on *Meloidogyne hapla* of excised tomato roots treated with α-methoxyphenylacetic acid. W. Wieser (*Science*, 1956, **123**, 374—375).—A foliar spray of α-methoxyphenylacetic acid induces in tomato roots the formation of an agent, repellent to the larvæ of *M. hapla*, that persists in the distal portion of the root after it has been excised and left in wet sand for 24 hr. T. G. MORRIS.

Control of weeds in padi fields. P. G. Coleman, D. W. M. Haynes and J. L. B. Hitchcock (*Malayan agric. J.*, 1956, **39**, 191—199).—Hormone-type herbicides (MCPA, 2 : 4-D, and 2 : 4 : 5-T) applied in various formulations at 1—3 lb. acid-equiv. per acre controlled 6 out of 10 species of weed commonly found in padi fields. Results were similar with all materials and indications were that less than 1 lb. acid-equiv. per acre would be effective. Rice grain yields were increased by all treatments. A. H. CORNFIELD.

Control of St. John's wort, *Hypericum perforatum*, L. var. *augustifolium* D.C. G. R. W. Meadly (*J. Agric. W. Aust.*, 1956, **5**, 661—664).—Cultural and biological methods of control are described. The weed was controlled by application of 2 : 4-D ester (3 lb. acid-equiv. per acre). A. H. CORNFIELD.

Effect of some substituted methylureas on the respiration of *Chlorella vulgaris* var. *viridis*. M. J. Geoghegan (*New Phytologist*, 1957, **56**, 71—86).—3-*p*-Chlorophenyl-1 : 1-dimethylurea and 2 : 4-dinitrophenol inhibit the growth of *Chlorella*, but less in the presence than in the absence of glucose. Endogenous respiration and the respiration of low concn. of glucose, fructose and glucose-1-phosphate and of pyruvic, acetic, succinic, fumaric, malic and citric acids are stimulated. L. G. G. WARNE.

Crop spraying for weed control in cereals. H. E. Orchard (*J. Agric. S. Aust.*, 1956, **59**, 458—462, 465).—Application rates and time of spraying of hormone-type weed killers in relation to stage of plant development and weather conditions are discussed. A. H. CORNFIELD.

Control of the spear thistle, *Cirsium vulgare*, Ten. H. E. Orchard (*J. Agric. S. Aust.*, 1956, **59**, 491—492).—The weed was controlled by spraying with 2 : 4-D or MCPA (1 lb. active ingredient per acre), 10% $NaClO_3$, 3% As_2O_5, or 2·5% Na_2AsO_3. A. H. CORNFIELD.

Control of thornapples, *Datura*, spp. G. R. W. Meadly (*J. Agric. W. Aust.*, 1956, **5**, 289—295).—Thornapples were relatively resistant to hormone-type weedkillers but were controlled with 5% $NaClO_3$. A. H. CORNFIELD.

Control of Cape weed. Anon. (*Tasmanian J. Agric.*, 1956, **27**, 312).—Cape weed was controlled by spraying with 2 : 4-D or MCPA (1 lb. per acre) in autumn or early spring. A. H. CORNFIELD.

Control of Berkheya (Hamelin) thistle, *Berkhaya rigida*, Thunb. Bol. and W. Dod. G. R. W. Meadly (*J. Agric. W. Aust.*, 1956, **59**, 453—455).—The thistle was controlled by applying 7·5% aq. $NaClO_3$ or Na_3AsO_3 (200 gal.) or 2 : 4-D ester (2 lb. acid-equiv. per acre). A. H. CORNFIELD.

Injury to plants by hormone herbicides. Anon. (*Agric. Gaz. N.S.W.*, 1956, **67**, 422—425).—Symptoms of injury by hormone herbicides to a no. of plants are described and illustrated. A. H. CORNFIELD.

Black root-rot, *Phytophthora cactorum* (Leb. and Cohn) Schroet, of the hop plant. T. Christie (*N.Z. J. Sci. Tech.*, 1956, **38A**, 277—284).—Good resistance to the disease is shown by the Fuggle variety, and to a less degree, by the Bumford and Golding varieties. The Californian variety is highly susceptible. The hop strain of *P. cactorum* grows fairly uniformly between pH 4·7 and 7·4. At pH 8·7 growth is restricted and ceases at pH 3·0. In soil sterilization tests no chemical gave complete control of the disease. Some degree of control was shown in early tests by DD (dichloro-propene and -propane) and chloropicrin, but this was not shown in later tests. Little or no control was obtained by : Capsine, Agrosan, $KMnO_4$, Hg_2Cl_2 and NaCl, CH_2O, NaCl, K_2SO_4, CS_2, fermate, Shirlan W.S., Cuprox, $CaCN_2$, $K_2Cr_2O_7$, Dithane Z78, Dithane D14, CuSO, or C.B.P. (chlorobromopropene). R. H. HURST.

Control of the brinjal shoot and fruit borer, *Leuconides orbonalis*, Guen. S. N. Banerjee and N. N. Basu (*Plant Prot. Bull.*, 1956, **5**, 7—8).—Of a no. of materials tested, 0·08% endrin, sprayed 3—4 weeks after transplanting and subsequently at intervals of 2—3 weeks, gave the best control of the pest. 0·5—1·0% DDT sprays were also effective, but were more expensive than endrin. A. H. CORNFIELD.

Senn pest, *Eurygaster integriceps*, in the Near East. C. Logothetis (*Plant Prot. Bull.*, 1956, **5**, 21—25).—A general account of the economic importance, life history and measures for control of the pest. A. H. CORNFIELD.

New disease of plantain. F. A. del Prado (*Plant Prot. Bull.*, 1956, **5**, 29—30).—A browning and rotting of rolled young central leaves of plantain is described. The disease is favoured by high humidity and usually occurred on plants showing low vigour due to poor drainage or nutrition or other factors. Several species of fungi and bacteria have been isolated from the diseased tissue. A. H. CORNFIELD.

Control of wattle tree insects. E. H. Zeck (*Agric. Gaz. N.S.W.*, 1956, **67**, 369—371, 377).—Insects attacking wattle trees are described. Caterpillars, leaf-eating beetles and their larvæ, leaf insects, and froghoppers were controlled with DDT (1 pint 20% emulsion per 25 gal.) or $PbHAsO_4$ (0·6 oz. per gal.). Scales and mealybugs were controlled with white oil emulsion (4 fl. oz. per gal.). A. H. CORNFIELD.

Control of coconut beetles and weevils in Thailand. E. F. Vestal (*Plant Prot. Bull.*, 1956, **5**, 37—44).—The red palm weevil, *Rhynchoplorus ferrugineus*, was controlled on young palms by spraying with 0·1% dieldrin. Mature trees infested with weevils and coconut beetles must be burned to prevent spread of the infestation. A. H. CORNFIELD.

Eradication of the khapra beetle, *Trogoderma granarium*, Evarts. D. R. Shepherd (*Plant Prot. Bull.*, 1957, **5**, 75—79).—A general account of the control of the pest in North America by methyl bromide fumigation. A. H. CORNFIELD.

Eradication of the Argentine ant. P. N. Forte (*J. Agric. W. Aust.*, 1956, **5**, 619—625).—A general account of the campaign for eradicating the Argentine ant in Western Australia. A. H. CORNFIELD.

Control of Paterson's curse, *Echium plantagineum*, L. and *E. italicum*, L. G. R. W. Meadly (*J. Agric. W. Aust.*, 1956, **5**, 549—552).—The weed was controlled in pastures by application of 2 : 4-D ester (1—2 lb.) and in cereals by 2 : 4-D amine (0·75 lb. acid-equiv. per acre). A. H. CORNFIELD.

Glasshouse test for control of antirrhinum rust. H. Jacks and A. J. Webb *N.Z. J. Sci. Tech.*, 1956, **38A**, 309—311).—Control of

the rust was best achieved by applying : zineb, wettable S, ferbam, maneb, dichlone, thiram, colloidal S, or lime-S plus colloidal S.
R. H. Hurst.

Seed disinfection. XIV. Effect of seed dressings on damping-off and nodulation of lucerne. H. Jacks (*N.Z. J. Sci. Tech.* 1956 **38A,** 312—315).—*Pythium ultimum,* Trow., caused damping-off of lucerne seed. Seed dressings based on dichlone, captan, thiram and ferbam increased emergence. They did not completely prevent post-emergence damping-off, but dichlone, captan and to a smaller extent, thiram, significantly reduced diseased seedlings. Development of *Rhizobium meliloti,* Dangeard, was not affected by the chemicals when seed was sown immediately after dressing.
R. H. Hurst.

Control of the nematode, *Meloidgyne incognita,* by crop rotation. H. W. Crittenden (*Plant Dis. Reptr,* 1956, **40,** 977—980).—Although one year of a resistant crop resulted in some measure of control of the nematode on a susceptible crop (cantaloupe) in the following year, much better control was obtained by growing a resistant crop for two consecutive years. Resistant varieties of soya-bean were particularly useful in this respect. A. H. Cornfield.

Control of tobacco black shank, *Phytophthora parasitica* var. *nicotianæ.* W. Lutz (*Plant Dis. Reptr,* 1956, **40,** 855—860).—The most effective soil treatments for controlling the disease were methyl bromide (1 lb. per 100 sq. ft.), Vapam (40% Na *N*-methyldithiocarbamate ; 1 gal. per 40 gal.), Dithane D-14 (19% disodium ethylenebisdithiocarbamate ; 2 lb. per 100 gal.) and 2% HCHO. Na azide (6 lb.) and urea (50—100 lb. per 100 gal.) gave control in some years only.
A. H. Cornfield.

Control of nematodes with Vapam. B. Lear (*Plant Dis. Reptr,* 1956, **40,** 847—852).—Root-knot nematodes, *Meloidogyne incognita* var. *acrita,* were controlled in soil by application of Vapam (Na *N*-methyldithiocarbamate ; 0·5 lb. per 100 sq. ft.). Best results were obtained by applying the material in a water drench or by applying conc. Vapam before and following application of water. Vapam diffused to a greater extent laterally than horizontally.
A. H. Cornfield.

Control of nematodes in rice soils with fumigants. J. G. Atkins and M. J. Fielding (*Plant Dis. Reptr,* 1956, **40,** 488—489).—Application of methyl bromide (3 lb. per 100 sq. ft.), ethylene dibromide (12 gal.), DD (40 gal.) or Nemagon (5 gal. per acre) five weeks prior to planting rice gave good control of *Tylenchorhynchus martini* and also resulted in higher yields, taller plants, and better tillering. Methyl bromide was somewhat more effective than the other treatments. A. H. Cornfield.

Sodium chloride as a protectant and chemotherapeutic against Dutch elm disease, *Ceratostomella ulmi,* Buisman, in American elm trees. A. W. Engelhard (*Plant Dis. Reptr,* 1956, **40,** 1005—1009).—Application of NaCl (0·5 lb. per in. of basal trunk diam.) in solution on each side of the rows of elm trees either before or after inoculation of trees with the fungus did not cure or prevent development of the disease. A. H. Cornfield.

Virus diseases of geraniums in the Pacific northwest (U.S.A.). F. R. McWhirter (*Plant Dis. Reptr,* 1957, **41,** 83—88).—Five virus diseases of geraniums and control measures are described.
A. H. Cornfield.

Control of wildfire, *Pseudomonas abaci,* in Burley tobacco plant beds. L. Shaw, G. B. Lucas and G. F. Thorne, jun. (*Plant Dis. Reptr,* 1957, **41,** 99—102).—Wildfire was controlled by six weekly sprayings of 100—200 p.p.m. streptomycin sulphate (3—5 gal. per 100 sq. yd.) or 200 p.p.m. streptomycin nitrate. Addition of 1% of glycerol had little effect on, whilst addition of " Tribasic Cu " decreased, the extent of control. A. H. Cornfield.

Animal Husbandry

Rumen metabolism of non-protein nitrogen. W. H. Hale (*J. agric. Food Chem.,* 1956, **4,** 948—951).—The rôle of non-protein N in the biochemical processes occurring in the rumen, and possible toxicity mechanisms are reviewed. (31 references.) S. C. Jolly.

Harvesting and storing silage. J. L. Butt (*Alabama agric. Exp. Sta.,* 1956, Circ. 117, 14 pp.).—A report of three years' tests at a no. of locations using various methods of harvesting and storing silage.
A. H. Cornfield.

Sericea (*L. cuneata*), with and without a nitrogen supplement, as a source of roughage for lactating dairy cows. G. E. Hawkins and K. M. Autrey (*J. Dairy Sci.,* 1957, **40,** 95—104).—Milk production by cows fed sericea, with and without supplementary urea to increase the digestible protein equiv., was less than that by similar animals fed good quality lucerne. As efficiency in utilizing available total digestible nutrients was similar with both roughages, sericea possibly either (i) contains a lactation depressant, (ii) is deficient in a non-energy-producing nutrient necessary for high milk production, or (iii) contains a factor that reduces digestibility of protein in concentrates. S. C. Jolly.

Grass-legume mixtures for irrigated pastures for dairy cows. G. Q. Bateman and W. Keller (*Utah agric. Exp. Sta.,* 1956, Bull. 382, 55 pp.).—Recommendations are given based on studies over 11 grazing seasons. A. H. Cornfield.

Nutritive value and palatability of some introduced and native forage plants for spring and summer grazing. C. W. Cook, L. A. Stoddart and L. E. Harris (*Utah agric. Exp. Sta.,* 1956, Bull. 385, 39 pp.).—Nutritive value and palatability of crested, tall, pubescent, intermediate, western, and beardless wheat-grasses, squirreltail grass, Indian ricegrass, Russian thistle and smother weed are presented. A. H. Cornfield.

Action of molybdenum on some copper enzymes. J. F. Scaife (*N.Z. J. Sci. Tech.,* 1956, **38A,** 285—292).—Cu-containing proteins were prepared from sheep's hide to demonstrate the functions attributed to Cu in producing the crimp and pigmentation of wool. One prep. catalysed the aërobic oxidation of L(—)-cysteine and the other was active towards L(—)-tyrosine and DOPA [β-(3 : 4-dihydroxyphenyl)alanine]. They showed several properties of true enzymes and were inhibited by MoO_4''. Molybdate influenced the catalysis of the oxidation of *p*-phenylenediamine by the Cu-containing protein, cœruloplasmin. R. H. Hurst.

Molybdenum excretion and retention in sheep. J. F. Scaife (*N.Z. J. Sci. Tech.,* 1956, **38A,** 293—298).—$(NH_4)_2MoO_4$ administered to sheep on a low-SO_4'' diet of chaffed oat hay is largely accumulated in the body and not excreted until the occurrence of a tissue degeneration. The Mo is present in the blood as MoO_4''. Sheep receiving Mo in a diet adequate in SO_4'' consisting of chaffed lucerne hay excrete nearly all the intake of Mo, much in the urine as MoO_4''. Renal Mo excretion is not related to diuretic activity but is stimulated by the administration of DL-methionine, Na maleate, Na fumarate and phloridzin. R. H. Hurst.

Reviews of the progress of dairy science. Section E. Diseases of dairy cattle. P. S. Blackburn (*J. Dairy Res.,* 1956, **23,** 384—406).—Various aspects of mastitis and contagious abortion are reviewed. (193 references.) S. C. Jolly.

Genetic and environmental factors in the development of American Red Danish cattle. N. R. Thompson, L. J. Cranek, sen. and N. P. Ralston (*J. Dairy Sci.,* 1957, **40,** 56—66).—The effect on butterfat production, lethal defects, calf mortality, sterility of cows and other factors of the introduction of Red Danish bulls into service with various dairy breeds in U.S.A. is reported. S. C. Jolly.

Bioassay for toxic factor in trichloroethylene-extracted soya-bean oil meal. V. Perman, C. E. Rehfeld, J. H. Sautter and M. O. Schultze (*J. agric. Food Chem.,* 1956, **4,** 959—963).—A bioassay using calves is described for estimating the toxic factor in trichloroethylene-extracted soya-bean meal (*M*) which is responsible for a fatal aplastic anæmia in the bovine. Using the thrombocyte count and % of lymphocytes as the principal and the total leucocyte count and death as secondary criteria of toxicity, it is possible to distinguish between daily doses of 1/100, 1/40, 1/20, 1/8, 1/6 and 1/4 or more lb. of a standard sample of *M* per 100 lb. of body wt. Acute or prolonged aplastic anæmia of different degrees of severity can be produced in calves by feeding different levels of *M*. (18 references.) S. C. Jolly.

Influence of milk yield on fertility in dairy cattle. E. J. Currie (*J. Dairy Res.,* 1956, **23,** 301—304).—Under New Zealand conditions of herd management, in which feeding often consists entirely of outside grazing unsupplemented by concentrates, high milk production during the month of service did not reduce fertility, as judged on conception at first service. S. C. Jolly.

Effect of various levels of dietary protein on the volatile fatty acids in the rumen of the dairy cow. R. F. Davis, N. S. Woodhouse, M. Keeney and G. H. Beck (*J. Dairy Sci.,* 1957, **40,** 75—80).—Lactating cows were fed low, medium and high levels of protein. Increased amounts of all volatile fatty acids, except those higher than valeric, occurred in the rumen liquor of animals on the highest protein intake. With increasing protein intake the proportion of acetic and higher acids decreased and that of butyric acid increased in the volatile fatty acids. Adjustment of the rumen to ration changes was not complete after two weeks. S. C. Jolly.

Effects of various hay-concentrate ratios on nutrient utilization and production responses of dairy cows. I. Relations among feeding level, predicted producing ability and milk production. S. Bloom, N. L. Jacobson, L. D. McGilliard, P. G. Homeyer and E. O. Heady (*J. Dairy Sci.,* 1957, **40,** 81—94).—Irrespective of the ratio of hay to concentrate in the ration, the inherent milk-producing ability of

Holstein cows exerted a greater influence on milk production than did intensity of feeding. Wt. changes during the first five weeks of the experiment were closely related to diet, but thereafter, particularly at lower feeding levels, they were largely independent of the ration. S. C. JOLLY.

Malathion in milk and fat from sprayed cattle. H. V. Claborn, R. D. Radeleff, H. F. Beckman and G. T. Woodard (*J. agric. Food Chem.*, 1956, **4**, 941—942).—Malathion (**I**) was not detected in the fat of cattle one week after the last of 16 sprayings with a 0·5% suspension or emulsion of **I**, but 0·08—0·36 p.p.m. of **I** was found in the milk of dairy cows sprayed 5 hr. earlier with 0·5 or 1·0% suspension or emulsion of **I**. Only traces of **I** were present in milk drawn 19 hr. later and **I** was completely absent after 3—7 days. Smaller residues were found in milk from cows sprayed with emulsions than when suspensions were used. S. C. JOLLY.

Some chromogens in cow urine. J. P. Mixner, H. L. Saunders, jun. and J. E. Johnston (*J. Dairy Sci.*, 1957, **40**, 67—74).—Cow urine contains $\not<6$ chromogens, apparently ionone derivatives of dietary carotenoids, which behave similarly to 17-ketosteroids during chromatography on alumina (benzene–ethanol system), but which give different absorption spectra in the Zimmerman, Pincus and Allen colour reactions. They are present in the conjugated state, but are hydrolysed by acids or by β-glucuronidase and are then extractable by lipin solvents. Their urinary excretion pattern shows a marked diurnal rhythm. Previously reported measurements of 17-ketosteroids in cow urine are unreliable because of the presence of these chromogens. S. C. JOLLY.

Microbiological assay of penicillin in feeding stuffs. J. S. Simpson and K. A. Lees (*Analyst*, 1956, **81**, 562—565).—A microbiological plate method with *Sarcina lutea* as test organism is described for the assay of penicillin in feeding stuffs. The samples are extracted with 25% v/v acetone in a PO_4''' buffer (pH 7·5), aliquots of the extract being diluted with the solvent to contain 0·1 and 0·05 units per ml. Standard reference solutions of sodium benzylpenicillin are prepared in the same solvent. The sample and reference solutions are used directly in a standard cup-plate microbiological assay with incubation at 30° for 18 to 24 hr. The limits of error ($P = 0·95$) of the method are of the order of 90 to 112% with six samples and two standards at two levels each on an 8 × 8 quasi Latin-square plate (J.A.C. Abstr., 1956, i, 566). A. O. JONES.

Effect of administering fluorocortisone with sodium sources to ruminants. A. C. Chung and J. C. Shaw (*J. Dairy Sci.*, 1957, **40**, 105—108).—As simultaneous oral administration of Na salts with intramuscular fluorocortisone (**I**) had a serious deleterious effect on the cow and four goats to which they were administered, the feeding of Na salts is contraindicated when **I** is used for the treatment of bovine ketosis; no adverse effects were noted in normal or ketotic cows on normal rations following the intramuscular injection of $\not>100$ mg. of **I**, which appears to be the optimum dose. In the bovine, **I** was $\sim$15 to 20 times as potent as hydrocortisone, and 4 to 5 times as potent as prednisone. S. C. JOLLY.

Endocrine factors influencing the intensity of milk secretion. I. Œstrogen, thyroxine and growth hormone. C. W. Turner, H. Yamamoto and H. L. Ruppert, jun. (*J. Dairy Sci.*, 1957, **40**, 37—49).—The daily feeding of 10 mg. of stilbœstrol for four weeks tended to arrest the normal decline, or even caused a slight increase, in milk yield with increasing lactation for up to 9—10 weeks. Injection of L-thyroxine (0·4 mg. per 100 lb. of body wt.) for 4—9 weeks sometimes produced a marked rise in milk yield; following cessation of thyroxine administration, milk yield declined rapidly for $\sim$2 weeks and then increased to expected normal levels as endogenous thyroxine secretion, which ceased during exogenous administration, took control again. Daily injection of 50 mg. of growth hormone for 1 week generally increased milk yield for 2—8 weeks. An analysis of the genetic–endocrine causes of the variability of milk production is suggested. S. C. JOLLY.

Fæcal elimination of hormones in sheep and cattle treated with synthetic œstrogens. M. Stob (*J. Anim. Sci.*, 1956, **15**, 990—996).—Œstrogens were detected (mouse test) in fæces of sheep and cattle receiving diethylstilbœstrol (**I**), dienœstrol or hexœstrol orally (or in sheep **I** subcutaneously. The reproductive performance of gilts following treatment was unaffected although there was evidence that the production of pituitary gonadotrophic hormone in boars was suppressed under these conditions. A. G. POLLARD.

Residual œstrogen in tissues of cattle treated orally with diethylstilbœstrol, dienœstrol, hexœstrol and chlortetracycline. M. Stob, T. W. Perry, F. N. Andrews and W. M. Beeson (*J. Anim. Sci.*, 1956, **15**, 997—1002).—Calves receiving diethylstilbœstrol (**I**) (10 mg. daily) up to the time of shipment for slaughter showed œstrogenic residues (mouse uterus test) in muscle, kidney, kidney fat, intestine and liver. Administration of dienœstrol (10 mg.) produced similar effects except in muscle. Hexœstrol (12 mg. daily) fed to 2-year-old cattle caused œstrogenic activity only in kidney and kidney fat. Chlortetracycline given in combination with hexœstrol did not affect the residual activity significantly. In the mouse uterus test for small amounts of **I** a 3-day period of administration is the min. needed for a satisfactory significant response. A. G. POLLARD.

Effect of aureomycin on young dairy calves raised in a new environment. F. T. Landagora, L. L. Rusoff and Barney Harris, jun. (*J. Dairy Sci.*, 1957, **40**, 50—55).—Irrespective of whether the animals were raised in a new uncontaminated environment or in an old environment, the administration of aureomycin significantly increased growth and efficiency of feed utilization at 12 weeks of age of calves. Growth stimulation occurred earlier in the animals in the new environment and when the antibiotic was administered orally [50 mg. daily in milk and 0·5% of Aurofac 2A (3·6 g. of aureomycin per lb.) in the starter] compared with intramuscular injection (400 mg. in oil weekly). S. C. JOLLY.

Rumen studies in sheep. II. Comparison between rumen microbial end-products in thrifty and ill-thrifty hoggets. V. J. Williams and K. R. Christian (*N.Z. J. Sci. Tech.*, 1956, **38A**, 268—276).—Comparison was made between the ruminal concn. of NH_3, volatile fatty acid, protein and free bacteria in six free-grazing hoggets on dairy farm pastures and six on sheep farm pastures. Intakes of herbage were measured concurrently. During the autumn the average weekly growth was 2·5 lb. for hoggets on the dairy farm, but those on the sheep farm grew slowly or lost wt. and two deaths occurred. Significant small differences between the groups in volatile fatty acids and NH_3 levels were found at the time of ill-thrift. The acetic : propionic acid ratio was lower in the dairy farm group. Levels of protein-N were much lower on the sheep farm until normal growth rates were resumed. No great differences were observed in ruminal micro-organisms. Daily feed intake during ill-thrift averaged 900 g. of digestible org. matter for the dairy hoggets and 580 g. for the sheep farm group. (14 references.) R. H. HURST.

Effect of various hormone implants on fattening lambs. R. M. Jordan and H. G. Croom (*J. Anim. Sci.*, 1956, **15**, 1003—1007).—Early spring treatment of lambs with stilbœstrol or a combination of œstradiol with testosterone or progesterone improved the growth rates and feed efficiency but lowered the grade and yield of carcass. Summer treatment with either combination had little effect on growth rates or feed efficiency but lowered carcass grades.
A. G. POLLARD.

Feeding trials with "Porcal" and "Mastpen." K. Tschiderer (*Bodenkultur*, 1956, **9**, 178—183).—Piglets in poor condition can be brought up to normal standards by feeding with a sour milk prep., Porcal. Reinforcement of an experimental feed with Mastpen and procaine penicillin does not improve performance except with regard to carcass quality. M. LONG.

Rearing trials with an artificial sow's milk "TOP" (A.-G. Esbjerg Milchinindustrie Dänemark).. K. Tschiderer (*Bodenkultur*, 1956, **9**, 184—190).—Piglets can be satisfactorily reared with TOP if the feed is supplemented with antibiotics; without antibiotics development is somewhat below average. M. LONG.

Effect of various levels of orally administered testosterone on growth and carcass composition of swine. T. W. Perry, W. M. Beeson, M. Mohler, F. N. Andrews and M. Stob (*J. Anim. Sci.*, 1956, **15**, 1008—1013).—Pigs were fed from 51 to 210—220 lb. live wt. a free-choice protein supplement providing 0—62 mg. of methyl testosterone (**I**) per head daily. A daily intake of $\not<27$ mg. of **I** daily diminished growth rates and thickness of back fat. Administration of **I** did not alter the consumption of protein but lowered the amounts of maize eaten. A. G. POLLARD.

Induction of œstrus in lactating sows with equine gonadotrophin. H. Heitman, jun. and H. H. Cole (*J. Anim. Sci.*, 1956, **15**, 970—977).—Injections of the gonadotrophin given to sows at various stages of lactation produced œstrus in about four days, the no. of positive results and the no. of sows farrowing being higher when injections were given 40—50 days than when 20—40 days after the beginning of lactation. A. G. POLLARD.

Pig feeding trials with Terramycin and Vigofac. K. Tschiderer (*Bodenkultur*, 1956, **9**, 191—196).—The average daily increase in wt., the efficiency of feed utilization and the daily food intake of fattening pigs were increased by supplements of Terramycin and still more by Terramycin + Vigofac (a growth stimulant extract from the fermentation product of *Streptomyces*). The mixed supplement tended to improve carcass quality. M. LONG.

High-energy turkey poult starting rations. W. C. Lockhart and R. L. Bryant (*N. Dakota agric. Exp. Sta., Bimo. Bull.*, 1956, **19**, 19—23).—High-energy rations with protein content varying from

28% to 30% and calorie : protein ratio varying from 43 : 1 to 42 : 1 were fed to poults from 10 to 56 days of age. Best results with respect to both growth rate and feed efficiency were obtained with the 28% protein–43 : 1 calorie : protein diet containing solvent-extracted soya-bean oil meal. A. H. Cornfield.

Manganese in poultry mashes. Anon. (*J. Agric. S. Aust.*, 1956, **59**, 326).—Where laying mashes contain a high proportion of crushed grain in place of bran or pollard there is a risk of Mn deficiency occurring, particularly in breeding stock. $MnSO_4$ (0·5 oz. per 100 lb. mash) should be added to the mash if its bran and pollard contents are low. A. H. Cornfield.

Control of cattle ticks in Central Africa. J. G. Matthysse (*Agric. Chemic.*, 1956, **11**, No. 11, 32—34, 117).—Spraying cattle with 0·17—0·25% toxaphene was effective in controlling *Amblyomma* and *Boophilus* ticks but not *Rhipicephalous* ticks. 0·14% DDT was effective only against *Boophilus*, whilst 0·02% γ-$C_6H_6Cl_6$ was effective only against *Amblyomma*. Dipping was usually more effective than spraying. A. H. Cornfield.

Insecticidal products. Murphy Chemical Co., Ltd. and M. Pianka (B.P. 750,471, 30.10.50).—Compounds CRR′ : N·[CH_2]$_n$·N:CR″R‴ are claimed as insecticides (especially ovicides). [R and/or R″ are H, or R—R‴ are (substituted) org. residues free from CO, *n* is O or an integer]. Thus, a mixture of diacetyl, aniline and alcohol is boiled during 2 hr., then cooled, with pptn. of the *di-anil*. This is dissolved in acetone and polyethylene glycol oleate, and the solution is diluted with water to 0·2% concn., to give an emulsion which effects a 98% kill of chrysanthemum aphids. F. R. Basford.

Pesticidal compositions. Rohm & Haas Co. (B.P. 750,757, 2.6.54. U.S., 19.6.53).—In the compounding of an aq. pesticidal spray, by admixing an aq. suspension (**I**) of an insol. polyvalent metal (Zn) salt (**II**) of ethylene bisdithiocarbamic acid [optionally containing a condensed naphthalene–formaldehyde Na sulphonate or Na lignosulphonate (0·1—2·5% on **II**)] with an emulsion concentrate of an insecticide (specifically DDT) in a hydrocarbon solvent containing an emulsifier, formation of undesirable greasy curd is prevented by addition of (hydroxyethyl)cellulose (0·4—3·5% on **II**). F. R. Basford.

Poly-bis-thiuram disulphides. Badische Anilin- & Soda-Fabrik A.-G. (B.P. 751,365, 14.1.54. Ger., 16.1.53).—Compounds (S·CS·NR·[CH_2]$_m$·NR′·CS·S)$_n$ (R and R′ are H, alkyl, or *cyclo*alkyl; *m* and *n* are integers), useful as fungicides, are prepared by oxidation of the corresponding bis-dithiocarbamates in aq. medium. Thus, CS_2 is added slowly to a mixture of ethylenediamine and 37·5% aq. NaOH at 25°, then after stirring during 10 hr., the solution is diluted with water, cooled to 0—5°, and treated with a mixture of 35% aq. H_2O_2, conc. H_2SO_4 and aerated water. After 2 hr., the pptd. *poly*(*ethylenebis*[*thiuram disulphide*]), m.p. 170—180°, is filtered off. F. R. Basford.

Tri-*n*-butylamine salt of αα-dichloropropionic acid. Dow Chemical Co. (B.P. 751,308, 9.7.54. U.S., 18.9.53).—The *salt*, m.p. 74—75·2°, useful as a herbicide, is prepared by direct addition of the amine to the acid in a petroleum fraction (b.p. 170—280°) in presence of an emulsifying agent (Triton X-155), the reaction being controlled by rate of addition of the reactants. F. R. Basford.

αα-Dichloropropionic esters of the polyhydric alcohols. Dow Chemical Co. (B.P. 750,545, 1.4.54. U.S., 6.4.53).—Compounds claimed as selective weed-killers and plant-growth stimulants comprise αα-dichloropropionic esters of polyhydric alcohols, specifically *ethylene glycol* (**I**), f.p. 16°, b.p. 125—129·5°/2·2 mm.; *propane-1 : 2-diol*, b.p. 127—134°/2 mm.; and (crude) *dipropylene glycol*, **di-(αα-dichloropropionate)**, b.p. 128—133°/1 mm., n_D^{25} 1·4624; also the *pentaerythritol tetra-*, m.p. 116—120°; and *glycerol tri*-esters, b.p. 167—170°/0·4—0·6 mm. They are made by boiling a mixture of the alcohol and αα-dichloropropionic acid during 30 min. at 125°, then distilling. F. R. Basford.

αα-Dichloropropionates of the haloaryloxy lower alkanols. Dow Chemical Co. (B.P. 750,655, 2.4.54. U.S., 6.4.53).—Compounds useful as selective weed killers comprise, e.g., 2-(2′ : 4′-*dichlorophenoxy*)*ethyl* (**I**), f.p. 34°, b.p. 150—152°/1 mm., 1-(2′ : 4′-*dichlorophenoxy*)*prop-2-yl*, f.p. 49°, b.p. 137—139°/0·5 mm., 2-(2′ : 4′ : 5′-*trichlorophenoxy*)*ethyl*, m.p. 49—50°, b.p. 161—164°/0·5 mm., 2-(4′-*chloro-o-toloxy*)*ethyl*), f.p. 36—39°, b.p. 162—172°/3·5 mm., and 1-(2′ : 4′ : 5′-*trichlorophenoxy*)*prop-2-yl* **αα-dichloropropionate**, b.p. 154—157°/0·08 mm. As an example of prep., a mixture of 2-(2′ : 4′-dichlorophenoxy)ethanol (162), αα-dichloropropionic acid (112 g.), conc. H_2SO_4 (0·5) and $(CH_2Cl)_2$ (150 c.c.) is heated during 12 hr. at 100—110° (with continuous removal of water by azeotropic distillation), then washed with water, and distilled, to give **I**. F. R. Basford.

[Treatment of seed] potatoes. T. G. Farish (Inventor: J. C. Sanders) (B.P. 750,349, 30.4.53).—The cropping quality of a seed potato is improved by implantation therein of complete fertilizer (0·5—6 g. per potato, e.g., a Plantoid). F. R. Basford.

Compositions for protecting seeds, etc., against nematodes. Directie van de Staatsmijnen in Limburg (B.P. 750,348, 17.9.53. Neth., 23.9.52).—Seed potatoes and seed beet are protected against attack by *Heterodera rostochiensis*, Wollenweber (potato eelworm) and *H. Schactii*, Schmidt (beetroot eelworm) by dusting or spraying with a composition containing $CH_2X{\cdot}CRO$ (X is halogen, R is H, Me, Et, vinyl, or alkyl), e.g., chloroacetaldehyde, monochloroacetone, monobromoacetone and ethyl chloromethyl ketone. F. R. Basford.

Preparing dry feed. F. Weiss and U. Weiss (B.P. 750,857, 29.3.54. Ger., 28.3.53).—A rotary drum has a horizontal travel for feeding stuffs to be comminuted in a dry condition. A central rotary shaft carries a helical array of fly-cutters. The inner surface of the drum is roughened and splash-plates keep material clear of the bearings. The drum can rotate at any desired differential speed of cutters and drum. K. Ridgway.

2.—FOODS

Mango cereal products. G. Lal and N. L. Jain (*Res. & Ind., India*, 1956, **1**, 229—230).—The production of cereal flakes, vermicelli and cereal flour from mango pulp is briefly described. J. S. C.

Formation of starch in young maize kernels. H. Fuwa (*Nature, Lond.*, 1957, **179**, 159—160).—Experimental results are reported from which it is clear that the endosperm of waxy maize is able to form amylose from glucose-1-phosphate by a reaction resembling that of phosphorylase, even though no amylose is detectable in the normal growing plant. J. S. C.

Effect of wheat bugs on maltose content of wheat. P. Nuorteva (*Cereal Sci.*, 1957, **2**, 16).—Since bugs which injure wheat kernels occur commonly and deposit salivary secretions possessing amylase activity into the kernels, the maltose content of wheat (or derived flour) may not be a reliable measure of the extent of germination of the kernels. J. S. C.

Problems and techniques in the analysis of plant material for hemicellulose. K. T. Williams and A. Bevenue (*J. Ass. off. agric. Chem., Wash.*, 1956, **39**, 901—918).—A review and discussion of procedures for the removal of lignin, pectic substances, starch and protein and for the separation and examination of hemicelluloses. For the removal of lignin, treatment with aq. acetic acid and sodium chlorite is recommended. Aq. sodium polymetaphosphate or ethylenediaminetetra-acetate is a good solvent for pectic substances that are not extractable by water. Starch is best removed by enzymic hydrolysis, but no enzyme system capable of completely hydrolysing all starches has yet been found. The effectiveness of proteolytic enzymes also is limited; treatment with a cationic detergent offers an alternative approach to the problem of removing protein. Conditions for the isolation of hemicelluloses, which are insoluble in water but soluble in aq. alkali, are considered together with their precipitation and separation. Cupriethylenediamine may be used to disperse the mixture of hemicellulose and cellulose, the latter being then precipitated with HCl at controlled pH. The graded hydrolysis of the resulting hemicellulose to determine the nature and mode of association of the monoses is also discussed, and there is a brief reference to the significance of the presence of water-sol. polysaccharides and α-cellulose. The review is freely documented. A. A. Eldridge.

Radiocative tracer technique for the study of dough mixing. D. W. E. Axford, J. B. M. Coppock, S. J. Cornford, S. Drysdale, A. Harrison and F. P. W. Winteringham (*J. Sci. Food Agric.*, 1957, **8**, 234—238).—Two methods of assay, a liquid suspension technique and a solid sample assay, were used to study the rate of dispersion of ^{131}I-labelled fat in a dough mixing process. The usefulness of isotopic tracer techniques and the speed at which such tests could be completed were demonstrated. For the sample size and dough mixer used, the fat was uniformly dispersed after 2 min. of mixing and the mixer was more efficient when fully loaded. E. M. J.

Microbial oxidation of pure carbohydrate in presence of calcium lignosulphonate. W. A. Lawrance and H. N. Fukui (*Sewage industr. Wastes*, 1956, **28**, 1484—1494).—The 15-day B.O.D. of "pure" Ca lignosulphonate (**I**) was found to be very low. No evidence was found to indicate that **I** is converted, in presence of carbohydrate, into compounds of high B.O.D. During the first five days of incubation, **I** did not affect the rate of microbial oxidation of hexoses: subsequently, **I** tended to depress oxidation with glucose and galactose. Arabinose and xylose were utilized by the organisms and **I** produced a marked retardation of the oxidation rate of xylose. J. S. C.

Chemistry and the sugar cane. L. F. Wiggins (*Int. Sug. J.*, 1957, **59,** 94—97).—See J.S.F.A. Abstr., 1957, i, 194. J. S. C.

Technical improvements in the beet sugar factory and distillery and the future of the beet-sugar industry. P. Devos (*Industr. agric. aliment.*, 1956, **73,** 873—875).—A review, mainly of the economic aspects. J. S. C.

Complexones in the estimation of sugars. M. Potterat and H. Eschmann (*Ann. Falsif.*, 1956, **49,** 464—479).—The Cu is precipitated quant. as Cu_2O, dissolved in HNO_3, the solution made alkaline with ammonia, and then titrated with standard complexone III. The accuracy of the method is about ±0·4%.

C. A. Slater.

Behaviour of monosaccharides in alkaline medium. R. Pieck (*Industr. chim. belge*, 1956, **21,** 1029—1052).—A review of the structure and chemistry of monosaccharides (particularly hexoses) with particular reference to mutarotation and degradation in alkaline solution. (160 references.) E. J. H. Birch.

Determination of pentosans. II. Use of barbituric acid for determination of furfural. P. O. Bethge and R.-M. Persson (*Svensk Papp. Tidn.*, 1956, **59,** 535—539).—The reaction of furfural with barbituric acid is slow and is influenced significantly by several factors such as temp., furfural concn., excess of barbituric acid, and impurities in the barbituric acid. Hydroxymethylfurfural (HMF) is not precipitated by barbituric acid, but is co-precipitated if furfural is present. At low furfural concn. (below 200 mg/l. reaction mixture) yields are low and are lowered further if HMF is present. Barbituric acid may be used for the determination of pentosans only if reaction conditions are carefully controlled and the use of empirical correction factors will frequently be necessary.

K. Wendtner.

Modifications of the aniline acetate–furfural method for determination of pentose. A. Borrow and E. G. Jefferys (*Analyst*, 1956, **81,** 598—601).—The pentose solution (1 ml. containing 10—80 μg. of pentose) is heated under reflux for 2·5 hr. in a tube calibrated at 7 ml. with 2 ml. of 5·55N-HCl. The aq. layer is then removed, xylene is added to the 7-ml. mark and the solution is dried with anhyd. Na acetate. A 5-ml. aliquot is cooled in ice, and treated with 5 ml. of cooled aniline reagent (4·5% in equal parts of ethanol and glacial acetic acid). The mixture is cooled in ice water for 18 min. and the colour is compared spectrophotometrically at 540 mμ. with that of a standard xylose solution (50 μg.) subjected to the whole procedure. A. O. Jones.

Relation between the solubilization of pectin and the fate of organic acids during maturation of apples. J. J. Doesburg (*J. Sci. Food Agric.*, 1957, **8,** 206—216).—Weekly data on the amount, degree of esterification and mol. wt. of pectins in apples before and after harvest were examined. The mol. wt. of pectin was constant during that period ; mol. wt. of insol. and sol. pectins in ripe fruits were nearly equal, so that there was no shortening of chains of pectin mol. during solubilization of part of the pectins in ripening. The correlation between changes in solubility of pectin and of Ca, changes in the mixture of org. acids in the fruits, in pH in the cell walls, indicate that solubilization of pectin during ripening is caused by movement of Ca in the cell walls. (35 references.) E. M. J.

Rôle of reducing sugars and amino-acids in the browning of potato chips. A. T. Habib and H. D. Brown (*Food Technol.*, 1957, **11,** 85—89).—The various types of sugars and free amino-acids present in potato tubers were separated and their amounts determined quantitatively by paper chromatography. Cold storage at 40°F. resulted in increase in glucose and maltose and slight changes in amino-acids ; reconditioning at 75°F. resulted in complete disappearance of maltose and decrease in other sugars, and disappearance of basic amino-acids, e.g., lysine, histidine and arginine. Low basic amino-acids and low reducing sugars, particularly pentose sugars, in potato tubers are associated with light coloured potato chips. (26 references.) E. M. J.

Relation of the calcium content of potato tubers to the quality of potato chips. Keng Chock Ng, H. D. Brown, R. H. Blackmore and J. Bushnell (*Food Technol.*, 1957, **11,** 118—122).—Total Ca determination of 15 individual samples (1954) and 32 variety samples (1955) indicated that high total Ca of raw potato tubers was correlated with high total solids, light coloured chips and low fat absorption. Data on the effects of moisture levels, planting dates and N levels on the Ca contents of potato tubers are reported. (14 references.) E. M. J.

Time–temperature tolerance of frozen foods. IV. Objective tests to measure adverse changes in frozen vegetables. W. C. Dietrich, F. E. Lindquist, J. C. Miers, G. S. Bohart, H. J. Neumann and W. F. Talburt (*Food Technol.*, 1957, **11,** 109—113).—In commercial lots of frozen peas, green beans and cauliflower held at temp. 0—30°F., reduced ascorbic acid was a reliable index of previous storage history, especially if the original content of samples is known ; a summation of reduced ascorbic acid and its oxidation products (dehydroascorbic and diketogulonic acids) is useful for green beans ; conversion of chlorophyll to pheophytin was well correlated with temp. and measurements of colour of peas and green beans by the Hunter Colour Difference Meter indicate progressive changes related to temp. (16 references.) E. M. J.

" COF effect " in frozen citrus products. J. S. Blair, E. M. Godar, H. G. Reinke and J. R. Marshall (*Food Technol.*, 1957, **11,** 61—68).—The occurrence of a peculiar flavour effect " cardboard off-flavour " or " citrus oxidized " flavour, in a small proportion of the frozen pack is reviewed. Evidence derived from a considerable amount of qual. laboratory work indicates that physiological immaturity is fundamentally the cause. (22 references). E. M. J.

Micro-organisms of significance in frozen citrus products. R. H. Vaughn, D. I. Murdock and C. H. Brokaw (*Food Technol.*, 1957, **11,** 92—95).—Review of six years of study indicates that lactic acid bacteria are the best index of sanitation of high quality frozen citrus products. (52 references.) E. M. J.

Stability of frozen concentrated orange juice. III. Effect of heat treatment in the production of high-Brix frozen concentrate. O. W. Bissett, M. K. Veldhuis, R. B. Guyer and W. M. Miller (*Food Technol.*, 1957, **11,** 96—99).—Frozen concentrates of 42° and 58·5° Brix were prepared from a uniform supply of Valencia oranges. Evaporator feed juices were heat treated to 150, 160, 170 and 180°F. held for 5 sec., cooled and concentrated. Cloud stabilities of all 58·5° Brix samples were superior to those of corresponding 42° Brix when stored at 40°F. Pectinesterase activity decreased with increase of temp. By mixing heated and unheated 68° Brix concentrates and cutting back to 58·5° Brix with unheated juice, only 50% of the evaporator feed juice needed to be heated to 150°F. to provide cloud stability for six weeks at 40°F. E. M. J.

Fragrance principles in fruit juices and their artificial substitutes. W. Schweisheimer (*Riechstoffe u. Aromen*, 1956, **6,** 376—378).—Lists are given of the various scent-impairing compounds that have been isolated from juices of raspberry, strawberry, peach, cherry, apple and coffee, together with commercial recipes of artificial replicas for use in drinks. Compositions for imparting the flavours of the following are also given : strawberry, raspberry and cherry. (15 references.) H. L. Whitehead.

Processing muscadine grapes. J. G. Woodroof, S. R. Cecil and W. E. Dupree (*Georgia agric. Exp. Sta.*, 1956, Bull. 17, 35 pp.).—Processing grapes for the production of juices, jams, preserves, etc., is described. Chemical composition of many of the products is presented. A. H. Cornfield.

Difference in chemical characteristics of grape musts and fruit musts and of their derivatives (wines and vinegars). F. Mecca (*Chim. e Industr.*, 1956, **38,** 1020—1023).—Grape juice products are differentiated from other fruit juice products on the basis of the polyhydric alcohol contents, determined by oxidation with KIO_4. The products are first treated with yeast to remove fermentable sugars, the acids removed by treatment with basic Pb acetate, and the polyhydric alcohols separated from glycerol by pptn. as a complex with $CuSO_4 + Ca(OH)_2$, which is redissolved in dil. H_2SO_4 and titrated with KIO_4. Grape wines gave an average oxidizability index of 1270 (range 1100—1500) and fruit wines of 3370 (2340—4850). The corresponding vinegars gave values of 460 (400—510) and 3440 (2500—4600) respectively. Adulteration of wines with water or ethanol, or of vinegars with acetic acid, will lead to a corresponding lowering of the oxidizability indices. L. A. O'Neill.

Characterization of various species of wine yeast. E. Peynaud (*Mitt. Wein-u. Obstbau, Wien*, 1957, **7A,** 1—15).—Methods of identification of yeasts are reviewed using *Saccharomyces acidifaciens* as an example. (20 references.) E. M. J.

Possible use of sorbic acid for preservation of wine. W. Saller and S. R. Kolewa (*Mitt. Wein -u. Obstbau, Wien*, 1957, **7A,** 21—26).—Concn. of sorbic acid required for the inhibition of the growth of (added) yeast in wine (containing 11% of sugar) during three weeks are 0·2 g./l. at 25°, and 0·15 g./l. at 12°. Sorbic acid at 0·3 g. per l. does not affect the flavour of wine. (14 references.)

P. S. Arup.

Determination of alkalinity of ash of wines and fruit juices. J. Schneyder (*Mitt. Wein -u. Obstbau, Wien*, 1957, **7A,** 26—30).—In comparison with other current methods, the method of Rancke-Madsen *et al.*, as applied by the author (cf. *ibid.*, 1956, **6A,** 309) gives the most accurate results for the " true alkalinity " of the ash, comprising that of the oxides, carbonates and silicates. (15 references.)

P. S. Arup.

Formation of glycerin in wine manufacture. Effects of adding sugar, sulphurization and clearing of grape must on glycerin formation. H. Grohmann and F. H. Mühlberger (*Dtsch. Lebensm. Rdsch.*, 1957, **53**, 51—59).—The relevant literature is reviewed. The proportions of alcohol and glycerin in natural must (of varied origin) and must with increased sugar content, the effects of treatment with 50 or 80 mg./l. of SO_2 are critically examined. Treatment with 50 mg./l. of SO_2 results in slight increase of glycerin formation. The mechanism of reactions involved are discussed. (34 references.)

E. M. J.

Determination of added distinctive cations in whisky. V. Spectrophotometric determination of molybdenum and nickel. M. J. Pro and R. A. Nelson (*J. Ass. off. agric. Chem., Wash.*, 1956, **39**, 945—951).—For the determination of Mo or Ni added to whisky as brand markers, the organic material is first destroyed by a wet ash procedure. For the determination of Mo the prepared solution, acidified with HCl, is treated with KCNS, and the Mo^{V} thiocyanate complex is extracted with *iso*amyl alcohol, the extinction being measured at 465 mμ. and read against a linear calibration curve prepared by using whisky containing μg. quantities of Mo. For Ni the ignited residue after wet oxidation is dissolved in water, treated with Br followed by NH_3, and then with dimethylglyoxine. The extinction is measured at 450 mμ. and read against a calibration curve. Cu, Cr, Mn and Al interfere only if present in quantity substantially >1 p.p.m.

A. A. ELDRIDGE.

Determination of distinctive added organic materials to whisky. VI. Spectrophotometric determination of citric acid. M. J. Pro and R. A. Nelson (*J. Ass. off. agric. Chem., Wash.*, 1956, **39**, 952—957).—The procedure involves the oxidation of the citric acid to acetone and conversion of the latter to pentabromoacetone, the reaction with $KMnO_4$ and KBr being carried out in presence of H_2SO_4 and HPO_3. The extinction of the pentabromoacetone in alkaline pyridine is determined at 535 mμ. A small blank is attributed to homologues of citric acid frequently present in whisky. The procedure is accurate, rapid and specific. For samples containing 10 p.p.m. of added citric acid, 9·5—10·2 p.p.m. were reported.

A. A. ELDRIDGE.

Use of centrifuges in brewery. M. Grünewald (*Brauereitechniker*, 1957, **9**, 33—37).—The construction and operation of centrifuges used in breweries are explained. The advantages of centrifuging over filtration in brewing practice are constancy of performance, economy in operation, reduced beer wastage, and (as shown by numerous comparisons) the production of beer with better flavour and aroma. As regards loss of CO_2, the foaming capacity of the beer, and removal of yeast, the merits of the processes are about equal. Centrifuging produces beer of inferior protein-stability at —0·5° as compared with filtered beer, but the retention of colloidal proteins is probably the reason for the superior organoleptic qualities of the centrifuged product. On balance, the replacement of filters by centrifuges is an economically sound proposition.

P. S. ARUP.

Judgement of quality of malt. W. Piratzky (*Brauwelt*, 1957, **97B**, 341—347, 398—402).—Current methods for evaluating malt are critically examined with special attention to the Hartong method. Hartong solubilities at 20° are of value only in relation to the solubilities at 45°; solubilities at 65° and 80° vary but little for different types of malt; values obtained at 65° agree essentially with those obtained by the Congress method. Solubilities at 45° show the greatest variations for different types of malt, and afford the best measure of enzymic activity. A simplified procedure is proposed for determining the Hartong solubilities in which mashes are made in 50-ml. graduated flasks from 5-g. portions of the sample, each with 25 ml. of water at the four appropriate temp. After shaking in the flasks immersed in the water-baths, the mashes are made up with water to 50 ml. at 20° and filtered; the refractometric solids are determined in the filtrates. With appropriate corrections, the values thus obtained are in good agreement with those obtained by the Hartong procedure. The results are calculated with reference to the solubility at 65° taken as 100. Results obtained by the Kolbach method, by determination of the η of the extract, or by solubility differences as between fine and coarse meal show no definite relationship to the Hartong values, and are supplementary rather than alternative to the Hartong results. (31 references.)

P. S. ARUP.

Effect of threshold preservative concentrations on the increase and the fermentation of yeast in malt wort. K. Raible (*Dtsch. Lebensm. Rdsch.*, 1957, **53**, 60—64).—In samples of malt wort to which ten different preservatives were added, each sample being inoculated with yeast and incubated at 25° for 10—17 days, a close relationship between cell growth obtained and preservative concn., but not between alcohol quantity and inhibiting dose of preservative, was found. Better correspondence in concn. up to about ¾ of a growth-inhibiting dose, was found in the alcohol quantities and those of the control tests. Complete inhibition of a fermentation, or growth, required the same concn. of preservative. In the case of K metabisulphite concn. up to 135 mg.-% influenced cell growth and fermentation very little, but at 157·5 mg.-% there was complete cessation of yeast development. (12 references.) E. M. J.

Evaluation of hops. V. Essential oil of hops. G. A. Howard. **VI. Influence of variety on the composition of the α- and β-acids.** G. A. Howard and A. R. Tatchell (*J. Inst. Brew.*, 1957, **63**, [New Series **54**], 126—138, 138—142).—V. An apparatus with which the aromatic constituents of hops were analysed by gas chromatography is described. The essential oil comprises two main fractions which were separated by chromatography on silica gel: (*a*) hydrocarbons, 70—80% of the total oil, consisting of myrcene, caryophyllene and humulene and (*b*) an oxygenated fraction, rapidly volatile in steam, containing esters of $\nless 12$ fatty acids of 4—12 C atoms. (16 references.)

VI. Examination of a wide range of hop varieties indicated that proportions of cohumulone in the α-acid and colupulone in the β-acid are primarily inborn characteristics, little affected by environment, and the ratio of the two substances is approx. constant. Male hop flowers contain humulone and lupulone together with their analogues; the co-fraction proportions vary with variety. (10 references.) E. M. J.

Determination of *iso*humulone and its analogues in beer. G. A. Howard and A. R. Tatchell (*J. Inst. Brew.*, 1957, **63**, [New Series **54**], 142—153).—The proportions of *iso*cohumulone, *iso*humulone and *iso*adhumulone were determined in the total bitter substances of beer by gas chromatographic methods. The *iso*-compounds were extracted with light petroleum together with contaminants ("background") which were removed with methanol + HCl; the *iso*-compounds were finally quant. extracted with NaOH solution. A portion of this extract was used to determine total bitter substances using u.v. spectroscopy; the remaining portion was oxidized with H_2O_2 and the fatty acids characteristic of each analogue were analysed by gas chromatography. "Background" material contains two fractions of mixtures of analogues, and a third fraction containing a mixture of humulinic acid and its analogues. (20 references.) E. M. J.

Analysis of hop oil by gas–liquid partition chromatography. F. L. Rigby and J. L. Bethune (*J. Inst. Brew.*, 1957, **63**, [New Series **54**], 154—161).—Apparatus for the analysis of hop oil by gas–liquid chromatography is described and analyses are reported from 16 varieties of hops. Myrcene and humulene comprise 70—80% of most oils, but methyl nonyl ketone and β and γ-caryophyllene are present in substantial quantities. Many additional components are present in small amounts. Highly significant correlations were found between the cohumulone content of the α-acid fraction and the myrcene content of the oil, and also between the humulone content of the α-acid fraction and the humulene content of the oil. European hop oils generally have a low myrcene and high humulene content; the reverse is true of North American hops. (14 references.) E. M. J.

Experiments on hop storage. R. M. J. Kenber and J. Schmid (*Brauwelt*, 1957, **97B**, 424—426).—The deterioration of hops was measured by the conversion of the humulones (α-acids) into resin. Hops packed in bales or sealed containers kept moderately well during the winter and early spring, but deteriorated rapidly during the summer (max. storage temp. 15°) and the following winter. Previous treatment with SO_2 did not improve the keeping quality. The hops kept very well in bales at —2° or at room temp. in sealed tins containing an atm. of CO_2. (14 references.) P. S. ARUP.

Hop addition and head retention [of beer]. H. Siegfried (*Schweiz. Brauerei Rdsch.*, 1957, **68**, 1—5).—Conditions of malting and brewing influencing head retention are reviewed. Complete analyses are given for the wort and beer in the various stages of four experimental brewings with varying (13—28 kg. per brewing) additions of hops. When tested by four different laboratory methods, the resulting beers showed moderately increasing head retention with increasing hopping, but no essential differences could be discerned when the beers were poured into glasses. In this series, observed differences in CO_2 contents of the samples were probably the decisive factor in the outcome of the laboratory tests. The ITT values of the beers decreased appreciably with the degree of hopping. (11 references.) P. S. ARUP.

Evaluation of beer by points and by comments. L. Macher (*Brauwelt*, 1957, **97B**, 373—383).—Three schemes for evaluating beer by its visual and organoleptic qualities are given, with general advice on suitable conditions for scoring. The first scheme is based on a series of positive point scales, the second on positive and (for defects) negative scales, and the third on positive scoring supplemented by brief comments. The advantages of the third scheme are pointed out. The various characteristics to be evaluated are dealt with in detail. P. S. ARUP.

Metallic turbidity of beer: action of tin on the colloidal stability of beer. G. Michel, B. Gagnaire and P. Lebreton (*Fermentatio*, 1956, No. 6, 275—288).—The formation of turbidity in beers in contact with Sn is examined. It is caused by the slow dissolution of metal to form Sn^{2+} ions, which is favoured by Sn–Pb couples, oxidizing agents, and high temp. The Sn^{2+} ion does not exert a catalytic or other effect on turbidity due to oxidation but contributes to it by combining, by adsorption or other forms of linkage, with a colloidal substrate in the beer, a combination which becomes more rapid at low temp. Generally, the overall effect of industrial Sn is highly destructive of colloidal stability and is intensified by the presence of oxidizing agents and by extreme temp. The intensity of its action varies with different types of beer. (16 references.) J. S. C.

Coffee and coffee substitute. II. Extraction of coffee with cold and hot water. H. Thaler (*Dtsch. Lebensm. Rdsch.*, 1957, **53**, 49—51).—Analytical examination of cold and hot water extracts of roasted coffee indicates that the cold-water sol. fraction constitutes 66—84% of the total extract, and there is good separation of the two groups of extracted substances. E. M. J.

A simple test for the detection of date and tamarind seed adulterants in coffee. C. P. Natarajan and B. S. Bhatia (*Bull. cent. Food technol. Res. Inst. Mysore*, 1956, **5**, 262—263).—The dry, extracted coffee powder is sprinkled on a filter paper and treated with drops of aq. NaOH (5%). By the colour diffusing from the residue particles (yellow, coffee; pink, tamarind; red, date), 10% of adulterant can be detected easily. G. B. Thackray.

Reactivation of phosphatase in high-temperature short-time pasteurized dairy products. H. Fram (*J. Dairy Sci.*, 1957, **40**, 19—27).—The possibility of phosphatase reactivation causing misinterpretation when the phosphatase test is applied to dairy products adequately pasteurized by the high-temp. short-time method can be eliminated either by testing immediately after treatment or by keeping the sample at a temp. >40°F. prior to testing. The min. pasteurization temp. above which reactivation occurred and the length of storage period before this took place were apparently functions of fat content. Reactivation occurred within 2 hr. at 88°F. in cream containing 20 and 30% of fat when heated for 16 sec. at $\nless$165° or at $\nless$180°F. respectively; longer storage at 88°F. (18 and 6 hr. respectively) and higher pasteurization temp. ($\nless$210° and $\nless$195°F. respectively) were necessary before reactivation occurred in skim milk and whole milk. Reactivated phosphatase in these products was not of bacterial origin. S. C. Jolly.

Come-up time method of milk pasteurization. III. Bacteriological studies. R. B. Read, jun., N. L. Norcross, D. J. Hankinson and W. Litsky (*J. Dairy Sci.*, 1957, **40**, 28—36).—Pasteurization of milk can be accomplished satisfactorily without adverse effect on milk quality by a process that uses rapid heating and cooling rates without any intended holding time at temp. easily attainable in modern equipment. A temp. of 175·6°F. was sufficient to kill suspensions ($\nless 1 \times 10^6$ micro-organisms per ml.) of *Escherichia coli*, *Streptococcus pyogenes*, *Shigella paradysenteriæ*, *Salmonella typhosa* and *Corynebacterium diphtheriæ* in milk within 0·25 sec.; a holding time of 0·05 sec. was inherent in the process and must be added to this period. S. C. Jolly.

Paper chromatographic detection of sucrose in milk. A. Castiglioni and R. Pilleri (*Z. anal. Chem.*, 1957, **154**, 1877).—The addition of sucrose as an adulterant to watered milk is not detectable by any alteration in the sp. gr. of the milk or whey, nor in the cryoscopic index or other constants which are an indirect measure of sp. gr. A paper chromatographic test was employed to separate milk sugar and sucrose. Whey, treated with acetic acid, on a strip of Whatman paper No. 1, is treated with butanol, EtOH and water. After 16—20 hr., the strip is withdrawn, dried and sprayed with α-naphthylamine. EtOH and conc. HCl. It is dried for 3—4 min. at 100°. If sucrose is present, it appears as a brown spot easily distinguishable from the rose-coloured spot given by milk sugar. W. J. Wright.

Applicability of xanthine dehydrase determination in milk in food inspection. F. Kiermeier and K. Vogt (*Z. LebensmittUntersuch.*, 1957, **105**, 194—198).—Studies on xanthine dehydrase determination in milk of cows at the beginning or during the lactation periods indicate the unsuitability of the test in milk control, or in differentiation between human and cow's milk. The views of certain authors regarding its use to differentiate between cooled and uncooled milk are discussed. (21 references.) E. M. J.

Preparation and amino-acid composition of enzymically dephosphorylated casein. T. A. Sundararajan and P. S. Sarma (*Biochem J.*, 1957, **65**, 261—266).—A dephosphorylated casein is prepared from casein by the action of a phosphoprotein phosphatase free from proteolytic activity which is obtained from ox spleen. The amino-acid composition of the dephosphorylated casein is approx. the same as that of casein, but it contains more serine, tyrosine and tryptophan, and less cystine and glycine. A mixture of acid-sol. peptides is also formed; these contain glutamic acid, *iso*leucine and serine. The advantages of enzymic as compared with alkaline dephosphorylation are discussed. J. N. Ashley.

Improved preparation of crystalline β-lactoglobulin and α-lactalbumin from cow's milk. R. Aschaffenburg and J. Drewry (*Biochem. J.*, 1957, **65**, 273—277).—An improved method is described for crystallization of the β-lactoglobulins and α-lactalbumin of cow's milk. The method is based on an initial separation of the two types of protein by a simple acidification process which allows simultaneous or independent working up of the fractions. The method is applicable to bulk milk and to the milk of individual cows that contains β_1- or β_2-lactoglobulin either singly or together. Improved yields of β_2-lactoglobulin are obtained by crystallizing it in the orthorhombic rather than the monoclinic form. J. N. Ashley.

Secondary factors in solubility of casein. H. G. Higgins (*Aust. J. appl. Sci.*, 1956, **7**, 379—382).—The effects of pH, temp. and protein concn. on the solubility of lactic casein in phosphate buffer (pH 6·8) at 5° and 25° after pre-treatment with aq. NaOH (pH 9—14) for 1 hr. at 25° are reported and discussed in relation to processes using casein dispersions. Most of the observed effects can be interpreted in terms of the formation and rupture of H bonds and side-chain or salt linkages. W. J. Baker.

Nutritive value of the proteins of stored instant nonfat dry milk. A. Z. Hodson and C. B. Miller (*Food Technol.*, 1957, **11**, 89—92).—The protein efficiencies as measured by rat growth of pasteurized milk proteins and those of nonfat dry milks stored under favourable conditions for one or two years, are similar. The lysine contents of the proteins of the two types of milk are similar. E. M. J.

Acid production in milk by starter cultures. Effect of peptone and other stimulatory substances. E. I. Garvie and L. A. Mabbitt (*J. Dairy Res.*, 1956, **23**, 305—314).—The rate of acid production in milk by a "slow" variant of *Streptococcus cremoris* was increased to that of the "fast" parent strain when peptone or acid-hydrolysed peptone was added; lactose utilization was similarly affected, but growth rate was not changed. Other sources of available N gave similar results. The change from a fast to a slow culture that occurs on continued transfer is the result of the loss of ability to utilize N compounds in milk, possibly due to loss of a proteolytic enzyme. Starter may best be maintained by storing active strains in the freeze-dried condition. S. C. Jolly.

Influence of cultural conditions on the characteristics of *Streptococcus cremoris*, strain HP. H. R. Whitehead, C. A. E. Briggs, E. I. Garvie and L. G. M. Newland (*J. Dairy Res.*, 1956, **23**, 315—318).—The main characteristics of two strains of *Streptococcus cremoris* HP maintained, one in New Zealand and the other in England, under slightly different cultural conditions differed little after 18 years. S. C. Jolly.

Some bacteriological aspects of commercially sterilized milk. I. Incidence and nature of spoilage. A. A. Nichols and M. R. Candy. **II. Type of spore-forming bacteria isolated.** M. R. Candy and A. A. Nichols (*J. Dairy Res.*, 1956, **23**, 319—328, 329—335).—I. The incidence of contamination by mesophilic and thermophilic spore-forming bacteria and the rate of spoilage at 30° of commercially sterilized milk varied with the season. The lower incidence of contamination in the summer was probably due to the introduction of fewer bacteria into the milk on the farms when the cows were not housed, and to increased processing times and temp. used in warm weather. Cleansed milk bottles were important sources of contamination by mesophilic spore-forming bacteria, but few thermophiles of the type commonly occurring in sterilized milk were found. Methods of reducing contamination are discussed.

II. The incidence of *Bacillus subtilis* and *B. licheniformis* strains tended to increase during winter and may be responsible for an increased rate of spoilage at 30°. *B. subtilis*, which rapidly digests milk under favourable conditions, was the most common spore-forming bacteria isolated; thermophilic spore-forming bacteria were frequently present and usually produced a firm acid clot in the milk. Classification of the thermophiles isolated is uncertain, and a general review of the classification of the aërobic thermophilic spore-forming bacteria is required. *Clostridium thermosaccharolyticum* was also isolated from the milk. S. C. Jolly.

Penicillin in milk. I. Rapid routine assay of low concentrations of penicillin in milk. II. Incidence of penicillin. N. J. Berridge and members of the National Agricultural Advisory Service. **III. Effect of low concentrations of penicillin on the rate of acid production by starter cultures.** N. J. Berridge (*J. Dairy Res.*, 1956, **23**, 336—341, 342—347, 348—354).—I. A simple rapid and sensitive microbiological method, based on acid production by *Streptococcus* *thermophilus*, is described for the determination of low concn. ($\not<$0·01 i.u. per ml.) of penicillin in milk. Pasteurization of milk that could not be tested on the day of collection was effected by heating at 62·5—65° for 30 min. or to 94° without affecting results of the assay.

II. The concn. of penicillin in 5000 samples of bulk milk taken in various parts of England and Wales rarely exceeded 0·1 i.u. per ml., although a significant proportion of samples contained more than one-third of the amount of penicillin that is believed likely to interfere in cheese-making. A 2- to 3- fold increase in the use of penicillin for treating dairy cows might cause difficulties.

III. Acid production by 6 of 12 strains of starter, both *Strept. lactis* and *Strept. cremoris*, currently used for cheese-making was not delayed until the concn. of penicillin in milk exceeded 0·096 i.u. per ml.; that by 3 strains was inhibited slightly by concn. of 0·096 i.u. per ml., and that by the remaining starters was reduced to $\sim$50% by concn. of 0·012 i.u. per ml. Cheese ripening was not affected by concn. of penicillin that failed to diminish starter activity. S. C. Jolly.

Interrelations of micro-organisms in cream. II. *Lactobacillus casei*, *Bacillus subtilis* and *Streptococcus lactis*. III. *Candida pseudotropicalis*, *Aerobacter aerogenes*, and *Streptococcus lactis*. L. G. Harmon and F. E. Nelson (*J. Dairy Sci.*, 1957, **40**, 1—10, 11—18).—II. When sterile cream was inoculated with *B. subtilis* (*B*) and incubated at 10°, 20° and 30°, population and amount of proteolysis increased with increase of temp., with max. tryptophan (**I**) concn. of 46 p.p.m. after three days at 30°; when *L. casei* (*L*) was used, max. **I** concn. was 9·4 p.p.m. after seven days. When *S. lactis* (*S*) was included with *B* substantial proteolysis occurred at all temp., but the population of *B* declined rapidly after max. population of *S* was attained. When *S* was inoculated with *L*, growth of *L* was unaffected but max. population of *S* was slightly reduced; max. **I** concn. was 29·6 p.p.m. after seven days at 20°. Bitter flavours suggestive of proteolysis occurred with $\not<$18—25 p.p.m. of **I**; cream containing $\not<$10 p.p.m. was usually of poorer quality, but not all samples of this quality contained $>$10 p.p.m. of **I**. Neither combination of micro-organisms produced amounts of water-insol. acids (**II**) sufficient to affect quality.

II. Neither *C. pseudotropicalis* (*C*) nor *A. aerogenes* (*A*), alone or in combination with *S*, when used to inoculate the cream produced as much as 400 mg. of **II** per 100 g. of fat; only *A* and *S* together increased **I** concn., giving 32 and 15 p.p.m. after three days at 20° and one day at 30° respectively. At 20°, *S* inhibited the growth of *C*; *A* inhibited the growth of *S*. At 10°, but not at 20°, *S* reduced the rate of organoleptic deterioration caused by *C*. Deterioration in organoleptic quality was apparently related more closely to increase in population than to chemical factors. S. C. Jolly.

Steam stripping of taints from liquids. V. Basic design of cream deodorizing equipment. J. K. Scott (*J. Dairy Res.*, 1956, **23**, 363—383).—The application of data presented previously (*idem, ibid.*, 1954, **21**, 354, 370; 1955, **22**, 302; 1956, **23**, 30) to the design of deodorizing equipment for cream is explained. Alternatively, the effect on volatile flavour components of existing or proposed plant can be determined, although the basic data cannot be used fully until the amounts and volatilities of the desirable and undesirable flavouring substances in cream used for butter making are known. S. C. Jolly.

Flavoured milks. V. L. S. Charley (*Dairy Sci. Abstr.*, 1957, **19**, *Rev. Art.*, No. 59, 267—276).—The prep., composition and nutritive value of chocolate milk products and fruit-flavoured milks are described. (26 references.) A. G. Pollard.

Structure of milk crumb. J. Saunders (*Chem. & Ind.*, 1957, 52—53).—Measurements of the power of dried mixtures of milk crumb constituents to reduce K ferricyanide provide clear evidence of a reaction between chocolate liquor and milk, and sucrose and milk which involves a blocking of reducing groups or a prevention of the exposure of reducing groups on heating. No such reaction takes place between chocolate liquors and sucrose. J. S. C.

Determination of diacetyl in Italian butters. M. Brogioni (*Ann. Falsif.*, 1956, **49**, 499—501; cf. *ibid.*, 1955, **48**, 56.)—The zone of max. absorption, according to the colour given by the reagent used, was at $\lambda = 350$ mμ. A graph was constructed with this λ and with increasing amounts of diacetyl of known strengths, brought up to 20 ml. to which were added 0·75 ml. of tetra-minodiphenyl hydrochloride and 0·75 ml. of conc. HCl. For determining the absorption of the base, a solution prepared from 20 ml. of water and of the reagent in amounts given above was used. The Lambert-Beer law was fully confirmed. Tabulated results for many sorts of butter gave 0·25—2 mg. per kg. Whether produced from sweet or acid cream, butter always contains diacetyl in the amounts mentioned. This contrasts with the assertion of Testoni, Ciusa and Cuppini. Their methods may have been insufficiently sensitive for the amounts in question. W. J. Wright.

Vitamin content of New Zealand butterfat. W. A. McGillivray (*N.Z. J. Sci. Tech.*, 1956, **38A**, 466—472).—Vitamin A, carotene, xanthophylls, tocopherol and iodine value were determined in factory butters from October to April and the seasonal trends previously reported are confirmed. (21 references.) J. S. C.

Limits of variation of indices of fats of butter. S. Kuzdzal-Savoie (*Ann. Falsif.*, 1956, **49**, 452—458).—Statistical analyses of iodine, saponification and volatile acidity values of butter and milk fat have been made. A wide variance in values depending on natural conditions and the breed of cow has been demonstrated. It is suggested that the limits of fat values admissible in France should agree with AVI × 100/AVS where AVI is the insol. volatile acidity and AVS the sol. C. A. Slater.

Minor fatty acid constituents of butter-fat. F. B. Shorland and R. P. Hansen (*Dairy Sci. Abstr.*, 1957, **19**, *Rev. Art.* No. 58, 167—189).—A review of fatty acid components occurring in proportions $<$2% of the total fatty acids in milk. (About 140 references.) A. G. Pollard.

Determination of carbonyl value. M. Barro-Raffel and G. Jacini (*Olii min*, 1956, **33**, 381—384).—A modification of the Terent'ev method for the determination of carbonyl value is described. Excess phenylhydrazine is titrated potentiometrically with some special precautions. The method is applied to a series of aldehydes and ketones with an average error of $\sim$0·5—0·6%. The method is suitable for the determination of volatile substances in rancid butters. C. A. Finch.

Critical examination of various methods for the determination of butterfat in the presence of coconut oil. H. Hadorn and H. Suter (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 512—535).—Various methods were used for the determination of butterfat in presence of coconut oil in prepared mixtures; that of Grossfeld gave the most reliable results for the semimicro determination of the butyric acid no. and the "residual no.". When much coconut oil ($>$30%) was present, an appropriate empirical formula was used to calculate the result. E. M. J.

Influence of penicillin on the manufacture and ripening of Cheddar cheese. H. R. Whitehead and D. J. Lane (*J. Dairy Res.*, 1956, **23**, 355—360).—Concn. of penicillin in milk $>$0·1 i.u. per ml. seriously affected the final quality of Cheddar cheese due to delaying acid production by the starter, which results in an abnormally high pH in the cheese and possibly a diminished moisture content. Penicillin had no direct effect on the ripening process. Concn. of 0·05 i.u. per ml. delayed cheese-making but did not affect adversely the final cheese quality. The addition of penicillinase neutralized any penicillin present, but a large excess of the enzyme was necessary if a contact time of $\sim$30 min. was used. S. C. Jolly.

Lactobacilli isolated from the surface of normal and slipcoat Stilton cheese. M. E. Sharpe and M. Brindley (*J. Dairy Res.*, 1956, **23**, 361—362).—Of 36 strains of lactobacilli isolated from surface scrapings of a large no. of normal and one slipcoat Stilton cheese at four different dairies, 26 were grouped serologically as *Lactobacillus plantarum*, one as *L. casei* and four as *L. brevis*. *L. plantarum* of serological type C3 was isolated from cheese, including the slipcoat, at each of the dairies, suggestive of a common source, which was not the milk. Conditions causing slipcoat and the prevailing pH apparently had no effect on species and type of lactobacilli on cheese coat. S. C. Jolly.

Control of accidental " blue " in cheese factories of the soft paste Camembert type. Influence of the medium, especially the rôle of salt. J. Jacquet (*C. R. Acad. Agric. Fr.*, 1957, **43**, 129—131).—The effects on the curds (pH 4—4·2) of the salting process, with NaCl or commercial salt containing small quantities of $MgCO_3$, and on the growth of micro-organisms, especially of the white mould *Penicillium caseicolum*, used in prep., and the blue mould *P. glaucum* are discussed. Increase in pH in the range to 8·2 by the presence of $MgCO_3$ inhibits the growth of *P. caseicolum* and favours that of *P. glaucum*. Control of " blue " is indicated by not using salts which modify the pH of the medium and selection of vigorous strain, e.g., of *P. caseicolum*. E. M. J.

Consumer trials for the determination of the acceptability of dehydrated beef. J. C. Hawke (*J. Sci. Food Agric.*, 1957, **8**, 197—205).—Acceptability of dehydrated beef was assessed more satisfactorily from large-scale consumer trials in Service establishments by means of questionnaires than by small-scale trials using the weight-of-rejection technique. Results of the questionnaire experiment indicated that minced beef which had been vac.-dried in edible fat, reconstituted and prepared under supervision was as acceptable as the fresh equivalent. Vac.-dried beef steak, similarly treated, was less acceptable than fresh steak, chiefly because it is slightly fibrous, but was about as acceptable as either fresh or dehydrated mince similarly flavoured and seasoned. E. M. J.

Fill and carcass changes of yard-fed and outwintered beef cattle turned on to spring pasture. J. C. Tayler, F. E. Alder and J. E. Rudman (*Nature, Lond.*, 1957, **179**, 197—198).—Cattle turned on to spring pasture show losses in live wt. or low rates of gain during the first few weeks. The results of a series of trials are reported and the patterns of live-wt. change observed are explained in terms of both fill losses and carcass gains. , J. S. C.

Analysis of fresh meat. E. J. Benne, N. H. Van Hall and A. M. Pearson (*J. Ass. off. agric. Chem., Wash.*, 1956, **39**, 937—945).—The prepared meat is rapidly passed through a grinding mill equipped with a fine mesh cutting plate and a fixed divider which rejects half the ground sample, the remainder being reground and divided until a homogeneous sample results. Technique for the determination of ash, crude fat (ether extract), moisture and protein is described in detail. For digestion (Kjeldahl) the sample is conveniently weighed on a tared piece of parchment paper, the meat being then rolled in the paper and the whole digested. For the determination of moisture, heating for 5 hr. for fat meat or 24 hr. for lean meat at 100—105° is recommended. A. A. ELDRIDGE.

Changes in water-holding capacity and cationic shifts during the heating, freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. E. Wierbicki, L. E. Kunkle and F. E. Deatherage (*Food Technol.*, 1957, **11**, 69—73).—A simplified centrifugal method was developed for the study of water-holding capacity (shrinkage) of meat. The technique was applied to the relationships of temp. of heating, added water, added salt, post-mortem age and freezing and thawing on the water-holding capacity of meat. Pronounced changes in proteins take place between 40° and 70°; pH values are increased; between 55° and 70° some reactions tend to promote protein hydration; dynamic shifts between meat and expressed juice involving Na, K, Ca ions occur. Drip losses on fresh and thawed frozen and aged meats are discussed. (12 references.) E. M. J.

Effects of added sodium chloride, potassium chloride, calcium chloride, magnesium chloride and citric acid on meat shrinkage at 70° and of added sodium chloride on drip losses after freezing and thawing. E. Wierbicki, V. R. Cahill and F. E. Deatherage (*Food Technol.*, 1957, **11**, 74—76).—Chlorides of Na, K, Ca and Mg added to meat prior to heating increase the water-holding capacity of the proteins at 70°; citric acid increased shrinkage. Beef was made more tender by treatment with solution containing NaCl and $MgCl_2$. When NaCl solution is added to meat prior to freezing, less drip is obtained on thawing. E. M. J.

Effect of reducing agents on cured meat colour. G. G. Kelley and B. M. Watts (*Food Technol.*, 1957, **11**, 114—116).—Cysteine, ascorbic acid and glutathione catalysed the production of nitric oxide hæmoglobin; regenerated the nitric oxide myoglobin on the surfaces of the light faded cured meat when residual nitrite was present and protected the surfaces of the cured meat from fading by light. (16 references.) E. M. J.

Effects of added chemicals on colour formation and maintenance of scalded sausages. M. S. Pohja and F. P. Niinivaara (*Dtsch. Lebensm. Rdsch.*, 1957, **53**, 64—68).—The colour formation in experimentally prepared scalded sausages, with and without polyphosphate (**I**), containing ascorbic acid (**II**), and containing **I** and **II** was studied. The following are discussed: pH values, NO_2 reduction, redox potential and colour formation. Sausages prepared without **I**, or with **II**, or with **I** + **II** were uniformly red; those containing **I** were flecked with grey colour and pH was increased. E. M. J.

Ion-exchange method of determining carnosine, anserine and their precursors in animal tissue. C. L. Davey (*Nature, Lond.*, 1957, **179**, 209—210).—The method involves the fractionation of basic amino-acids and the dipeptides, carnosine and anserine, by buffered elution from a 50-cm. column of 0·9 cm. diameter, packed with "Dowex-50" resin, 4% cross-linked. The amino-acids were estimated by a photometric ninhydrin method. J. S. C.

Use of tetrazolium salts for assessing the quality of iced white fish. J. M. Shewan and J. Liston (*J. Sci. Food Agric.*, 1957, **8**, 222—226).—A moistened test paper impregnated with a tetrazolium salt (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) is applied to the fish surface and after a stated interval the resultant red-coloured formazan is eluted and determined colorimetrically. Quality assessment, as judged by a taste panel, viable bacterial counts, and the contents of trimethylamine and volatile bases, can be correlated with formazan concn. E. M. J.

Volatile bases as quality indices of iced North Sea cod. J. M. Shewan and A. S. C. Ehrenberg (*J. Sci. Food Agric.*, 1957, **8**, 227—231).—Chemical and sensory variables measured at intervals during the spoilage of different catches of fish stored up to 22 days in ice indicated that different catches spoil at different rates as far as the deterioration in their sensorily perceptible qualities and their increasing content of volatile bases is concerned. Laboratory tests indicate that the volatile bases content of fish muscle could at best serve as a much less precise index of eating quality than at first seemed possible, and that different indices might have to be used for different fishing-grounds. E. M. J.

Expressible fluid of fish fillets. V. Cell damage in fillets frozen from one side: the general picture. R. M. Love (*J. Sci. Food Agric.*, 1957, **8**, 238—242).—Cell damage, as measured by the escape of deoxypentose nucleic acid (DNA) into the intercellular spaces, was studied in cod fillets frozen from one side at different speeds, the freezing time being taken as the time taken for the centre of the fillet to cool from 0 to − 5°. Three types of damage were revealed by peaks in the DNA/freezing time curves, at freezing times of ~27, 75 and >125 min. Photomicrographs of the muscle frozen in ~75 min. indicated that at this rate of freezing the change from intra- to extra-cellular freezing was occurring. Previous work under these conditions, but in fillets frozen from both sides, indicated the escape of much DNA. E. M. J.

Amino-acid composition of fish collagen and gelatin. J. E. Eastoe (*Biochem. J.*, 1957, **65**, 363—368).—Sturgeon, cod and shark collagens have an amino-acid content and distribution similar to that of mammalian collagen, but they have decreased amounts of proline and hydroxyproline and increased amounts of serine, threonine and, in some cases, methionine and hydroxylysine. Gelatin prepared from cod bone has a very low rigidity at 10°, whilst sturgeon, shark and Australian lung fish collagens yield gelatins with rigidities approx. the same as, but sometimes less than, those of mammalian gelatins. Generally, the shrinkage temp. of a collagen and the gel properties of the derived gelatin decrease with decreasing hydroxyproline content. Variations in properties and composition of fish collagens seem to be related to the water temp. of the normal habitat rather than to considerations of broad zoological classification. J. N. ASHLEY.

Transport and preservation of fish from fishing ports to consumers. J.-B. Verlot (*Industr. agric. aliment.*, 1956, **73**, 885—888).—A review of the distributive organization of the French fish trade. J. S. C.

2-Thiobarbituric acid method for the measurement of rancidity in fishery products. T. C. Yu and R. O. Sinnhuber (*Food Technol.*, 1957, **11**, 104—108).—The procedure described is made on the intact sample, eliminating the fat extraction step. The results on fish oil, fish meal, etc., are reproducible and the method can be used as a measure of oxidative rancidity in fishery products. (27 references.) E. M. J.

Seasonal fluctuations of colour and of vitamin A-activity of carcass fat. O. Dahl (*Z. LebensmittUntersuch.*, 1957, **105**, 180—188).—The colour intensity was measured in a photocell colorimeter which is described. In beef- and horse-fat there is a great seasonal fluctuation of colour intensity, increasing rapidly after 1½—2 months at pasture and decreasing during the stall period, the max. being 50% higher than the min. In comparison of horse- and beef-fat, the former has the stronger colour and greater activity of vitamin A; the fat of a yearling heifer has vitamin A activity similar to that of commercial beef tallow, but less than that of pure cow's fat, and has less colour. Analytical findings are discussed. (26 references.) E. M. J.

Polarographic estimation of vitamin A in cod-liver oil. W. Keller and F. Weiss (*Z. anal. Chem.*, 1957, **154**, 241—245).—Following the authors' previous communication (*Z. anal. Chem.*, 1955/56, **148**, 26), the application of this method to cod-liver oil is reported. Prep. for polarography involves, in the case of vitamin-A concentrates, saponification and ether extraction, and, in the case of cod-liver oil, a further chromatographic purification on specially pptd. alumina. A 5% correction has to be added to compensate for oxidation, the results then being in good agreement with colorimetric and biological methods. G. BURGER.

Simplified method for the determination of water, fat and starch in margarine. G. Lindstedt (*Mitt. Lebensm. Hyg., Bern*, 1956, **47**, 506—511).—Wachmuth's method, slightly modified, is described for the determination of water and fat in margarine and a colorimetric method is given for the determination of starch. All three tests can be made on 1 g. of margarine; there is saving of time and reagents and the accuracy is satisfactory for the practical control of margarine. E. M. J.

Technology of plasticizing of fatty materials and of margarine manufacture. E. Luotti (*Olii min.*, 1956, **33**, 384—388).—The need to improve production of margarine and shortenings by the use of closed and pressure machines for continuous production is emphasized. Modern German machinery is described. The necessary properties for a suitable raw material for margarine or shortening are described. C. A. Finch.

Processing, storage and nutritive value of cottonseed oil. V. Subrahmanayan, M. Narayana Rao, K. Krishnamurthy, S. Kuppuswami, M. Swaminathan and D. S. Bhatia (*J. Proc. Oil Technol. Ass., India,* 1954, [1955], **10,** 64—69).—Crude cottonseed oil has a high stability which is attributed to the antioxidant properties of gossypol. Refined cottonseed oil rapidly develops peroxide value. Feeding experiments (with rats) showed no significant difference in growth promotion, digestibility or Ca and P utilization of crude, refined and hydrogenated cottonseed oil, refined groundnut oil, and cow's ghee. (14 references.) J. S. C.

Phosphatides of Indian vegetable oils. II. Determination of phosphatide contents spectrophotometrically. K. V. Rao, B. Appu Rao and K. S. Murti (*J. sci. industr. Res.,* 1956, **15C,** 224—226).—The phosphatide contents of 10 samples each of Indian cottonseed and groundnut oils have been determined spectrophotometrically. The phosphatide contents of groundnut oil samples vary from 0·15 to 0·87% and of cottonseed oil samples from 0·46 to 2·40%. O. M. Whitton.

Mbocaya or Paraguay cocopalm—an important source of oil. K. S. Markley (*Econ. Bot.,* 1956, **10,** 1—32).—*Acrocomia totai* is a common native palm of Paraguay. The mesocarp of the fruit contains 16 to 30% of oil and the kernel 55 to 65%. Pulp oils have an iodine value of about 60 and a saponification value of 197—209 and kernel oils, iodine values of about 30 and saponification values of 240. Descriptions of the palms and methods of utilization are given. L. G. G. Warne.

Biological effects of polymeric residues isolated from autoxidized fats. H. Kaunitz, C. A. Slanetz, R. E. Johnson, H. B. Knight, D. H. Saunders and D. Swern (*J. Amer. Oil Chem. Soc.,* 1956, **33,** 630—634).—The introduction into liberally vitaminized diets of 10% of the residues obtained after the molecular distillation of highly oxidized lard or cottonseed oil causes stunted growth and serious digestive disturbances in young rats. The distillates obtained at 200—280° are harmless, whilst that obtained at 280—300° has moderate effects of the above type. Diets containing 20% of the residues cause rapid death. Smaller proportions of the residues are tolerated without permanent injury after normal diets have been restored. The polymeric residue from cottonseed oil is more injurious than that from lard. Addition of fresh fat to the residues decreases their growth-stunting effect. Addition of the residues (4—10%) to restricted diets nullifies the normal reduction in calorific requirement for wt. maintenance. In this respect, the residues from both fats are equally effective. (20 references.) P. S. Arup.

Use of differential curves in the dilatometry of fats. H. Jasperson and A. A. McKerrigan (*J. Sci. Food Agric.,* 1957, **8,** 46—54).—Differential dilatometric curves in which the rate of expansion is plotted against temp. were used to assess the amount of melting of an individual fat or mixture of fats, through a range of temp. (10—50°), covering their commercial usefulness. Characteristic curves are given of single fats, hydrogenated fats and blended fats and they are discussed in relation to their predominant glycerides. E. M. J.

Effect of high-energy radiation on fats. J. R. Chipault and O. S. Privett (*A. R. Hormel Inst.,* 1955—56, 22—29).—The effects of temp. of irradiation and of subsequent storage temp. on the development of peroxides and carbonyl compounds in fatty acid esters differ with the ester; several low irradiation doses produced greater changes than did an equiv. large single dose. The odour of ozonized fats resembles that of irradiated fats and the colours of reduced ozonides and irradiated Me oleate and linoleate are also similar. Ozonides at high concn. can be differentiated from hydroperoxides by i.r. spectroscopy of the products formed by reduction with a Lindlar catalyst. Irradiation of Me linoleate, but not of Me oleate, in the solid condition resulted in the formation of solid insol. polymers containing *trans* double bonds; liquid sol. polymers, also with *trans* double bonds, were formed from Na oleate and linoleate. I.r. examination of polymers formed by irradiation of Me linoleate-urea inclusion compounds showed the presence of sec. amides. Monocarbonyl compounds with >8 C atoms and dicarbonyl compounds were formed by irradiating Me oleate. S. C. Jolly.

Determination of structure and analysis of highly unsaturated and saturated acids of fish oils. O. S. Privett (*A. R. Hormel Inst.,* 1955—56, 59—61).—Modifications are reported for improving the accuracy of the alkali-isomerization spectrophotometric technique for the analysis of fatty acid mixtures. Concentrates of polyunsaturated fatty acids (I vol. >400) have been prepared from tuna and menhaden oils by urea fractionation of the Bu esters; the fatty acids may be obtained from these concentrates by a combination of distillation and chromatography. The action of lipoxidase on fish oils is reported. The majority of the double bonds in the polyunsaturated fatty acids of menhaden oil are arranged in the methylene-interrupted type of unsaturation. S. C. Jolly.

Odour problem in fish oils. J. R. Chipault (*A. R. Hormel Inst.,* 1955—56, 61—65).—Oxidation of menhaden oil by air at either 100° or 40° yielded appreciable amounts of strongly odoriferous compounds which were separated by vac. steam distillation. The ether-sol. compounds in the volatile fraction were more odoriferous than were the water-sol. compounds; an appreciable portion of these materials was acidic. Carbonyl compounds in the volatile fractions have been identified tentatively as (i) acrolein or acetaldehyde or a mixture of both, (ii) crotonaldehyde or propionaldehyde or a mixture of both, (iii) butyraldehyde, (iv) valeraldehyde and (v) heptaldehyde. S. C. Jolly.

Chemistry of inclusion-type complexes. H. Schlenk (*A. R. Hormel Inst.,* 1955—56, 66—71).—Deoxycholic acid forms complexes with fatty acids, binding preferentially long-chain and saturated acids from mixtures. The method is unsuited to analytical and preparative techniques due to incomplete recovery of fatty acids from the choleic acids formed. Fatty acids from menhaden oil are made up of C_{14} (13·8), C_{16} (33·6), C_{18} (20·6), C_{20} (19·4), C_{22} (10·6) and possibly C_{24} and higher (2%). Approx. 1% of the total fatty acids consisted of two unidentified, but possibly C_{15} and C_{17}, acids not previously reported in this oil. The C_{18} and C_{16} fatty acids contained from 0 to 4 double bonds, octadecatetraenoic and hexadecatetraenoic acids representing $\sim$5% of the respective fractions. The C_{14} acids are less unsaturated. S. C. Jolly.

Fish oil oxidation products formed under conditions of chamois tannage. I. Formation of polymerized products and changes in composition on autoxidation. II. Chromatographic separation of 2 : 4-dinitrophenylhydrazones of volatile oxidation products. A. Küntzel and T. Nungesser (*Leder,* 1956, **7,** 73—78; 115—119).—I. Cotton-wool impregnated with oil is subjected to a stream of air at 80°: volatile products are condensed and aldehydes determined with 2 : 4-dinitrophenylhydrazine. The residue is extracted with CCl_4, leaving an insol. polymer. Oils classified by amount of polymer formation gave: light cod, seal, dark cod, herring (in order of decreasing amount).

II. The presence of aldehydes is established in the volatile products from the oxidation of fish oils by air: they include dialdehydes. S. E. Fox.

Isolation of piperonyl butoxide from oils, fats and waxes. H. L. Williams and J. P. Sweeney (*J. Ass. off. agric. Chem., Wash,* 1956, **39,** 975—980).—With the chromatographic procedure described recoveries of 92·6—106·6% of piperonyl butoxide added to various materials (grains, nuts, paper) were obtained. Use of Florisil as absorbent gives uniformly good recoveries and the eluate is free from turbidity. Mixtures of Skellysolve F and ethyl ether are used as eluting solutions. Colour is developed with tannic acid in a mixture of acetic and phosphoric acids and the absorbance read in a photoelectric colorimeter. A. A. Eldridge.

Application of chromatography to separation problems in the lipin field. R. T. Holman (*A. R. Hormel Inst.,* 1955—56, 32—35).—The application of displacement chromatography to the separation of natural lipin mixtures for analytical and preparative purposes is reported. The products of the action of *Mycobacterium phlei* and *Pseudomonas fluorescens* on Me linoleate have been investigated. Using this method, the isolation of 100—300-mg. quantities of apparently pure polyunsaturated fatty acids from $<1·0$ g. of concentrate has been effected for the following: octadecatetraenoic acid and Et octadecatetraenoate from cod-liver oil; eicosadienoic acid from rape-seed oil; Me eicosapentaenoate and Et arachidonate from hog liver; and Me docosahexaenoate from hog brain. Displacement chromatography of tall oil fatty acids showed (in order of displacement) the presence of a dieneconjugated substance, linoleic acid, a non-polyunsaturated fatty acid, and another diene-conjugated substance. S. C. Jolly.

Separation and identification of lipins. H. Schlenk (*A. R. Hormel Inst.,* 1955—56, 54—58).—The quantitative analysis of mixtures of stearic, oleic, linoleic and linolenic acids and their deriv., including their monoacid triglycerides, with an error of $< \pm 10\%$ is reported using paper chromatography. Different chain lengths (C_{12}—C_{22}) in a mixture can be determined by this method with the same accuracy; samples consisting of components with different chain lengths and unsaturation require separation according to chain length before chromatographing. Pure authentic samples are necessary for identifying the components of mixtures, but the possibility of overcoming this disadvantage is discussed. S. C. Jolly.

Phospholipins in foods. I. Commercial production of phospholipins and their uses in foods. II. Classes and formulæ of phospholipins. H. H. Hutt (*Chem. & Ind.,* 1956, 1409—1411, 1412—1415).

—I. Recent developments in the application of lecithin to food manufacture are reviewed. (28 references.)

II. A table of general formulæ and relevant literature references for: phosphatidic acid, phosphatidyl-ethanolamine ("Cephalin"), phosphatidyl-choline ("Lecithin"), phosphatidyl-serine, β-lysolecithin, α-bis-phosphatidic acid, phosphatidyl-inositol, diphosphoglyceroinositide, phosphatidyl-inosityl-phosphonyl-ethanolamine glycoside, phosphatidyl-ethanolphosphoramidyl-inositol glycoside, ethanolamine-phosphoglyceracetal, choline-phosphoglycero-hemiacetal, cardiolipin, sphingomyelin (lignoceramido-*N*-sphingosine choline-phosphoric acid diester), ceramide phosphate, cerebronyl-*N*-sphingosine (hydroxylignoceramido-*N*-sphingosine), cerebrosides and phytosphingosine. (20 references.) J. S. C.

Deteriorative reactions involving phospholipins and lipoproteins. C. H. Lea (*J. Sci. Food Agric.*, 1957, **8**, 1—13).—Changes which are liable to occur in the phospholipin constituents of foods during processing and storage such as (i) denaturation of lipoprotein, usually with liberation of lipin, (ii) hydrolysis of phospholipin by tissue or microbial enzymes and spontaneous decomposition, (iii) autoxidation of unsaturated fatty acid constituents of phospholipins followed by fission, browning, polymerization, etc., (iv) lipin browning reactions between phospholipin free amino-groups and aldehyde groups of sugars, etc., (v) lipin–protein co-polymerization reactions are reviewed. Deterioration in individual commodities, e.g., commercial "lecithin," milk and butter, eggs and dried blood plasma is discussed. (66 references.) E. M. J.

Surface force and stability of lecithin sols. P. H. Elworthy and L. Saunders (*J. chem. Soc.*, 1957, 330—335).—The stability of lecithin sols in presence of electrolytes depends on the method of prep. of the lecithin; lecithin which has been subjected to harsh chemical treatment gives very unstable sols. Small quantities of soaps in the lecithin greatly increase the stability. Both uni- and bi-valent metal chlorides give surface forces at the sol–water interface. The production of surface force is governed by the degree of instability of the sols. The lecithin sol–water interface and the surfaces of some simple cells are similar in many respects. I. Jones.

Experiments on cocoa fermentation in West Africa. G. R. Howat, B. D. Powell and G. A. R. Wood (*J. Sci. Food Agric.*, 1957, **8**, 65—72).—Experiments on cocoa fermentation using heaps, boxes and steel vessels are reported. Different methods of fermentation were studied to include changes in the moisture content of the beans, pH of the cotyledons and pulp during fermentation and drying, and of the composition of the gas surrounding the beans. A small fermentation vessel for beans from 150 to 500 pods was tested, found to be satisfactory and is recommended for use. Beans from pods which have been harvested a week before breaking, and beans from unripe pods, can be fermented without modification of the standard box method. Transportation of wet beans and delays of up to 24 hr. before beginning of fermentation had no adverse effects on the fermented beans judged by appearance and chocolate flavour. E. M. J.

Soluble carbohydrates of raw materials of the chocolate industry. II. Oligosaccharides of walnuts and almonds. H. Thaler (*Z. LebensmittUntersuch.*, 1957, **105**, 198—200; cf. J.S.F.A. Abstr., 1957, i, 259).—Examination of hazelnuts, walnuts, almonds, groundnuts, coconuts, and seeds of *Cardamomum racemosun* by paper chromatography indicated stachyose in all cases, richly occurring in cardamom seeds, as traces only in walnuts. Raffinose was found in traces in walnuts, chiefly not found in coconuts and in the remaining seeds was found in measurable quantities. Sucrose was richly occurring in all, glucose in sparing quantities and not in cardamom seeds; fructose was found in hazelnuts and groundnuts only. E. M. J.

Shark liver residue: determination of B-vitamins and preparation of proteolysed extracts. K. V. Rajagopalan and P. S. Sarma (*J. sci. industr. Res.*, 1956, **15C**, 227—231).—Four samples of dry oil-free shark liver residue have been prepared from liver residue remaining after aq. extraction of whole liver for the liver oil. Two whole-liver preparations have been similarly obtained. Proteolysed extracts have been prepared from the oil-free dry liver residue using crude papain and crude pancreatin. The extracts obtained thus as well as the various prep. of liver residue have been analysed for their contents of the B vitamins, viz., thiamine, riboflavin, nicotinic acid, vitamin B_6, pantothenic acid, biotin, folic acid, vitamin B_{12}, choline, and inositol. Shark liver is a good source of B-vitamins and can be used for the prep. of cheap and nutritionally potent liver extracts. O. M. Whitton.

Thermal destruction rates of thiamine in pureed meats and vegetables. E. Feliciotti and W. B. Esselen (*Food Technol.*, 1957, **11**, 77—84).—The rate of destruction of thiamine in aq. phosphate-buffered solutions and selected low-acid foods was studied in the temp. range of 228—300°F. The reaction of cleavage of the thiamine mol. was of the first order, and the energy of activation for the breakdown was 27—28·8 kg.-cal./mole in the different media. The rate of thiamine destruction in phosphate buffered solutions in the pH range of 4·5 to 7·0 increased with increasing pH; the most pronounced change occurred between pH 6·0 and 6·5. Thiamine in foods is more resistant to thermal breakdown than is the pure vitamin in aq. of buffered solutions. (34 references.) E. M. J.

Circular paper chromatography. X. Separation, identification and quantitative estimation of riboflavin and flavin compounds. K. V. Giri and P. R. Krishnaswamy (*J. Indian Inst. Sci.*, 1956, **38A**, 232—244).—A method is described for separation, identification and quant. determination of riboflavin and its deriv. (flavin mononucleotide, flavinadenine dinucleotide, lumiflavin and lumichrome) using a solvent mixture of *n*-butanol–acetic acid–water in the proportions 4 : 1 : 5. Quant. determination was made by cutting the bands from the chromatogram, elution with water and measurement of fluorescence. The use of the method for studying the riboflavin metabolism of a mutant yeast is described. (23 references.) J. S. C.

Content of essential amino-acids in some foods and fodders. K. Nehring and E. Schwerdtfeger (*Z. LebensmittUntersuch.*, 1957, **105**, 12—21).—Detailed analytical results, obtained by paper chromatographic methods, of % of essential amino-acids in proteins of animal origin, cereals, leguminous seeds, green fodder, cabbages, oil-seed residues, potatoes and yeast are given. These tests provide a distinction between the various groups of proteins and a rough survey over the various food proteins with corresponding valuation and possibility of completion, in consideration of essential amino-acid contents of the various species and also the quality. The state of development, effects of weather and manuring are significant influences and the importance of these factors in plant breeding is indicated. (11 references.) E. M. J.

Compleximetry in organic analysis. II. Determination of amino-acids and peptides. B. Buděšínský (*Chem. Listy*, 1956, **50**, 1236—1240; *Coll. Trav. chim. Tchécosl.*, 1957, **22**, 230).—The simple method developed consists in treating the amino-acid or peptide with a suspension of copper phosphate and titrating the Cu in the soluble complex formed with EDTA, using 1-(2-pyridylazo)-2-naphthol as indicator. The complexes of various peptides and amino-acids were isolated and analysed, and their structure is discussed. J. Zýka.

Binding amino-acids on strongly acid cation-exchangers. G. Naumann (*Chem. Tech., Berlin*, 1956, **8**, 715—718).—An experimental study is made of the adsorption of amino-acids of varying degrees of acidity on a Wofatit KPS 200 exchanger (a sulphonic acid resin) and of their desorption by various media and by titration. All acids are adsorbed equally well, and their desorptions on treatment with HCl are very similar. Desorption by elution with water however falls with the basicity of the acid. Desorption with aq. NaOH in suitably low amount gives the free amino-acids in good yield; excess aq. NaOH produces the Na salts. There is apparently some loose binding of the amino-groups to the SO_3H groups of the exchanger in adsorption but no formation of a carboxylic acid resin. H. L. Whitehead.

Synthesis of some amino-acids and related products. I. P. B. Mahajani and J. N. Ray (*J. Indian chem. Soc.*, 1956, **33**, 455—458).—Amino-acids are prepared by condensing methyleneimino-acetonitrile with aldehydes in alcohol in the presence of NaOEt, the nitrile first formed being hydrolysed with 10% H_2SO_4. Thus, methyleneimino-acetonitrile and paraformaldehyde in EtOH and NaOEt are heated at 50—60° and then left at 40° for 12 hr. Acidification with AcOH gives *2-cyano-2-methyleneaminoethanol*, m.p. 64°, hydrolysed to DL-serine with 10% H_2SO_4. DL-Threonine is similarly prepared from paraldehyde. The method is used for the preparation of 3-aminocoumarin deriv., *8-methoxyaminocoumarin*, m.p. 201°, being prepared from *o*-methoxysalicylaldehyde. I. Jones.

Quantitative determinations of amino-acids in undesalted hydrolysates by buffer filter-paper chromatography. B. E. Baker and N. A. Khan (*J. Sci. Food Agric.*, 1957, **8**, 217—222).—The use of five newly developed solvent systems for quant. chromatographic separation of amino-acids of undesalted casein hydrolysates is discussed. The values obtained were in agreement with those reported in the literature. The coeff. of variation of individual amino-acids ranged from 7·4% for glycine to 1·3% for arginine. E. M. J.

Limit test for fluorides in foods. E. H. Vogelenzang (*Pharm. Weekbl.*, 1956, **91**, 905—910).—A limit test which is to be included in Pharm. Dutch is based on the colour change from red to yellow which occurs on the addition of F′ to a reagent containing $Zr(NO_3)_4$ and Na alizarinesulphonate in acid solution. The test solution (10 ml. of distillate) is added to the acidified reagent, and the colour produced is compared with that given by 10 ml. of a standard solution containing 50 mg. of F′. Starting with 1 g. of the sample, a limit of 50 mg. of F′ can be detected. This test is more convenient than the similarly based titration methods of the U.S.P. and Pharm. Dan. (26 references.) P. S. Arup.

Effect of fluoride on dental conditions. J. Forrest (*Chem. & Ind.*, 1957, 64—66).—Fluoridation studies in U.S.A., Canada and the U.K. are reported and discussed. J. S. C.

Automation for the food plant. K. W. Moseley (*Food Technol.*, 1957, **11,** 117—118).—A discussion of specific problems common in plants. E. M. J.

Stainless steel in the food industries. P. Chave (*Ind. agric. aliment.*, 1956, **73,** 869—870).—A general review of the applications of the stainless steels for storage and transport of food products, and in processing, particularly of dairy produce, vegetable conserves and vinegar. J. S. C.

Making of free-flowing table salt. G. I. Finch (*J. sci. industr. Res.*, 1956, **15A,** 539—542).—Free-flowing salt is produced by controlled crystallization to produce an optimum grain size and coating the crystals by warm tumbling in an atmosphere of paraffin wax vapour. J. S. C.

Red pepper. I. New method for the determination of pungency in red pepper. J. I. Suzuki, F. Tausig and R. E. Morse (*Food Technol.*, 1957, **11,** 100—104).—To eliminate the difficulty encountered in measuring the pungency of hot red peppers, a method, proved reliable and reproducible, is reported. Capsaicin is isolated, its spectrophotometric characteristics are established and chromatographic techniques are developed for its separation and measurement in red pepper and pepper oleoresin. (25 references.) E. M. J.

Food and colour. M. R. Verma and R. Dass (*J. sci. industr. Res.*, 1956, **15A,** 425—431).—A review of synthetic and natural colours used in food preparation. (33 references.) I. Jones.

Proc. 2nd Int. Congr. Surface Activity, Lond., 1957, 2 (Preprints). Identification of surface components on the bacterial cell wall. A. M. James (pp. BB 138—144). **Action of lytic agents on surface structures of the bacterial cell.** M. R. J. Salton (pp. FI 274—282).—A review with 28 references. **Surface chemical studies on the protoplast membrane of** *Micrococcus lysodeikiticus*. A. R. Gilby and A. V. Few (pp. FA 488—496).—A review with 19 references. **Interaction of molecular layers of fatty acids with urea-treated [-denatured] serum albumin.** H. Sobotka, R. Santamaria and M. Demeny (pp. DB 175—177). **Film formation from phosphatide sols.** L. Saunders (pp. AE 56—60). H. S. R.

Chemical preservatives. II. Determination of antimicrobial activity of chemical preservatives. K. Raible and G. Busch (*Z. LebensmittUntersuch.*, 1957, **105,** 174—180).—When wine yeast was grown in samples of malt wort containing varying concn. of chemical preservatives in quantities sufficient to arrest, but not to inhibit growth completely, the course of the growth curves was in close relationship to the quantity of preservative used. A close connexion exists between the applied preservative concn. and the finally attained cell density. Practical examples are given and discussed in the development of a process of preservative evaluation. E. M. J.

Storage of roasted and salted cashew kernels. M. Prasad, N. S. Kapur and P. B. Mathur (*J. Proc. Oil Technol. Ass., India*, 1954, [1955], **10,** 55—63).—Cashew kernels were roasted in 11 vegetable oils to find a suitable medium conferring long storage life. By roasting in "Vanaspati," the product can be preserved for six months in sealed cans under ordinary atmosphere. The optimum quantity of "Vanaspati," added prior to roasting, is 6—9 g./200 g. kernels. Deterioration of the roasted kernels arises mainly from rancidity in the surface film of the medium. (15 references.) J. S. C.

Foodstuffs from starch-containing powders. Nibb-it Products Ass., S.A. (Inventor: D. R. d'A. Gerkens) (B.P. 750,217, 31.7.52).—Dry powder made from starch-containing tubers (potatoes) is admixed with water (≯35, preferably 10—20%), and the mixture is pressed, to form a tough, hard, storage-stable product which can be fried rapidly in oil without further drying. F. R. Basford.

Baked and/or toasted cereal products. P. O. Stokkebyes Kvarn A.-B. (Inventors: G. H. Blomqvist and A. I. Malm) (B.P. 751,122, 26.2.54).—A baked and/or toasted cereal product containing a high % of oats, e.g., toasted oat-flakes, oat biscuits, etc., is compounded with 0·1—12% of coconut oil, to minimize formation of bitter substances (from the oats) during storage. F. R. Basford.

Manufacture of hard sugar. Svenska Sockerfabriks A.-B. (B.P. 750,055, 12.4.54. Sw. 24.4.53).—The production of hard (cube) sugar of improved properties, by effecting crystallization of a sugar solution in batch-handling vacuum pans then separating the sugar crystals from the mother liquor (massecuites), is characterized by yielding crystals of predetermined average size and mixing, prior to centrifuging, the massecuites from strikes in such proportions that the crystals display a considerably higher percentage mean deviation from their average size than do the crystals in any single one of the different massecuites mixed. F. R. Basford.

Trimming [of butt and crown of pineapples]. Food Machinery and Chemical Corp. (B.P. 750,283, 25.2.54. U.S., 9.6.53). K. Ridgway.

[**Removal of shells, skins, etc. from nuts, etc.**] Slasto Co. of Africa (Pty), Ltd. (Inventor: O. Thaning) (B.P. 750,345, 31.8.53).—The (cashew) nuts are soaked in 3% aq. NaCl, gradually cooled to −20° and then passed through a high-frequency electric field (~15 Mc/sec.) whereby the frozen outer shells are burst off by instantaneous evaporation of the liquid between the shell and the nut meat. The nuts are then submerged in a saline solution acting as a carrier for sonic waves (~27,000 cycles per sec.) so that the inner rind is detached and may be removed, e.g., by flotation. J. M. Jacobs.

Curing of meat. R. H. Starr (B.P. 751,255, 19.3.54).—Curing of meat with NaCl in aq. solution is accelerated in presence of (hydrated) Ca lactate (~15% on NaCl) and lactic acid (~5 wt.-% on lactate), at pH 5·8—6·3. F. R. Basford.

Extraction of non-fatty materials from animal or vegetable materials. British Glues and Chemicals, Ltd. (Inventor: I. H. Chayen) (B.P. 750,230, 20.2.53).—Extraction of non-fatty substances (e.g., vitamin B_{12} or vanilla) from animal or vegetable material (of low fat content), e.g., liver or vanilla pods, is effected by subjecting to repeated intense impact (hammer mill) in presence of 1—30 (5—10) pt. of a fluid, e.g., water, hydrocarbon, glycol, ketone, halogenohydrocarbon, or alcohol, to disrupt the structure of the material, so that a substantial proportion of the solid residue is not reduced to colloidal size. F. R. Basford.

Chocolate flavouring material. General Foods Corp. (B.P. 751,121, 25.2.54. U.S., 10.7.53).—Water-sol., full-bodied chocolate flavouring material is obtained by extracting unroasted cacao beans with 5 pt. of water at 175—325°F. during 30—120 min., then concentrating *in vacuo* and roasting the extract (optionally in presence of other taste ingredient), e.g., spray-drying during 3—12 min. at 190—350°F. F. R. Basford.

Solvents and/or fixatives. Chemodemica Chemikalien u. Arzneimittel-Vertriebsges. Creutzberg & Co. (B.P. 748,301, 19.8.53. Austria, 26.8.52).—Solutions of natural or synthetic aromatic or flavouring substances or of pure substances isolated therefrom used in the cosmetic, perfumery, pharmaceutical, beverage and food industries are produced by dissolving the substances (preferably 10—50%) in *iso*propylidene glycerol or mixtures with one or more solvents (e.g., water, ethylidene glycerol) miscible with the glycerol. I. Jones.

Antibiotic product having a specific action against microbes causing fermentation and decomposition of foods. Industrie Prodotti Agricoli, I.p.A. (B.P. 750,797, 26.3.53. It., 25.6.52).—The desired antibiotic product is prepared by permitting spontaneous growth of the noxious organisms on the kind of food to be preserved, then subjecting them to treatment to cause the slow death of the organisms, viz., radiations from a source located at a distance progressively decreasing and for a time decreasing proportionally to said distance, or chemical treatment with increasing amounts (during decreasing periods) of toxic agents, the treatment being such that the organisms, while still alive, are capable of producing antibacterial substance which is toxic to the organism. F. R. Basford.

Azulenes. K. Ziegler (B.P. 750,209, 23.7.54. Ger., 7.8.53 and 30.6.54).—Azulenes are obtained in improved yield by heating $\mathrm{R{:}CH{\cdot}CH{:}CH{\cdot}CH{:}CHX}$ in presence of liquid diluent, with rapid removal of the product (R is *cyclo*penta-2 : 4-dien-1-yl, X is halogen, OH, alkoxy or NH_2). Thus, a solution of 1-(*N*-methylanilido)penta-1 : 3-dien-5-al in abs. EtOH is added with di*cyclo*pentadiene to Na–EtOH with cooling. The resulting magma is filtered, and the solid is washed with dil. aq. AcOH, then dried, to give 5-(N-*methylanilido*) *pentadienylidene*-cyclo*pentadiene*, m.p. 111—112°. This is heated to 150—200° *in vacuo*, the resulting distillate is cooled to −80° during 5 hr., and a solution of the pptd. solid in ether is washed several times with 0·1N-HCl, then evaporated, to give azulene, m.p. 96—98°. F. R. Basford.

3.—SANITATION

Identification of stored products insects by the microbiology of the Exoskeleton. III. Identification of larval fragments and their significance in sanitation averages. O'D. L. Kurtz (*J. Ass. off.*

agric. Chem., Wash., 1956, **39**, 990—1014).—Morphological characteristics useful in identification of various insects are described and illustrated. A. A. ELDRIDGE.

Determination of methyl bromide in foodstuffs treated by this insecticide. P. Desbaumes and J. Deshusses (*Mitt. Lebensm. Hyg., Bern.*, 1956, **47**, 550—561).—The sample (e.g., of grain) is heated at 70° and the MeBr is driven by a current of air through a quartz tube heated to 1000°. The decomposition products are led into a solution of NaOH containing H_2O_2 and the Br is titrated according to the method of Bognar and Vereskoï. MeBr disappears very quickly from foodstuffs that have been treated, with the exception of dry fruits in shells (e.g., nuts). (34 references.) E. M. J.

Resistance to organic phosphorus insecticides of the housefly. J. Keiding (*Science*, 1956, **123**, 1173—1174).—Houseflies have been increasingly resistant to org. P insecticides following the use of parathion (*OO*-diethyl *O*-*p*-nitrophenyl thionophosphate), Diazinon (*OO*-diethyl *O*-2-*iso*propyl-4-methylpyrimid-6-yl thionophosphate), and Resistox (3-chloro-4-methylumbelliferone *OO*-diethyl thionophosphate) used over a period of three years. T. G. MORRIS.

Energy relationships in physical toxicity [of narcotics to warm-blooded animals]. D. J. Crisp and D. H. A. Marr (*Proc. 2nd Int. Congr. Surface Activity, Lond.*, 1957, **2**, Prepr. FJ 429—438).— H. S. R.

Importance of chemical research of drinking water. J. Müller (*Z. LebensmittUntersuch.*, 1957, **105**, 211—216).—Based on the experience of many years of analysis of drinking water, the importance of chemical research on water was discussed, including the absolute necessity for establishing reliable judgement of drinking- and useful waters. E. M. J.

Colorimetric method for determining dissolved oxygen. C. S. Oulman and E. R. Baumann (*Sewage industr. Wastes*, 1956, **28**, 1461—1465).—The use of a spectrophotometer for measuring the colour intensity of the iodine solution obtained in the Winkler method is described. J. S. C.

Evaluation of broad field disposal of sugar beet wastes. G. J. Hopkins, J. K. Neël and F. L. Nelson (*Sewage industr. Wastes*, 1956, **28**, 1466—1474).—A further evaluation of broad field disposal of sugar beet wastes (cf. J.S.F.A. Abstr., 1956, i, 240) in which the overall efficiency of disposal, the purification obtained in various segments of the grassland area, detention time and flow patterns were particularly studied. J. S. C.

Biological processes in estuarine environment. VIII. Iron bacteria as gradient organisms. L. G. M. Baas Becking, E. J. Ferguson Wood and I. R. Kaplan. **IX. Observations on total base.** L. G. M. Baas Becking (*Proc. K. Ned. Akad. Met.*, 1956, **59B**, 398—407; 408—420).—VIII. The work of Pringsheim (*Biol. Rev.*, 1949, **12**, 200) and van Beneden (*Hydrologia*, 1951, **3**, 1) on the development of iron bacteria is confirmed using a freshwater as well as a marine medium. Aëration of water after percolation through a mud layer gives a development of iron bacteria at the point of aëration in a short time. If sulphate reduction occurs in the mud and the effluent contains H_2S and/or SH, *Thiothrix*, *Thiovulum* and *Beggiatoa* may appear. These organisms also require a steep change of the electrode potential, but at a lower level than the iron bacteria. H_2 is generated during the oxidation of the percolates. The pH–E_h limits of iron bacteria, based on 23 sets of measurements, are outlined.

IX. The empirical K_1 of the carbonic acid–bicarbonate system (K_1 = first dissociation constant of H_2CO_3 $\sim 4 \times 10^{-7}$ at 20°) approaches closely the value for pure water given by Harned and Davis (*J. Amer. chem. Soc.*, 1943, **65**, 2030) but is significantly different from the values derived from ionic activity in sea water (10^{-6} for 20% Cl at 20°) by Buch *et al.* (*Inst. p. l'Explor. de la Mer, Rapp et Proc. Verb.*, 1932, **5**, 79). Other buffering systems are observed in estuarine waters, the most important being the $H^{\cdot} + SH' \rightleftharpoons H_2S$ system which may cause a hundredfold increase in the total base (**I**). The presence of ammonia, amines, phosphate and org. acids might influence **I**. **I** may be considerably lowered at a high, constant salinity. Shaking sea water with air, with several types of mud, with purified sand or with $CaCO_3$ causes no significant changes in **I**. Apart from dilution, changes in **I** in the estuarine environment are most probably caused by biological processes. I. JONES.

Destruction of micro-organisms in water, sewage and sewage sludge by ionizing radiations. H. N. Lowe, jun., W. J. Lacy, B. F. Swikiewicz and R. F. Jaeger (*J. Amer. Wat. Wks Ass.*, 1956, **48**, 1363—1372).—Samples of double-distilled water and autoclaved sewage were inoculated with *Bacillus subtilis*, *Mycobacterium smegmatus*, *Micrococcus pyogenes* var. *aureus*, *Escherichia coli* and *E. coli* phage T3, and exposed to γ-rays from ^{60}Co. Samples of settled sewage and sewage sludge were similarly irradiated. All the tests organisms were destroyed, the dosage for 100% destruction ranging from $7{\cdot}5 \times 10^4$ to $2{\cdot}0 \times 10^6$ rad (1 rad = radiation dose of 100 ergs per g. of material). (20 references.) J. S. C.

Derivatives of chrysanthemum-monocarboxylic acid. Union Carbide & Carbon Corp. (Assees. of H. A. Stansbury, jun. and H. R. Guest) (B.P. 744,268, 17.7.53. U.S., 18.7.52).—Chrysanthemum-monocarboxylic acid (**I**) anhydride, prepared, e.g., by heating the acid with 1·1—10 mol. of a low-mol. (2—3 C) fatty acid anhydride, is converted into a product of high biological (insecticidal) activity by esterification with a 4-hydroxy-3-methyl*cyclo*pent-2-en-1-one substituted in the 2-position by unsaturated org. radical of 3—4 C (allyl or but-2-enyl), aralkyl of 7 C, e.g., benzyl, or by furyl. Esterification is effected at 50—200 (150—175)°, and the product is recovered by removing **I** with aq. alkali, then freeing the residue from volatile material. The prep. of allethrin is detailed. F. R. BASFORD.

Halogenated Diels–Alder adducts having pesticidal activity. Arvey Corp. (B.P. 745,079, 17.6.53. U.S., 5.7.52).—Compounds, useful in the immediate or residual control of insect pests, comprise adducts of furan (or an alkyl derivative thereof) with (1 : 2 : 3 : 4 : 7 : 7-)hexachloro (**I**)- or (1 : 2 : 3 : 4 : 7 : 7-)hexabromo-*bicyclo*[2 : 2 : 1]hepta-2 : 5-diene (or the corresponding epoxide). Thus, Furan (25 c.c.) is introduced during 4 hr. below the surface of **I** 59·8 g. at 165°, the next day the pptd. adduct, viz., 1 : 2 : 3 : 4 : 10 : 10-*hexachloro*-1 : 4-*methano*-5 : 8-*dioxa*-1 : 4 : 4a : 5 : 8 : 8a-*hexahydronaphthalene*, m.p. 138·7—139·6° (from methanol). F. R. BASFORD.

Organic compounds containing halogen and phosphorus. Ciba, Ltd. (B.P. 744,360, 18.9.52. Switz. 25.9.51 and 26.8.52).—Compounds $XR(X'R'){\cdot}PO{\cdot}CR''Y{\cdot}COR'''$, active against animal and vegetable pests [R is alkyl; R′ is aryl, optionally carrying a substituent; X and X′ are O or N-alkyl; R″ is H, halogen, or halogenoalkyl; R‴ is H, alkoxy,.(substituted) alkyl, phenyl, or dialkylamino; Y is halogen], are obtained by interaction of $XR(X'R')P{\cdot}OR^{IV}$ (R^{IV} is as R or R′) with $CR''Y_2{\cdot}COR'''$ [e.g., chloral, $CO(CHCl_2)$, or $CCl_3{\cdot}CO_2H$] in an inert solvent. Thus, interaction of diethyl *p*-chlorophenyl phosphite with chloral in benzene gives, after working up, a viscous oil [*ethyl* p-*chlorophenyl* (*dichloroformylmethyl*)*phosphonate*. F. R. BASFORD.

Dialkylamidophosphoryl halides, hexa-alkylphosphorylamides and octa-alkylpyrophosphorylamides. Monsanto Chemical Co. (Inventors: G. A. Saul and K. L. Godfrey (B.P. 744,484, 2.3.53).—The compounds named, useful as insecticides, are prepared by interaction of POX_3 or PSX_3 (X is halogen) with a *sec.*-amine in inert solvent (benzene, chlorinated aliphatic hydrocarbon) in presence of an inorg. acid acceptor (Na_2CO_3, baryta, etc.). The prep. of tetramethylphosphorodiamidic chloride from $POCl_3$ and $NHMe_2$ in trichloroethylene in presence of $Ca(OH)_2$ is given as an example. F. R. BASFORD.

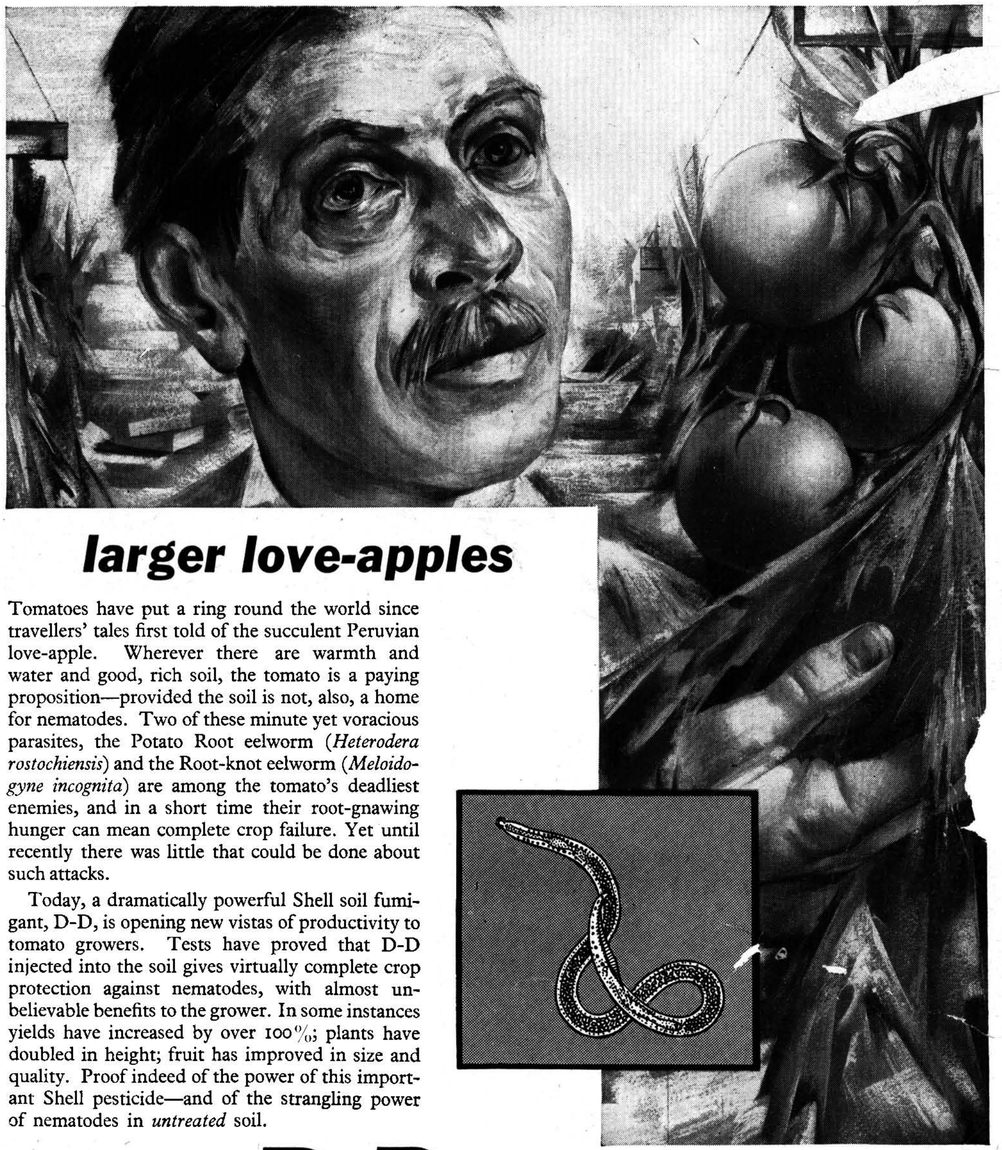
Sewage treatment. Dorr-Oliver, Inc. (B.P. 745,295, 6.3.53. U.S., 1.4.52 and 2.2.53).—The effluent from a primary clarifying tank is aërated in presence of a high-rate activated sludge for 2—3 hr. and the product allowed to settle in a secondary clarifying tank. The primary sludge is withdrawn at 3%, and the secondary at 0·1%, solids content. Half of the latter sludge is thickened with the former to give after settling a sludge of 8—24% solids content after a retention time of 5 hr. in hot or 30 hr. in cold weather. No offensive odour develops in the bed. K. RIDGWAY.

4.—APPARATUS AND UNCLASSIFIED

Colorimetric determination of diphenyl in biological materials. R. B. Bruce and J. W. Howard (*Analyt. Chem.*, 1956, **28**, 1973—1975).—Diphenyl is nitrated in acetic acid solution with conc. HNO_3 to 4-nitrodiphenyl. This is reduced and the resulting amine is coupled with *N*-(1-naphthyl)ethylenediamine to give a purple dye which is measured spectrophotometrically. Diphenyl in biological samples is solvent extracted prior to the above procedure. Recoveries of added diphenyl in blood, urine and fæces averaged 94, 100 and 91% respectively, over the range 2·5 to 75 μg. of diphenyl. G. P. COOK.

Monolayers of bile acids. P. Ekwall and R. Ekholm (*Proc. 2nd Int. Congr. Surface Activity, Lond.*, 1957, Prepr. **I**, DP 310—317).—Force/area data are presented for a no. of bile acids at pH2 on water at various temp. and on aq. NaCl solutions of various concn. at 20°. A. JOBLING.

Proc. 2nd Int. Congr. Surface Activity, Lond., 1957, 2 (Preprints). Sorptive nature of the olfactory stimulus. R. W. Moncrieff (pp. BC 160—167). **Molecular shape, size and adsorption on olfaction.** J. T. Davies and F. H. Taylor (pp. BV 358—369).—A review with 20 references. H. S. R.



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