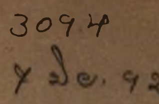

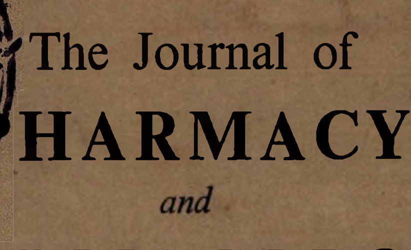

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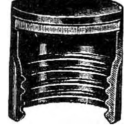

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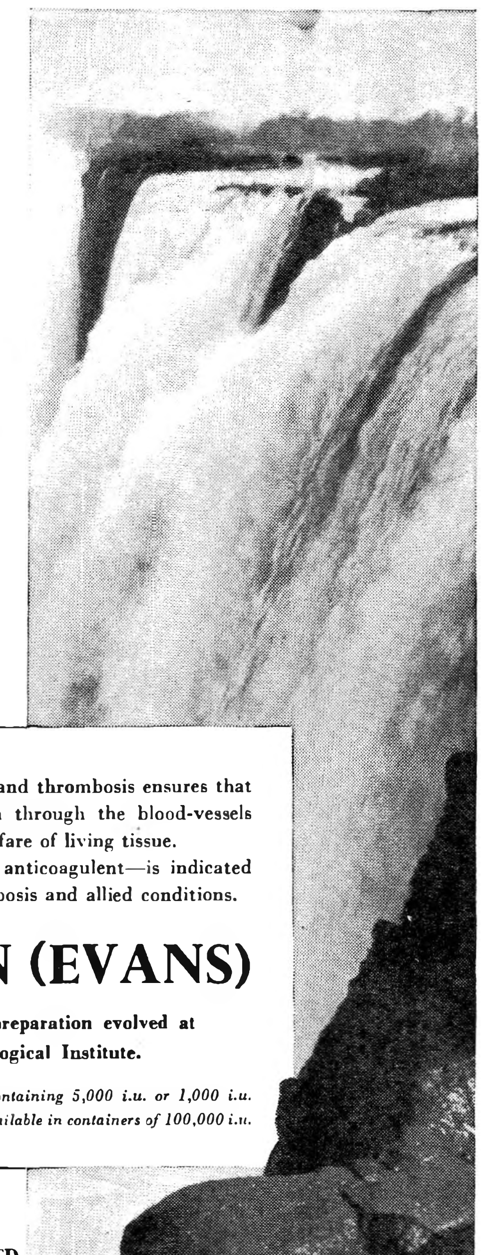

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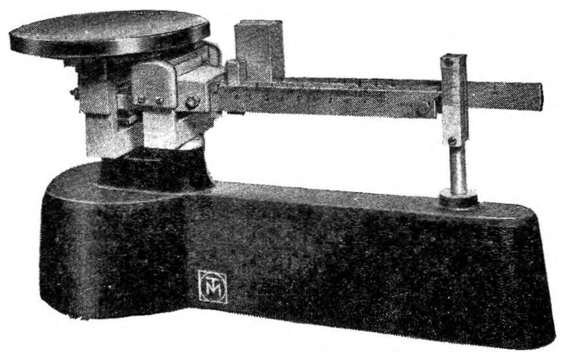

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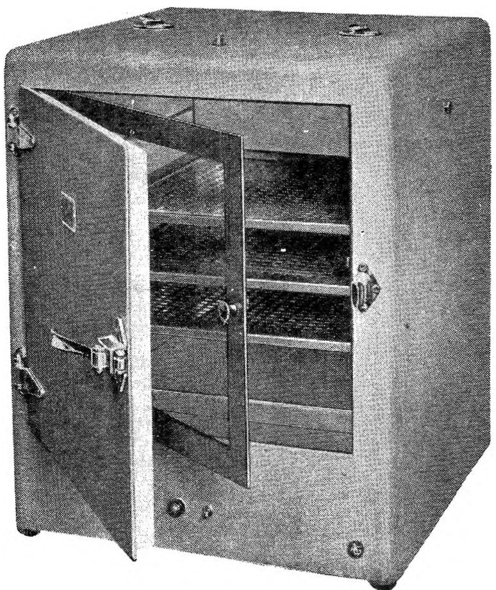

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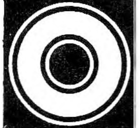

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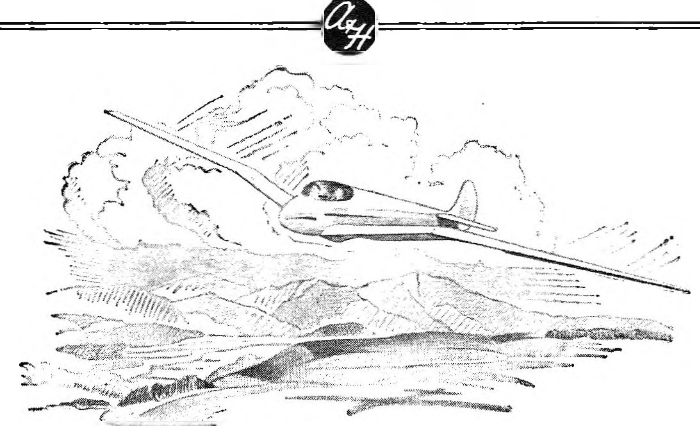

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REVIEW ARTICLE

THE PHARMACOLOGY OF CURARE AND CURARISING SUBSTANCES

By W. D. M. Paton, B.A., B.M.

National Institute for Medical Research, Hampstead, N.W.3

Historical

Eighty years have now elapsed since Crum Brown and Fraser[1,2], in a paper still worth close study, laid the foundations of our knowledge of the relation between the chemical structure and the pharmacological action of quaternary salts. Even at that early date, they referred to the therapeutic possibilities of the new compounds they had described, and to their advantages over curare in being "readily obtained in a state of perfect purity, and, therefore, of constant strength." It is only in recent years, however, that either a natural alkaloid or a synthetic curarising substance has emerged in good supply and of the required properties. This rapid progress has been greatly stimulated by King's determination of the structure of *d*-tubocurarine chloride[3,4,5] by the success of Bennett[6] in softening therapeutic convulsions with a curare extract prepared by MacIntyre and standardised by Holaday's rabbit head-drop method; by Griffith and Johnson's pioneer demonstrations of the usefulness of curare in anæsthesia[7]; by Wintersteiner and Dutcher's isolation[8] of a potent curarising extract (intocostrin) rich in *d*-tubocurarine chloride, from a known botanical species (*Chondrodendron tomentosum*); and by the discovery of Bovet and his colleagues of synthetic compounds with a potency comparable to that of the natural alkaloids[9,10,11,12,13]. As a result of this and other work, the clinician now commands a choice of reliable and well-studied compounds, and the pharmacologist has been enriched by many stimulating (and often bewildering) additions to his knowledge.

Recent Literature

Four important reviews should be consulted for detailed references: Ing has reviewed the relation of the chemical structure of onium salts to their pharmacological action in an authoritative article[14]. MacIntyres' "Curare"[15] is a very valuable source-book, particularly on the history of the subject: Bovet and Bovet-Nitti[12] have reviewed recent work, particularly their own studies of "curares de synthese": and Craig[16] has compiled and discussed a very extensive list of compounds tested up till 1947 for curarising action. There are many reports of use in medicine, surgery and anæsthesia, for which reference should be made to the clinical journals. A recent discussion by Kuffler, Acheson, Welsh and Harvey[17] of theories of neuromuscular transmission also deserves attention.

The present article is not intended to be a comprehensive survey of the subject, but attempts to review important features of the work of

recent years in the light of previous knowledge, and to indicate some of the problems remaining unsolved.

Modes of " Curarisation "

The term " curarisation " is no longer restricted in current use to that form of neuromuscular block caused by curare, but is sometimes used where similar effects are produced by other drugs, even where no more than muscular relaxation is meant (e.g., the action of myanesin). This implies, of course, a considerable widening of the meaning of the word, and a corresponding risk of confusion. But such extension of meaning is not altogether inappropriate; for block at the neuromuscular junction can be regarded as a special case of synaptic block, which may also occur at the ganglionic or central nervous synapse. Dale[18] and Feldberg[19] have discussed the evidence that the transmissions at each of these synapses possess features in common, with particular reference to the possibility of a common mechanism of chemical transmission. It should be noted that if this evidence is accepted, the failure of curarising substances to exert similar actions at each type of synapse is a fact of the first importance. For the time being, however, the interests of clarity are best served by simply describing, with particular reference to the neuromuscular junction, the different kinds of synaptic block that can be referred to as curarisation. The opportunity will be taken, in doing this, of mentioning some of the recent additions to knowledge in this field.

1. The Neuromuscular Junction. (a) *The action of curare alkaloids.* Classical experiments have established that after paralysis of a muscle by curare to excitation through its nerve, conduction in the nerve trunk is unaltered and the muscle can still give a propagated contraction in response to direct electrical stimulation. The site of the paralysis is thus localised to the nerve terminals and motor end-plate. Dale and his colleagues have further shown that the terminal nerve endings of the curarised muscle still liberate acetylcholine, and that arterially injected acetylcholine is antagonised as much as, or more than, the effect of a nerve volley. Apart from the relevance of these facts to the mechanism of neuromuscular transmission, they are also important criteria of curare-like action. To them may be added the inhibition by curare of the contracture of frog's rectus due to acetylcholine; the antagonism of anticholinesterases to the actions of curare on the neuromuscular junction; the failure of the partially curarised muscle to sustain a tetanus; and the revealing, under suitable conditions, of a transient potential at the end-plate excited by nerve stimulation, which has been closely studied and termed the " end-plate potential " by Eccles and his colleagues. These, among other characteristics, constitute a highly specific picture.

The normal conception of neuromuscular transmission at present is as follows: the wave of excitation reaches the nerve terminals, and there causes the discharge of acetylcholine in close relation to the motor end-plate. This discharge depolarises the end-plate (giving rise to the end-plate potential) and the depolarisation of the end-plate in turn excites the

muscle fibre. If this conception is accepted, then the characteristic actions of curare lend themselves very readily to the belief that curare acts by raising the threshold of the motor end-plate to excitation by acetylcholine, and that it exerts this action by competing for the acetylcholine receptor sites on the end-plate. But although this view of its action is both plausible and widely held, it cannot be said to be established beyond all question, for there are many phenomena of curarisation that still remain unexplained. It certainly provides, however, a most useful working hypothesis.

It is unfortunate that the specific tests mentioned are rarely used to verify that a substance is " curare-like "; proof of excitability of nerve and muscle is commonly omitted, and investigation of effect on acetylcholine release is a rarity. One test sometimes used, that of antagonism by anticholinesterases, is, by itself, useless, since these substances may greatly increase the tension of the twitch of the normal uncurarised muscle.

(b) *The action of* bis*trimethylammonium decane diiodide* (*C*10). This compound, which will be further discussed below, is curare-like in four respects: during its action, conduction in nerve is unaltered, direct excitability of muscle is retained, injected acetylcholine is rendered ineffective, and release of acetylcholine by motor nerve stimulation is not prevented. But there are also important differences; C10 itself elicits a contraction of frog's rectus; it is not antagonised by anticholinesterases, although C5 (the pentane homologue) is an effective antagonist; its activity varies very greatly with species of animal used for test; and it produces a depolarisation of the muscle membrane[20]. None of these effects is shown by curare. The mode of action of C10 is still uncertain, but these and other differences from curare are sufficiently great to make it necessary to distinguish the actions of the two drugs.

(c) *The action of anticholinesterases.* Eserine has long been known as a depressant of the muscular contraction caused by a tetanus of the motor nerve, although it usually augments the tension of single twitches. This depressant action exerted both by eserine and by other anticholinesterases, is due to the accumulation of paralysing concentrations of acetylcholine at the end-plate (Brown, Dale and Feldberg[21]). It is not possible, however, to exclude entirely some direct action by the anticholinesterase itself (*cf.* Riker and Wescoe[22]).

(d) *The action of substances depressing the release of acetylcholine.* Harvey[23] has presented evidence suggesting that some of the neuromuscular block caused by procaine is due to interference with release of acetylcholine by the nerve-ending. A similar block is caused by botulinus toxin (Burgen, Dickens and Zatman[24]) after which progressive failure of transmission occurs, although nerve and muscle remain excitable: acetylcholine injected is still effective, but release of acetylcholine is depressed. Brown and Harvey[25] and Brown and Vianna Dias[26] have reported that such a failure of acetylcholine release also results from calcium deficiency or from injection or perfusion with solutions rich in phosphate.

2. The Ganglionic Synapse. Depression of transmission at the synapse of the superior cervical ganglion by curare, without loss of excitability of preganglionic fibres or of ganglion cells, or abolition of the release of acetylcholine has been shown by Brown and Feldberg[27]. Brown and Feldberg[28] have also demonstrated block of transmission due to accumulation of acetylcholine in the presence of eserine. Harvey[23] found that procaine blocked transmission by preventing release of acetylcholine at preganglionic nerve terminals, and Harvey and MacIntosh[29] have shown that calcium lack in the perfusion fluid leads to the same result. Block by injection of large doses of potassium has also been described (Brown and Feldberg[30]).

The same types of "curarisation" may, therefore, be seen in the ganglion as at the neuromuscular junction. One of the most interesting developments recently, however, has been the observation that a "curarising" compound which is active at the neuromuscular junction may be relatively inactive on the ganglion and *vice versa.* It has, of course, been known for some time that tetraethylammonium iodide, although of neglible activity at the neuromuscular junction, is a powerful paralyser of ganglionic transmission (Burn and Dale[31], Acheson and Moe[32]). Depierre[33] studied this point on certain of Bovet's ethyl-choline ethers of phenol and polyphenols, using the contraction of the cat's nictitating membrane excited by stimulation of the cervical sympathetic. These compounds can be arranged in a series in which curarising activity increases as ganglionic activity diminishes (so that the ratio of curarising dose to ganglion-paralysing dose ranges from *c.* 30 to 0·02). A similar dissociation occurs in the *bis*trimethylammonium series: here C5 or C6 injected intravenously into the cat requires only 1·0 mg./kg. to affect the superior cervical ganglion, but 10 to 20 mg./kg. or more are required to depress neuromuscular conduction; C10, on the other hand, active at the junction in a dose of 30μg./kg. requires more than 3 mg./kg. to depress ganglionic transmission. The ratio just mentioned is thus more than 100 for C5 and less than 0·01 for C10. It is clear from these and similar results that activities in paralysing neuromuscular transmission and ganglionic transmission can be very widely dissociated.

3. The Central Nervous Synapse. No method yet exists adequate to determine whether a given depression of activity by the spinal cord or brain is due to paralysis of transmission at the synapse rather than to failure of conduction in neurone or axon. The action of a drug such as myanesin however, suggests some such action, since it can depress reflex activity without depressing conduction in a peripheral nerve. The point most relevant, at the moment, is the remarkable lack of central action of drugs such as *d*-tubocurarine chloride or C10. Some of this inactivity may be due to failure to pass through the capillaries of the central nervous system, which cations traverse but slowly (Krogh[34]). But even when curare is administered more directly, it appears most commonly to exert a central stimulant action. It is possible, therefore, that the central synapse is, like the neuromuscular and ganglionic synapses, relatively specific as regards the agents which block it; we have to contrast drugs

like curare (notably paralytic on the neuromuscular junction and ganglionic synapse, but centrally stimulant); tetraethylammonium iodide (almost inactive at the junction, highly active on the superior cervical ganglion, and of mixed action on the central nervous system) (Salama[35]); and myanesin (inactive on neuromuscular conduction but centrally depressant).

In referring, therefore, to "curarisation" in its extended sense, it is necessary to specify both the synapse at which paralysis of transmission of excitation occurs, and the mode of that paralysis. A tentative summary of the modes of paralysis of transmission due to various drugs described can be attempted. (a) Competition block (e.g., curare) in which the threshold of the end-plate to acetylcholine is raised; (b) depolarisation block (e.g. potassium chloride and possibly C10); (c) block by accumulation of acetylcholine (e.g. anticholinesterases); (d) block by transmitter failure, either by immobilisation of acetylcholine (e.g. procaine, calcium lack), or by deficiency of acetylcholine (e.g. botulinus toxin). This variety of modes of "curarisation" makes it essential to define clearly which is being used, even if the fundamental mechanism of the particular type of block is not fully understood. These distinctions become all the more necessary if any attempt is to be made to relate "curarising" potency to chemical structure. For the purpose of this review, the distinctions just made will not be pursued, and discussion will be confined to the pharmacology of compounds producing block of curare-like or C10-like character.

Natural Alkaloids

1. The Curare Group. The total number of such alkaloids is very large. For practical purposes three should be carefully distinguished: (a)

OCH_3 — O — HO — CH_2 — $(CH_3)_2Cl$ N — CH_2 — O — N $(CH_3)_2.Cl$ — HO — OCH_3

Fig. 1. Structure of *d*-Tubocurarine chloride.

curarine chloride; this is the material isolated by Boehm from calabash curare, much used in experimental work before the isolation of *d*-tubocurarine chloride: (b) *d*-tubocurarine chloride (*d*-T.C.), isolated by King[2] from tube curare, whose structure was finally determined by him[4]: (c) intocostrin, an extract prepared by Squibbs, rich in *d*-tubocurarine chloride; the intocostrin unit is equivalent to 1 mg. of a standard preparation from *Chondrodendron tomentosum*, and the activity of 6·5 units is equal to that of 1 mg. of *d*-tubocurarine chloride, on the rabbit head-drop test. "Curare" is used in this review as a generic term when the distinction between the above substances is not important.

The main features of the pharmacology of these substances at the neuromuscular junction have already been described; further details will be found in the references cited. But other properties of the compounds have recently come into prominence arising particularly with reference to the possibility of side-actions in clinical use.

(a) *Liberation of histamine.* The observation that curarine liberated histamine from muscle by Alam, Anrep, Barsoum, Talaat and Weininger[36] has been repeated by Gregory and Schild[37]; the latter showed that *d*-T.C. exerted this action both on the perfused tongue of the cat and on the rat's diaphragm. Grob, Lilienthal and Harvey[38] have extended these results to man, showing that *d*-T.C. injected into the brachial artery causes flushing, œdema, and itching of the arm, which is lessened by anti-histamines, and that injected intradermally it causes a typical " triple response." Striking confirmation of this work was provided by Landmesser[39], who showed that *d*-T.C. caused fall of blood pressure in spinal dogs and bronchoconstriction of guinea-pig's lung similar to that caused by peptone, and that these effects were prevented by antihistamines. Further, he observed that occasional dogs were refractory to the effects of *d*-T.C. (just as they are to peptone), and that sensitive animals could be made refractory to peptone by previous injection of adequate doses of *d*-T.C., or *vice-versa.* *d*-T.C. thus resembles peptone rather closely, and it is highly probable that, as with peptone, the liberation of heparin accompanies the liberation of histamine by the drug. As Landmesser points out, the practical application of these results is difficult, since, for instance, anæsthetics depress the release of histamine. But it is clearly desirable, at least, that compounds less active in this respect should be investigated.

(b) *Actions on the C.N.S.* That *d*-T.C. has some central stimulant property has already been mentioned. The experimental results in this field, however, are still somewhat confused. References to the stimulant action of curare when applied directly to the central nervous system may be found in the reviews mentioned. Salama[35] has recently verified these results, administering *d*-T.C. directly into the ventricles of cats. It is common to see a stage of excitement preceding curarisation with *d*-T.C., particularly with smaller animals. Everett[40] has described the convulsant action of *d*-T.C. given intracisternally to rabbits. On the other hand, it also has been claimed that curare given intravenously may have a narcotic or anæsthetic action. Whitacre and Fisher[41] report an illustrative case from their surgical experience. The report by Prescott, Organe and Rowbotham[42], however, and the careful and courageous experiment by Smith, Brown, Toman and Goodman[43] fail to substantiate this. In the latter paper, an account is given of the curarisation of a volunteer, under artificial respiration, so deeply that not even the most trivial muscular movement could be made; nevertheless, a full and intelligent narrative of his experience was furnished by the subject after recovery. Kellgren, McGowan and Wood[44] found no alteration in sensation after small doses of *d*-T.C. Paton and Zaimis[45], studying

the respiratory depression by *d*-T.C., found that the discharge down the phrenic nerve of the cat was not depressed by an intravenous dose of *d*-T.C. sufficient to abolish spontaneous respiration. It seems unlikely from this and other work, therefore, that the central actions of *d*-T.C. are important after intravenous doses, probably because (as mentioned above) it would not be expected to pass very readily through the capillaries of the central nervous system.

(c) *Action on autonomic ganglia.* Curare has long been known to depress ganglionic transmission, as has been already mentioned. Such action has, indeed, been suggested as the basis of an assay method, in which inhibition of the peristaltic reflex of isolated intestine is used to assay *d*-T.C. or kindred drugs (Feldberg and Lin[46]). Gross and Cullen[47] have shown that in the dog, curarising doses of intocostrin or *d*-T.C. cause inhibition of peristaltic activity by stomach and small intestine, with some loss of tone; fall of blood pressure was also sometimes observed. Heymans[48,49] found that rapid injections of intocostrin cause a fall of blood pressure and depression of the cardiovascular reflexes, but that with slow injection of the same dose these actions did not appear. Prostigmine did not abolish these effects.

(d) *Antagonism by certain dyes.* An old observation that certain dyestuffs antagonise curare has been reinvestigated by Kensler[50]. Congo red, chlorazol fast pink and Evans Blue are highly effective at both preventing and relieving paralysis of frogs by *d*-T.C. The action is due to the formation of a precipitable complex, in which form *d*-T.C. is not active. The phenomenon promises to be a useful tool in suitable circumstances.

(e) *Anticholinesterase action.* Some of the earlier preparations of intocostrin contained material with an appreciable power of inhibiting cholinesterase (Harris and Harris[51]). Pure *d*-T.C., however, has slight activity in this respect; the materials responsible were tertiary bases of negligible curarising activity. The finding is of interest, in view of the extent to which anticholinesterase action has been observed among synthetic compounds.

2. Dimethyl Ether Of *d*-Tubocurarine Chloride. It has been known for some time that methylation of *d*-T.C. increases its potency. Further studies (Collier, Paris and Woolf[52]) have shown that the dimethyl ether is about 10 times as active as *d*-T.C. in rabbits, and that it displays certain species differences in potency and duration of action. In the main, it is very similar to *d*-T.C., but considerably more active. Successful clinical trials have been reported by Stoelting, Graf and Viera[53].

3. The Erythrina Alkaloids. Exceptional interest attaches to these compounds, of which erythroidine (from the seeds of the legume *Erythrina Americana*) and β-dihydroerythroidine (obtained by hydrogenating erythroidine) are the most important. (Erythrina extracts are said to have been used in the treatment of convulsions as long ago as 1887.) They are at present unique in being highly active and yet possessing only trivalent nitrogen atoms; on converting the latter to quaternary nitrogen, the compounds diminish greatly in potency. So far as their

action at the neuromuscular junction is concerned, they resemble the curare alkaloids, having 1/5th or less the activity of *d*-tubocurarine chloride, and they are antagonised by anticholinesterases. In other respects, there are important differences; the erythroidines are active by mouth; they do not possess anti-esterase activity (Harris and Harris[51]); they do not share the ability of *d*-tubocurarine to liberate histamine (Landmesser[39]); they are not antagonised by congo red (Kensler[50]); they possess a feeble atropine-like action. Clinical trial has been reported (Harvey and Masland[54], Dripps and Sergent[55]); the most serious disadvantage appears to be a depression of the blood pressure with effective doses; respiratory depression is also common. It is to be hoped that other members of this series may be discovered which are free from these defects.

4. Other Natural Alkaloids And Their Derivatives. The comprehensive study of quinine methochloride by Harvey[56] requires mention; this is one of the few compounds investigated whose action on the release of acetylcholine has been tested; in paralysing doses, it fails to prevent such release in the superior cervical ganglion of the cat. It is curare-like in most respects, is about 1/40 times as active as *d*-T.C., and is active by mouth. Trials in man have been reported by Harvey and Masland[54]. A large number of other related alkaloids of the cinchona group have also been studied but are not promising clinically.

Among compounds related to *d*-T.C., the *iso*chondrodendrines (Marsh and Pelletier[57]) and the chondrodendrines (Marsh, Sleeth and Tucker[58]) have been investigated; in both groups variation of potency with species, and increase in potency by methylating free hydroxyl groups were found, analogous with the effect of methylating *d*-tubocurarine chloride. Another related compound, N-methyl oxyacanthine (Marsh, Herring and Sleeth[59]) is of interest, since in man it lessens salivary secretion, and has a weak atropine-like action (antagonising the depression of dog's blood pressure by acetylcholine).

The most potent known curarising substances are the toxiferines, isolated by Wieland from calabash curare; they are effective in doses of the order of 10μg./kg. in frogs and rabbits. So far as is known, however, they do not appear to be sufficiently free from side-actions of various kinds to be suitable for therapeutic use.

On the whole, therefore, only two serious rivals to *d*-tubocurarine chloride have emerged from the natural alkaloids and their derivatives—the methyl ether of *d*-T.C. itself, and the β-erythroidines. Apart from questions of potency the compounds do not differ significantly from curare, and do not, for instance, eliminate the need for careful control of respiration. Information is not yet adequate to assess their side-actions in clinical use.

From the pharmacological side, *d*-T.C. is clearly not the ideal curarising agent, because of its ganglionic action and its power of liberating histamine. Its depression of the respiratory minute volume is probably less important, since modern methods of anæsthesia are fully adequate to maintaining artificial respiration without inconvenience. A

technical disadvantage of it is that some preparations cannot be given simultaneously with pentothal owing to mutual precipitation. Finally, it is a drug that is relatively expensive and difficult to prepare in the pure state.

Against all this, it is important to realise that it is with this compound that the use of curarising substances in anæsthesia has established itself; this rapid success, and the widespread search for substances of similar action are, indeed, sufficient testimony to its value.

Synthetic Curarising Substances

1. **Early Work.** The first synthetic compound exhibiting curare-like activity, tetramethylammonium iodide, ($N(CH_3)_4.I.$) was made by Crum Brown and Fraser[2] in 1869, and provided them with a striking confirmation of their theories. Its curariform action is not strong and its power of stimulating autonomic ganglia is its most prominent action in the cat, followed by a weaker paralysing action on ganglia; it also possesses appreciable muscarine-like action (Burn and Dale[31]). Bacq and Brown[60] found that it could also elicit a contraction from mammalian striated muscle, and it is known to cause a contracture of frog's rectus. It is typical of the many related compounds that they possess, besides curariform activity, some or all of these other activities in some degree—some of them also possessing anticholinesterase potency. But none of the synthetic monoquaternary salts, whether simple tetralkylammonium halides or choline or betaine derivatives, proved to be sufficiently potent or free from side-actions even to approach the natural alkaloids.

2. **Bovet's Compounds.** The work of Bovet and his colleagues marked an important advance. Taking as a model the structure of *d*-tubocurarine chloride (which had been almost completely defined by King at that time).

N — $C_2H_5 \cdot I$ $O \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot O$ N — $C_2H_5 \cdot I$

(a) 3381 R.P. Head drop dose = 0.25 mg./kg.

$O \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot O$

$N(CH_3)_3 \cdot I$ $N(CH_3)_3 \cdot I$

(b) 3565 R.P. Head drop dose = 0.2 mg./kg.

$O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_3$

$O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_3$

$O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_3$

(c) 3697 R.P., 2559 F, flaxedil: Head drop dose = 0.5 mg./kg.

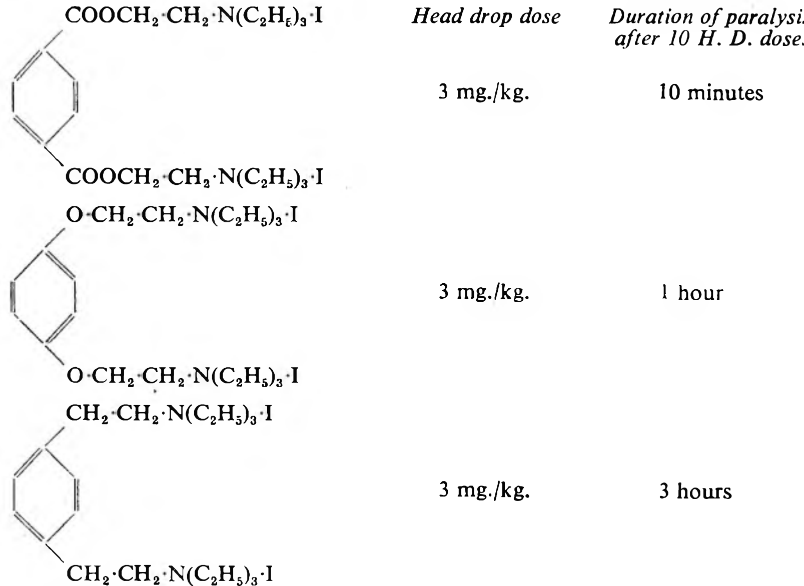
Fig. 2. Synthetic curarising compounds (Bovet).

simpler related structures were synthesised. Using this basic structure, variations of chain-length and quaternary substituents were studied. The first compound reported (Bovet, Courvoisier, Ducrot, and Horclois[9]), 3381 R.P., is also the first synthetic compound that resembles curare at all closely, in being potent and sensitive to anticholinesterases (Fig. 2(a)). It possesses, in addition, some anticholinesterase potency (Halpern, Benda and Bourdon[61]).

The next step was a further simplification of structure, as a result of which 3565 R.P. (see Fig. 2b) was described (Bovet, Courvoisier, Ducrot and Horclois[11]). This compound, too, is highly active, but does not possess anti-esterase action; it is antagonised by anti-esterases, and has some nicotinic action.

The third main series were the choline ethers of phenols and polyphenols (Bovet, Depierre and Lestrange[10]), of which the ethyl-choline triether of pyrogallol proved to be the most important (2559 F. or 3697 R.P., flaxedil. See Figure 2c). This possessed much the same actions as the other compounds with fewer side-actions.

Finally, an interesting series of choline esters has been built, also of considerable potency, but of transient action (Bovet, Bovet-Nitti, Guarino and Fusco[13]). Figure 3 gives a comparison of one of these with the corresponding ether and the directly substituted compound, together with their effective doses and duration of action.



	Head drop dose	*Duration of paralysis after 10 H. D. doses*
Ester	3 mg./kg.	10 minutes
Ether	3 mg./kg.	1 hour
Directly substituted	3 mg./kg.	3 hours

FIG. 3. Variation of duration of action with chemical structure.

Courvoisier and Ducrot[62] have shown that flaxedil (2559F) possesses little power to liberate histamine, although 3381 R.P. and 3365 R.P. are active in this respect. Depierre[33] has shown that it possesses little ganglionic action.

3. THE *bis*TRIMETHYLAMMONIUM SERIES. The most potent synthetic compound so far described is the decane derivative (C10) of an even simpler series, the *bis*trimethylammonium polymethylene salts. The curarising action of the series was reported independently by Barlow and Ing[63] and by Paton and Zaimis[64], and the latter authors have studied the pharmacological actions of the series in considerable detail (Paton and Zaimis[64,45]). Some of these actions of C10 have been already mentioned above, and will not be further discussed. But three points require further discussion.

(a) *Variation of potency with species and test object.* C10 is highly active in cat, 0·03 mg./kg. causing full neuromuscular paralysis; in rabbit 0·1 mg./kg. is required; in mouse 1 mg./kg., and in rat 5 mg./kg. In man a total dose of 3 mg. usually causes almost complete paralysis, and man thus corresponds very closely to the cat in sensitivity (Organe, Paton and Zaimis[65]); the equivalent dose of *d*-T.C. in a man is about 15 mg.

$$I{\cdot}N(CH_3)_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}N(CH_3)_3{\cdot}I$$

FIG. 4. Structure of *bis*Trimethylammonium decane diiodide (C10)

The comparison of *d*-T.C. with C10 further varies with the muscle used, and with the rate of stimulation. In the cat, using single twitches every 10 sec., C10 is 10-20 times as active as *d*-T.C. But if tetani are used this figure is at least halved, since *d*-T.C. depresses a tetanus much more than a twitch and C10 allows a tetanus to be fairly well sustained. In addition, the two compounds differ in their actions on different muscles; thus, in the cat, C10 paralyses tibialis more readily than soleus, while *d*-T.C. does the reverse. Finally, a test method such as that used by de Jalon[66], in which the antagonism of curare to acetylcholine on frog's rectus is employed, cannot be used at all, since C10 itself elicits a contracture and (by reason of its anti-esterase activity) actually potentiates acetylcholine. The findings on species difference are paralleled in other compounds. Collier, Paris and Woolf[52] have reported a species difference with the dimethyl ether of *d*-T.C., and Wien[67] has done so, using the isolated diaphragm, for some of Bovet's compounds. From such results, it is obvious that investigations of new compounds should be made by several test methods, and that in an assay the method used should be fully sensitive to all the substances likely to occur in the material under test. The fact that man closely resembles the cat in his sensitivity to C10 is an important argument for the use of cats at an early stage in any investigation. The species difference, and the different action on different muscles are fascinating problems for which there is no explanation at present.

(b) *Effect on respiration.* One of the remarkable features of the action of C10 in the cat is the failure to depress respiration significantly at a time when tibialis twitch is more than 95 per cent. paralysed (Paton and Zaimis[45]). This appears to be due to the fact that tetani are well-sustained, and that red muscles are less affected than white. The contrast with *d*-T.C. is sharp, for in the cat respiration may be depressed before any action on the twitch has appeared. But it is difficult to predict

the same sparing of respiration in relation to other muscular activities (e.g., abdominal relaxation in anæsthesia or the softening of therapeutic convulsions), in the absence of information about the physiological characteristics of the muscles involved and of the rate at which their motor units are excited.

(c) *Side actions.* Curarising compounds may depress the blood pressure by liberating histamine, by paralysing ganglia or by exerting a muscarine-like action. Curarising doses of C10 have no effect on the blood pressure of the anæsthetised cat, and large doses (at least 100 times the effective curarising dose) must be given to show such an action. It is at least 5 times less active in releasing histamine or causing ganglionic depression than the same weight of *d*-T.C., and its muscarine action is negligible. C10 has some anticholinesterase action, but this does not appear to cause any undesirable effect. In man, there is no interference with sensation or consciousness after an intravenous dose of C10 sufficient to cause almost complete paralysis (Organe, Paton and Zaimis[65]).

Discussion

There is much that is confusing in recent developments, which cannot be discussed here. But one feature may be stressed; this is the remarkable diversity of actions among the various compounds studied. Their variation in activity on different species, on different muscles, and on different synapses; their differences in side-actions, and their wide disparity in chemical structure:—all these are hopeful prognostics of yet other compounds with useful selective actions Before these are likely to be discovered, however, fundamental work must be done on the reasons for these diversities of action, about which we know little, and such fundamental research is the most urgent need. There are, further, many more practical requirements to be satisfied, such as a satisfactory method of prolonging the action of these drugs; compounds active by mouth and safe to use; or an antagonist to the activity of curarising agents which is free of side-actions.

To the pharmacologist, however, the most absorbing question remains that of the relation of the structure of these compounds to their pharmacological action. No field has proved more hazardous than that of the 'onium salts, in which to venture generalisations. Many of the important anomalies are reviewed by Ing[14]. Certain broad statements, however, can be made.

(1) Crum Brown and Fraser's generalisation of 1868 still remains remarkably true. The only important exception to it is that of the properties of the *Erythrina* alkaloids; and in ignorance of their structure it is impossible to say how much of an exception they represent. There is little doubt that the most promising approach, in devising substances of curarising potency, is still by way of quaternary salts; and among these, there is still no rival to the salts of quaternary nitrogen.

(2) The introduction of a second or even a third quaternary group into the molecule appears to be an important element in producing compounds of high curarising potency: all the compounds of known

chemical structure active in a dose of 1 mg./kg. or less have two or more such groups. Possible reasons for this enhancement of potency are not far to seek in the favourable effects such additions have on the attachment of the drugs to a receptor surface.

(3) The introduction of further quaternary groups has another effect: the monoquaternary compounds active on the neuromuscular junction are commonly like tetramethylammonium iodide, particularly in possessing stimulant nicotine-like and muscarine-like actions. In *bis*-quaternary salts these actions are replaced by weak anti-esterase activity, ganglionic depression, and histamine liberation. These latter actions would, indeed, be a serious disadvantage; but *bis*quaternary or *ter*quaternary compounds have been obtained in which they are slight, although the compounds still possess high potency (C10 and flaxedil).

(4) A further significant point appears to be the spatial separation of the two quaternary groups. It can hardly be coincidental that in such active compounds as *d*-tubocurarine chloride and its methyl ether, Bovet's compounds 3381 R.P. and 3565 R.P., and C10, the quaternary groups should be separated by 10 to 11 atoms, particularly since shortening the chain in the *bis*trimethylammonium series to less than 7 carbon atoms almost completely abolishes activity. The implications of this require further study, but the suggestion certainly appears that the ability to interfere with neuromuscular transmission depends not only on certain characteristic polar groups but also on their characteristic spatial location. It is certain that other considerations than distance are also concerned; thus, King[4] reports that *l*-tubocurarine chloride is 30 to 60 times weaker than the dextro-rotatory isomer.

Finally, it is worth pointing out that the diversity of structure exhibiting curarising activity suggests that the specific characteristics of such compounds differ in some important way from those of (for instance) muscarine-like substances. Pfeiffer[68] has drawn some striking analogies between the distances separating certain prosthetic groups (the nitrogen atom and two oxygen atoms) in muscarine-like and atropine-like substances, and the distances between the same groups in acetylcholine. But no such analogy is at present visible among curarising substances. It is, indeed, possible that their specific activity is not dependent solely on a relationship to acetylcholine, but that resemblance to some other physiologically active cation must be considered. The papers of Ing and Wright still represent the most critical approach to the subject; recent attempts to apply modern concepts of atomic structure to the problem (Holmes, Jenden, Taylor[70]) do not advance much beyond the position of Ing and Wright.

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RESEARCH PAPERS

THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART I

THE LIBERATION OF PHOSPHORUS AS PHOSPHATE FROM VITAMIN B_{12} BY ACID HYDROLYSIS

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WORK in these laboratories on the anti-pernicious anæmia factor present in liver has led to the isolation from anahæmin[1] of a clinically active red crystalline compound which we have characterised by:—

(i) lack of a definite melting-point, the crystals darkening at 190° to 250°C.

(ii) an R_F value of 0·1 when run on unidimensional paper-strip partition chromatograms using *n*-butyl alcohol as a solvent.

(iii) characteristic light absorption, an aqueous solution showing maxima at 550 mμ (shoulder at *ca.* 520 mμ), 361mμ, and 278 mμ, with inflections at 322 mμ and 304 mμ.

(iv) the presence of nitrogen, phosphorus and cobalt in the molecule (see also Rickes *et al.*[2]; Smith[3]).

(v) a cobalt content of 4·0 per cent. for material dried *in vacuo* at 76°C.

(vi) the presence of one ninhydrin-reacting substance detected in hydrochloric acid hydrolysates run on paper-strip partition chromatograms.

The identity of our compound with the substance to which the name vitamin B_{12} was first applied[4] has now been established by a direct comparison with an authentic specimen, kindly carried out for us by Merck Laboratories, Inc., through the courtesy of their Vice-President and Research Director, Dr. Randolph T. Major.

More detailed examination of the paper-strip chromatograms obtained in (vi) above revealed the presence of phosphate on them, an observation which led us to undertake a quantitative study of the liberation of this ion from vitamin B_{12} during its hydrolysis with hydrochloric acid. The solutions obtained on hydrolysis, however, are deeply coloured owing to the survival of a pigmented cobalt-containing fragment of the B_{12} molecule to which we have already referred in an earlier publication[1]. The problem of estimating phosphate liberated on hydrolysis thus resolved itself into a search for a micro-method which would permit quantitative separation of the inorganic ion from the pigmented material both of which are present in the hydrolysate. We ultimately achieved this by a new application of the unidimensional paper-strip chromatography technique. In addition, we have carried out some exploratory experiments on the behaviour of

phosphates on chromatograms irrigated with a number of solvents, the results being reported below (Experimental (i)).

The method finally adopted for the estimation of phosphate consisted in spotting the vitamin B_{12} hydrolysate on to strips of filter-paper, irrigating with *iso*butyric acid, locating phosphate on the guide strips by means of the ammonium molybdate and benzidine spot test reagents[5], and estimating the phosphate colorimetrically after its elution from the paper.

We were unable to obtain evidence for the liberation of phosphate from vitamin B_{12} when the compound was hydrolysed with 20 per cent. hydrochloric acid at room temperature for periods as long as 17 days. Phosphorus must therefore be present in a fairly stable form. At 100°C., however, liberation of phosphorus as phosphate took place and appeared to be essentially complete after about 6 hours, values corresponding to a phosphorus content in vitamin B_{12} of *ca.* 2·0 per cent being obtained. This was equivalent to all the phosphorus present in vitamin B_{12}, as total phosphorus determinations gave results ranging between 1·9 and 2·3 per cent. of phosphorus.

We have previously reported that vitamin B_{12} contains 4·0 per cent. of cobalt[1]. This figure, taken in conjunction with a phosphorus content of 2·1 per cent., shows that the atomic ratio cobalt:phosphorus in the vitamin B_{12} molecule is 1 : 1. The anti-pernicious anæmia factor isolated by Smith and Parker[6], it may be added, is reported by Smith[3,7] to have a cobalt:phosphorus ratio of 1 : 3. It follows from this that the compound described by Smith and Parker[6] must differ from vitamin B_{12}, a conclusion supporting the view that at least two hæmatopoietic factors containing cobalt may be obtained from liver. Further work is required to establish whether in fact both these compounds co-exist in liver or whether, as is possible, one of them is a clinically-active artefact produced from the other during the process of isolation.

Experimental.

Whatman No. 1 filter paper was used for all chromatograms. Solvents used for irrigation were saturated with water, with the exception of *iso*butyric acid which was employed as a 65 per cent. aqueous solution. Phosphorus was estimated colorimetrically by the method of Fiske and Subbarow[8]. A "Spekker" photoelectric absorptiometer with an Ilford Spectrum filter No. 608 was used throughout for colour intensity comparisons, in conjunction with at least two phosphate standards. These were freshly prepared on each occasion and gave absorptiometer readings satisfactorily coinciding with points on a previously constructed calibration curve.

(i) *Detection, behaviour, and quantitative estimation of the phosphate ion on paper-strip chromatograms.* Disodium hydrogen phosphate, potassium dihydrogen phosphate, diammonium hydrogen phosphate, and phosphoric acid were employed as sources of phosphate ions. Phosphate was readily detected on the chromatograms using the ammonium molybdate and benzidine reagents employed in spot-test techniques[5]. For this purpose, the chromatogram, after irrigation with the solvent, was dried

and lightly sprayed with a reagent consisting of a solution of 5 g. of ammonium molybdate in a mixture of 100 ml. of water and 35 ml. of concentrated nitric acid. The presence of phosphate on the paper, in quantities exceeding 2μg. of phosphorus, was indicated by the appearance of a yellow spot or zone, which changed to blue after spraying with a solution prepared from 50 mg. of benzidine (or benzidine hydrochloride) in 100 ml. of 10 per cent. acetic acid, followed by exposure of the area to ammonia vapour. Diffusion or running of the coloured spot or zone was minimised by spraying very lightly with the two reagents, whilst excessive exposure to ammonia was avoided as this tended to reduce the intensity of the blue colour.

n-Butyl alcohol, *iso*butyric acid, collidine, and phenol were employed as solvents in the present investigation.

n-*Butyl alcohol.* Migration of phosphate did not occur.

iso*Butyric Acid.* Slightly elongated spots falling within the region R_F 0·20 to R_F 0·25 were obtained with phosphoric acid and with all the phosphates. The diammonium hydrogen salt gave, in addition, a second smaller spot at R_F 0·3.

Collidine. Elongated tapering zones, extending from the source to points determined by the nature of the cations present were obtained. The phosphoric acid and diammonium hydrogen phosphate zones were longest, terminating at R_F 0·20, whilst those formed by the potassium and disodium salts terminated at R_F 0·17 and R_F 0·13, respectively.

Phenol. The major portion of each phosphate migrated as a spot to *ca.* R_F 0·1, but a small fraction resisted migration and remained as an annular ring at the source. It was important that all traces of phenol be removed from these chromatograms before application of the ammonium molybdate reagent, otherwise the chromatograms turned black or brown.

Experiments directed to the quantitative estimation of phosphate were limited to chromatograms irrigated with *iso*butyric acid as, when used for the chromatography of acid hydrolysates of vitamin B_{12}, complete separation of the phosphate and pigmented material occurred.

The technique used for locating phosphate and preparing " cuts " for elution followed that described by Consden, Gordon and Martin[9]. Twenty microlitres of an aqueous solution containing a known amount of phosphate (equivalent to 10 to 20μg. of phosphorus) were distributed on 8 spots, 1 cm. apart, along a starting line drawn 6 cm. from one end of a paper strip 12 cm. wide. After irrigation of the paper with *iso*butyric acid a " cut " containing the phosphate was trimmed to a point at one end and eluted by the method of Dent[10]. Phosphate in the eluate was estimated colorimetrically by the method of Fiske and Subbarow[8]. Substantially quantitative recoveries (96 to 102 per cent.) were obtained in all of 14 estimations using both phosphoric acid and its salts.

In current paper-strip chromatography technique, acid hydrolysates are evaporated to dryness to remove hydrochloric acid and the residues dissolved in distilled water before being spotted on the paper strips. In quantitative work such a procedure involves the risk of errors arising during evaporation and re-solution. In order to establish whether this

procedure could be eliminated during estimation of phosphate in acid hydrolysates of vitamin B_{12}, control experiments were carried out in which phosphoric acid dissolved in 20 per cent. hydrochloric acid was spotted directly on the paper strips. The spots so obtained were allowed to dry at room temperature for at least 1 hour before irrigation with *iso*butyric acid. Chromatograms prepared in this way showed fairly uniform areas of waterlogging within which, however, the phosphate could be located in approximately its normal position. Elution, followed by estimation, resulted in substantially quantitative recoveries as before. Some degree of waterlogging could therefore be ignored in these estimations. It followed from this that acid hydrolysates of vitamin B_{12} could be employed *directly* for phosphate estimations and that the usual procedure involving removal of hydrochloric acid by evaporation and re-solution of the residues was unnecessary.

(ii) *The liberation of inorganic phosphorus from Vitamin B_{12} during hydrolysis with hydrochloric acid.* Sealed tubes containing 3 to 3·5 mg. of vitamin B_{12} dissolved in 200 microlitres of 20 per cent. hydrochloric acid were, with one exception, heated at 100°C. for increasing periods of time, cooled to room temperature, and opened. Twenty microlitres of each hydrolysate were withdrawn with a micropipette and dispensed on to a paper strip (12 cm. wide) in a series of 8 spots. An additional 2 to 3 microlitres of solution were placed near one edge of the paper to serve as a " guide " for the location of phosphate. The papers were irrigated overnight with 65 per cent. *iso*butyric acid and air-dried. The phosphate was located by means of the guide strip and fell within the region R_F 0·2 to R_F 0·3, well removed from the pigmented zones which appeared further down the chromatograms. The area containing the phosphate was cut out, eluted, and phosphorus determined colorimetrically[8]. The main results of duplicate phosphorus estimations obtained from pairs of chromatograms arising from each hydrolysate are given in the table below.

Experiment	(a) Phosphorus content of vitamin B_{12} per cent.	Time of hydrolysis	Temperature	(b) Phosphorus liberated as percentage of vitamin B_{12} used	Ratio (b)/(a)
1	1·9	17 days	18-23°	Nil	0
2	2·1	1 hour	100°	0·6	0·29
3	2·1	2 hours	100°	1·0	0·48
4	2·3	3 "	100°	1·7	0·74
5	—	6 "	100°	1·9	—
6	1·9	12½ "	100°	2·0	1·05
7	2·0	25 "	100°	2·1	1·05

(Specimens of vitamin B_{12} used in experiments 1, 5, 6 and 7 were dried *in vacuo* at room temperature. Crystals dried *in vacuo* at 76°C. were employed in experiments 2, 3 and 4.)

Total phosphorus was estimated by dispensing aliquot portions of each hydrolysate on to small pieces of filter paper and determining phosphorus on them by the perchloric acid digestion method of King[11]. Colour intensities, which reached a maximum 20 minutes after the addition of the aminonaphtholsulphonic acid reducing agent, were measured with a

"Spekker" photoelectric absorptiometer as before. These estimations were carried out in triplicate, the mean results being given in the above table.

Summary and Conclusions

1. Phosphate is found in acid hydrolysates of vitamin B_{12}.
2. Such hydrolysates are deeply coloured and, in order to determine phosphate in them colorimetrically, it has been necessary to develop a new application of the unidimensional paper-strip partition chromatogram technique.
3. The atomic ratio of cobalt: phosphorus in vitamin B_{12} is found to be 1:1.
4. From a comparison of this result (3 above) with the values recorded by Smith[3] for the anti-pernicious anæmia factor of Smith and Parker[6], it is concluded that the latter compound is not identical with vitamin B_{12}.

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THE PHARMACOGNOSY OF RAUWOLFIA

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Introduction

The leaves and roots of *Rauwolfia serpentina* Benth. (*Ophioxylon serpentinum* Linn.), family Apocynaceæ, are mentioned in Sanskrit works describing Ayurvedic medicines under the name "sarpagandha" and are commonly known in India by the Hindustani name of "choota chand." This plant is indigenous to India, Burma, Malaya, Siam and Java, and the drug derived from it (hitherto described as root, but actually consisting of root and rhizome mixed) has been in use in indigenous medicine for several centuries in India. Its reputed successful use has attracted the attention of numerous writers on Indian Materia Medica, including Dymock[1], Watt[2], Chopra[3], Nadkarni[4], Khori and Katrak[5].

Habitat

The plant is described[6,7] as a large climbing or twining shrub, found in the foot-hills of the Himalayas and in the neighbouring plains from Sirhind and Moradabad to Sikkim. It occurs in Assam, Pegu, Tennasserin (at altitudes up to 4,000 feet), in the Deccan peninsula along the ghats to Travancore and in Veylong. It is also widely distributed in the Malaya peninsula and in Java.

Constituents

In 1931, Sen and Bose[8], in Calcutta, isolated from the root two alkaloids having different melting-points. Siddiqui and Siddiqui[9] in the same year, working in Delhi, found five alkaloids and arranged them in two groups, which they named the ajmaline and the serpentine groups respectively; Van Itallie and Steenhauser[10] confirmed these findings in 1932. Other constituents in the roots are oleo-resins, sterols, unsaturated alcohols, oleic acid, fumaric acid, glucose, sucrose, an oxymethylanthraquinone derivative, a fluorescent substance and mineral salts. J. C. Gupta *et al.*[11] state that the roots contain about 1·21 to 1·36 per cent. of total alkaloids, and that a standardised alcoholic extract containing 0·5 per cent. of total alkaloids is being made commercially.

Pharmacological Action

According to Siddiqui and Siddiqui[9], the alkaloids of the ajmaline group, as tried on frogs, act as general depressants to the heart, respiration and nerves; while those of the serpentine group paralyse the respiration and depress the nerves, but stimulate the heart. Sen and Bose[8] found from their experiments on cats that the alkaloids isolated by them brought about a slight fall in blood pressure and the respiration was slightly stimulated. The heart muscle was depressed and the plain muscle, like that of the intestines, uterus, etc., was relaxed. None of these actions represents the characteristic sedative and hypnotic effect for which the drug is valued. For this reason Gupta *et al.*[11] in 1947,

working in India, reinvestigated the drug, giving their attention more especially to the pharmacological action of the oleo-resins present in the root. They report that these oleo-resins possess the specific sedative and hypnotic action of the drug which the alkaloids failed to produce.

Description

A description of the root, with brief notes upon its microscopical structure, was given in Dymock's "Materia Medica of Western India,"

Fig. 1.—*Rauwolfia serpentina* Benth. (= *Ophioxylon serpentinum*, Linn.). Drawing of a flowering plant (about ½ natural size) from Wight, *Icones plantarum Indiæ orientalis*, 1840-53, plate 849. The flowers are in cymes; they have shining red pedicels and calyces, and pure white corollas. The fruits are black berries.

2nd edition, as long ago as 1885. Quite recently the root has been included in the Indian Pharmacopœial List of 1946, where the drug is represented by a brief monograph containing particulars giving information somewhat similar to that in the earlier description of Dymock. These accounts appear to be the only two available descriptions of the structure of the root. It is, moreover, obvious that the data given are not sufficient to characterise the drug properly, either in the unground condition or in the form of powder; also no reference is made to the presence of rhizome in the drug. In view of its promising therapeutic value and increasing importance as an article of commerce, it was decided to make a systematic pharmacognostical study of the drug.

Material

Four samples from different sources were examined, *viz.*:—

1. Sample from the Museum of the Pharmaceutical Society of Great Britain. (July, 1947, S. K. Crews.)
2. Sample from a drug supplier in Saharanpur, U.P., India, obtained in December, 1947.
3. Sample received from the drug market in Calcutta in November, 1947, the identity of which was confirmed by S. N. Bal, Curator, Botanical Survey of India, Calcutta.
4. A fresh plant with the rhizome and a few fibrous roots attached, collected from the foot of the Himalayas near Saharanpur in December, 1947, by a drug dealer. This plant was examined by Dr. Chatterji, in charge of the Indian section of the Herbarium at the Royal Botanic Gardens, Kew, and was confirmed as belonging to the family Apocynaceæ and agreeing in all particulars with the specimen of *Rauwolfia serpentina* Benth. in the Herbarium at Kew.

Macroscopical Characters

Rauwolfia roots (Fig. 2, A) occur in commerce in pale brownish-grey pieces about 4 to 10 cm. in length and 4 to 17 mm. in diameter, cylindrical or slightly tapering, rather tortuous, rarely branched, with occasional small oval or rounded scars of rootlets, usually in a tetrastichous arrangement; the rootlets themselves are very few and when present are broken off short, their diameters varying from 0·5 to 1 mm.; outer surface of the root dull, with somewhat coarse and irregular longitudinal ridges; the outer layers of the cork are soft and tend to scale off from the harder somewhat prominently ridged, yellowish-brown inner bark; patches of entire bark sometimes exfoliate exposing a hard, compact, pale yellowish wood which has a fairly smooth surface and a low density (about 0·3); occasionally pieces of aerial stem or rhizome occur attached to the root. A smoothed transversely cut surface (Fig. 2, B) shows a large pale-yellowish wood, which is compact and finely radiate and usually shows 3 to 8 growth rings. The xylem itself is very finely porous and the woody core occupies about 4/5th of the diameter of the root; surrounding the wood is a narrow yellowish-brown bark about 0·5 to 2 mm.

wide; the root is starchy throughout and the fracture is short. The drug is almost odourless and has a bitter taste.

Pieces of the rhizome closely resemble the root, but are less uniform in diameter; they are somewhat knotty and tortuous. They are best

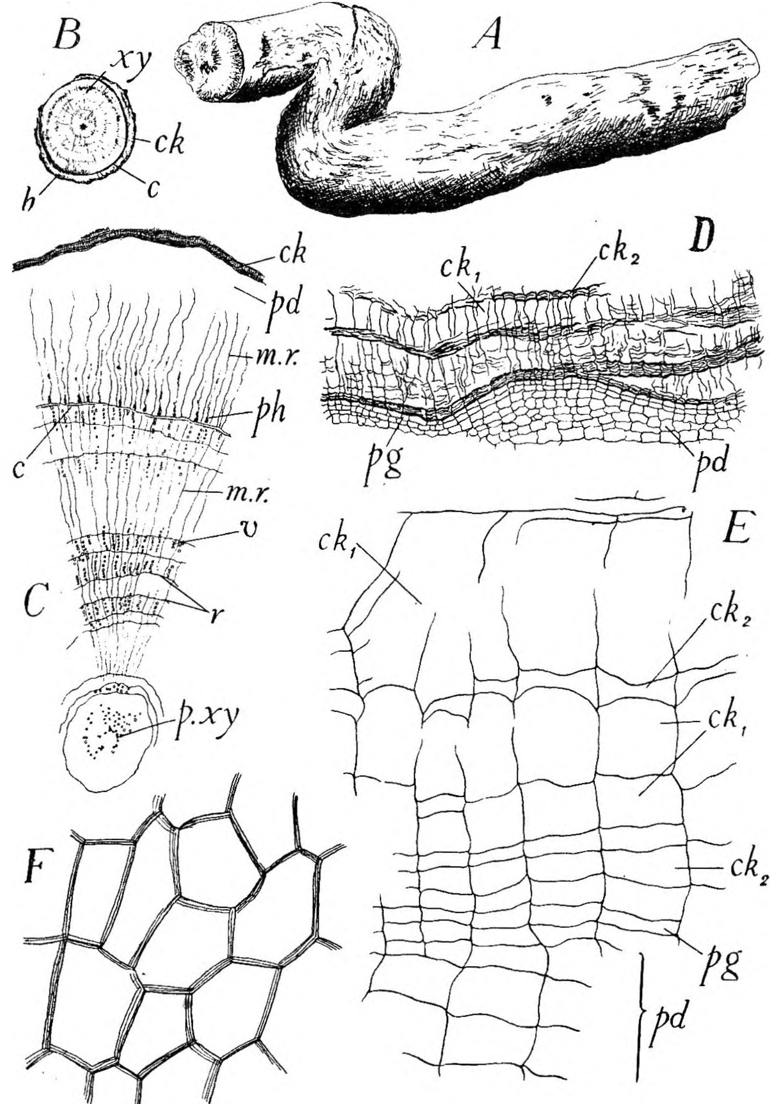

FIG. 2.—*Rauwolfia serpentina* Benth. Root. A, habit sketch of a piece of root ×1. B, smoothed transverse surface of root ×1. C, diagrammatic transverse section ×8. D, transverse section of the tegumentary tissues ×40. E, a portion of D to show details of the cells ×200. F, cork in surface view ×200. *b*, bark; *c*, cambium; *ck*, cork; ck_1, wide cork cells; ck_2, narrow cork cells; *m.r.*, medullary ray; *pd*, phelloderm; *pg*, phellogen; *ph*, phloem; *p.xy.*, primary xylem bundle; *r*, growth ring; *v*, vessel; *xy*, xylem.

distinguished by the smoothed transversely cut surface which exhibits a central pith having a very small diameter (about 1 to 2 mm.).

HISTOLOGY

Root: Externally there are several layers of approximately polygonal-tabular cork cells (Fig. 2, D, E and F) of two kinds, occurring in layers which in transverse sections form bands alternating with each other. One type of band consists of 1 to 5 layers of narrow cells with suberised but unlignified walls measuring about *R = 7 to **11** to 18μ, L = 18 to **35**

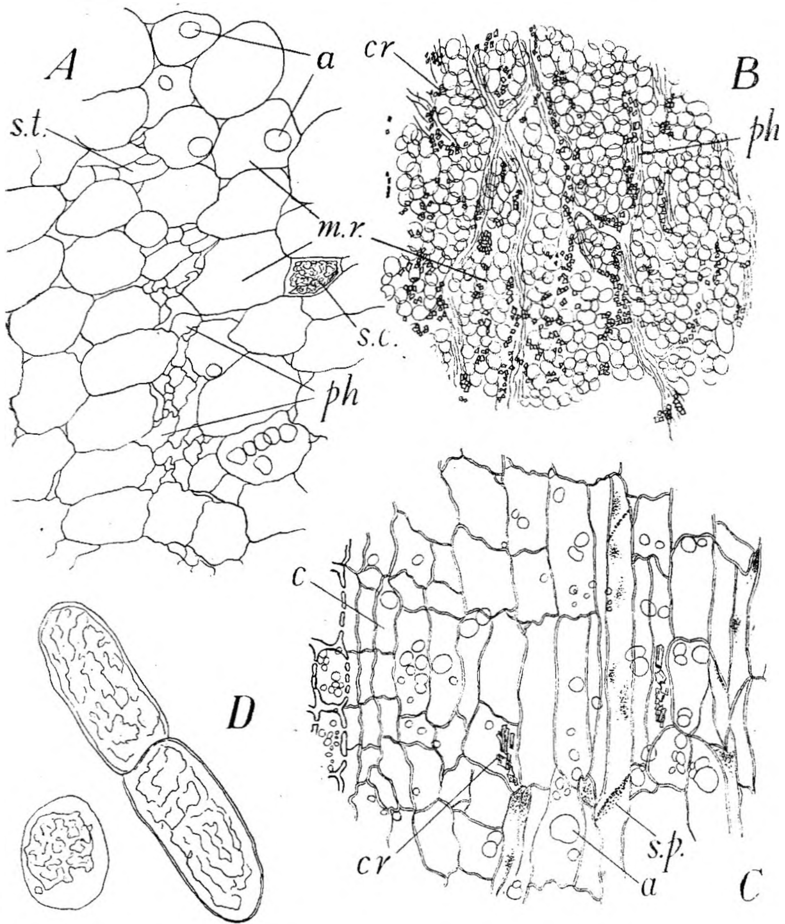

FIG. 3.—*Rauwolfia serpentina* Benth. Root. A, transverse section of the phloem × 200. B, tangential longitudinal section of the phloem × 40. C, radial longitudinal section of the phloem and cambium × 200. D, isolated secretion cells. *a*, starch grains; *c*, cambial cells; *cr*, crystals of calcium oxalate: *m.r.*, medullary ray; *ph*, phloem; *s.c.*, secretion cell; *s.p.*, sieve plate; *s.t.*, sieve tube.

* R, T and L indicate the measurements made in the radial, tangential and longitudinal directions respectively; the use of these symbols is suggested by Moll and Janssonius in their "Botanical Pen Portraits 1923."

to 56μ and T = 49 to **56** to 70μ. Each of these bands is followed by a band consisting of 1 or 2 layers of broad cells with suberised and lignified walls, measuring approximately R = 28 to **42** to 49μ, L = 18 to **35** to 56μ and T = 49 to **56** to 70μ. The radial walls of the broad cells tend to break, and as a consequence the cork frequently peels off in layers. The broad lignified cork cells stain red with phloroglucin and hydrochloric acid, whereas the narrow ones remain yellowish with the same treatment; all the cork cells are insoluble in 80 per cent. sulphuric acid showing that they are suberised.

The cork is followed by a layer of phellogen cells, which have thin cellulosic walls. The phelloderm sometimes consists of about 12 layers of cells, the layers near the phellogen having smaller rectangular tabular cells regularly arranged, whereas the inner layers have larger irregularly shaped cells which appear to have been displaced due to gliding growth during development. The phelloderm cells have walls which are comparatively thicker and more highly refractive than those of the parenchyma of the phloem; they contain numerous starch grains. A few cells of the phelloderm in some specimens contain yellowish granular contents which stain brown with iodine. The fairly wide band of inner bark (Fig. 2, C) consists of numerous broad medullary rays running radially and alternating with comparatively narrow rays of secondary phloem. The cells of the medullary rays (Fig. 3) have thin cellulosic walls and contain numerous starch grains. The medullary rays are 2 to 4 cells wide, the cells being rounded rectangular or ovoid as seen in a transverse section and have small intercellular spaces. The cells measure R = 18 to **28** to 49μ, L = 32 to **63** to 123μ and T = 35 to **63** to 70μ. The path of the ray becomes irregular and indistinct as it approaches the phelloderm. Occasional cells are partly or completely filled with yellowish granular contents which stain brown with iodine or sudan III.

The sieve tissue (Fig. 3), consisting of sieve tubes, companion cells, and phloem parenchyma, lies closely packed in the narrow rays of the phloem between the broad medullary rays, as seen in transverse sections. The majority of the cells of the phloem parenchyma contain starch grains, a few cells contain crystals of calcium oxalate. In a tangential longitudinal section of the bark, the sieve tissue forms irregular wavy lines, the interspaces being filled with the cells of the medullary rays as shown in Figure 3, B. The numerous cells containing calcium oxalate crystals are best seen in this section; some of the crystals are well-formed prisms with which are usually associated numerous irregularly shaped angular crystals of different sizes forming groups or clusters. The clusters or groups usually measure about 7 to **11** to 18μ and the well-formed prisms 11 to 14μ.

The simple starch grains of the inner bark are smaller than those of the wood; they measure 4 to **10** to 20μ and are rounded or ovoid in form with a central hilum. Occasional grains are 2- to 3- occasionally 4-compound. Mounted in lactophenol and observed under polarised light they show a well-defined maltese cross.

The cambiform tissue (Fig. 3, C) consists of 2 or 3 layers of well-defined rectangular cells forming a complete ring between the bark and the wood.

Most of the roots exhibit a tetrarch primary xylem (Fig. 4, A), but occasional roots are triarch; the primary xylem groups form narrow

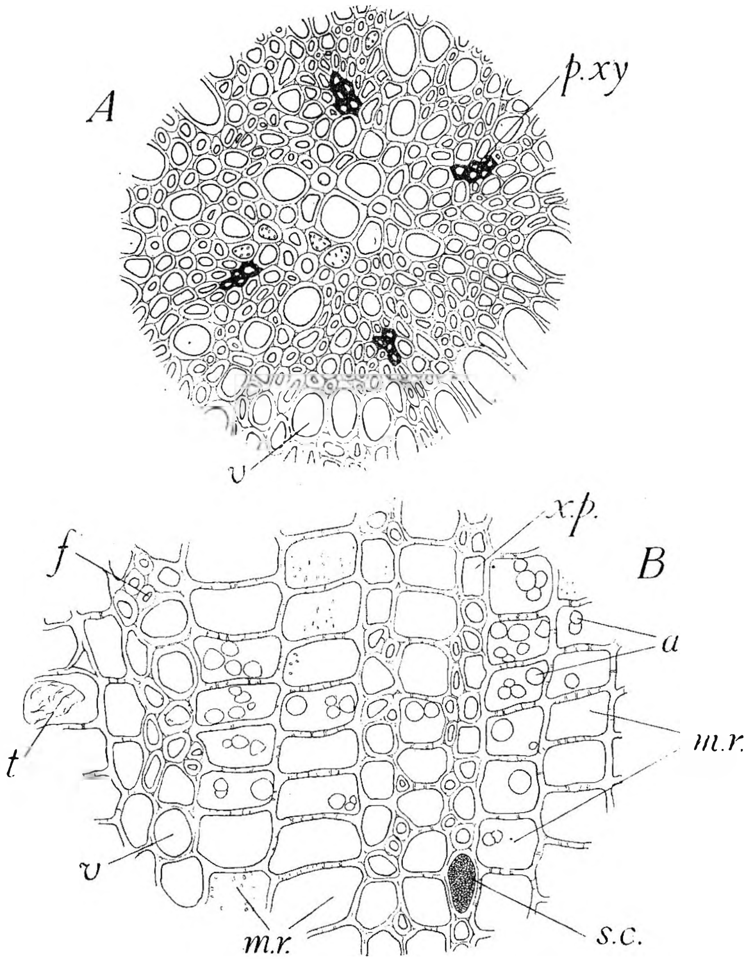

FIG. 4.—*Rauwolfia serpentina* Benth. Root. A, central core of the wood showing primary xylem bundles ×200. B, transverse section of the secondary wood ×200. *a.* starch grains; *f*, xylem fibres; *m.r.*, medullary ray; *p.xy.*, primary xylem bundle; *s.c.*, secretion cell; *t*, resin-like mass; *v*, vessel; *x.p.*, xylem parenchyma.

triangles as seen in a transverse section. The wood consists of strongly lignified cells (Fig. 2, C), the secondary xylem being arranged in growth rings of varying width, most commercial specimens showing about 3 to 6 seasons' growth. All the cells of the xylem parenchyma (Fig. 4, B) and of the medullary rays contain abundant starch, the grains being simple or 2- to 4-compound; the simple grains measure about 6 to **20** to 46μ,

the components of the compound grains being rather smaller. Occasional cells of the xylem parenchyma are either completely or partly filled with a yellowish granular substance which stains brownish with iodine or sudan III. In a transverse section the medullary rays are well marked, straight, and continuous with the medullary rays of the phloem. The narrow rays of xylem form anastomosing longitudinally undulating bands. The medullary rays are 1 to 5 cells wide alternating with narrow rays of secondary xylem which consists of vessels, fibres and xylem parenchyma. The longest medullary rays start from a point opposite the apices of the protoxylem groups, others originate at points nearer the circumference making the rays more numerous in the outer growth rings. The cells of the medullary rays measure approximately R = 14 to **63** to 86μ, L = 17 to **42** to 70μ and T = 14 to **42** to 60μ, their walls as well as those of the xylem parenchyma having simple pits except when they adjoin a vessel when they bear half-bordered pits.

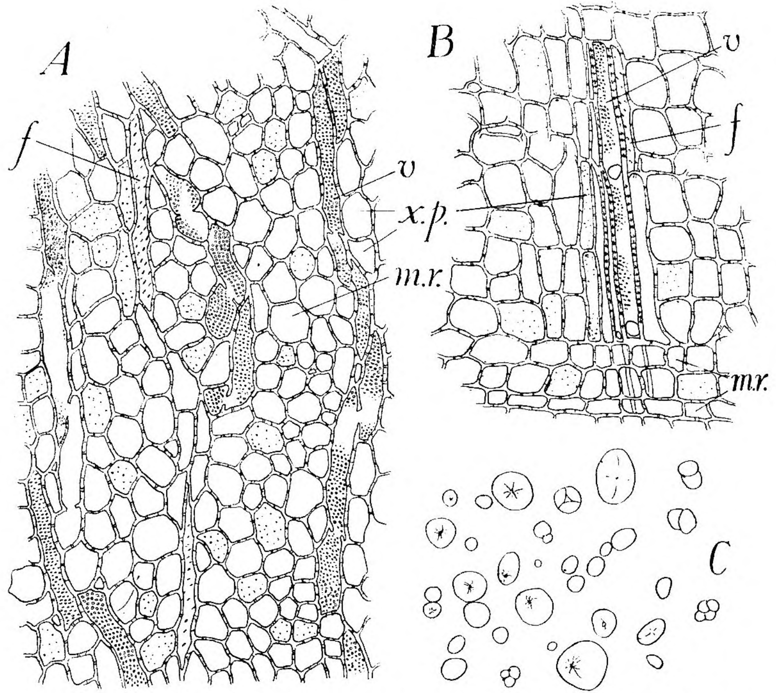

FIG. 5.—*Rauwolfia serpentina* Benth. Root. A, tangential longitudinal section of the wood ×100. B, radial longitudinal section of the wood ×100. C, simple and compound starch grains from powdered drug × 200. *f*, xylem fibre; *m.r.*, medullary ray; *x.p.*, xylem parenchyma; *v*, vessel.

The cells of the scanty xylem parenchyma measure about R = 14 to **46** to 70μ, L = 46 to **53** to 77μ and T = 14 to **42** to 56μ.

The vessel elements (Fig. 6), which bear numerous bordered pits, measure about 180 to **234** to 432μ in length and 36 to 54μ in diameter. The transverse or oblique articulations of each element form two rounded

or oval openings either at opposite ends or on the side walls; in the latter case the openings are usually diagonally opposite to each other. The planes of junction of the vessel elements are at right angles to the tangential plane and often lie in the radial plane as shown in Figure 5. Many vessel elements are prolonged at their ends into fibre-like projections. The vessels are associated with numerous xylem fibres having strongly thickened walls and spirally arranged slit-like simple pits. The fibres measure approximately 432 to **576** to 774μ in length and 18 to

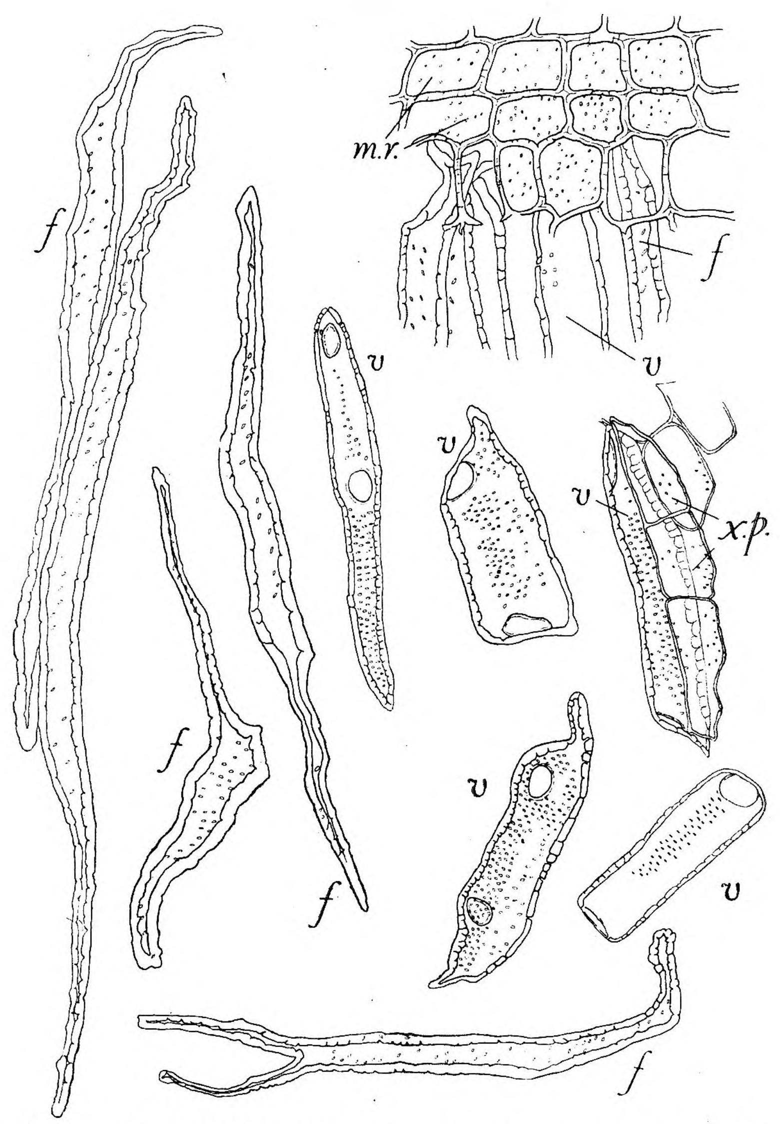

FIG. 6.—*Rauwolfia serpentina* Benth. Root. Isolated elements of the wood from chromic-nitric acid preparation ×200. *f*, xylem fibre; *m.r.*, medullary ray; *x.p.*, xylem parenchyma; *v*, *vessel*.

36μ in diameter. Many fibres possess one, or sometimes two, long tapering and often contorted ends. There are very small intercellular spaces between the elements of the xylem.

Structure of the Rhizome

Histologically, the rhizome differs from the root in certain respects only. The cork of the rhizome (Fig. 7, B) closely resembles that of

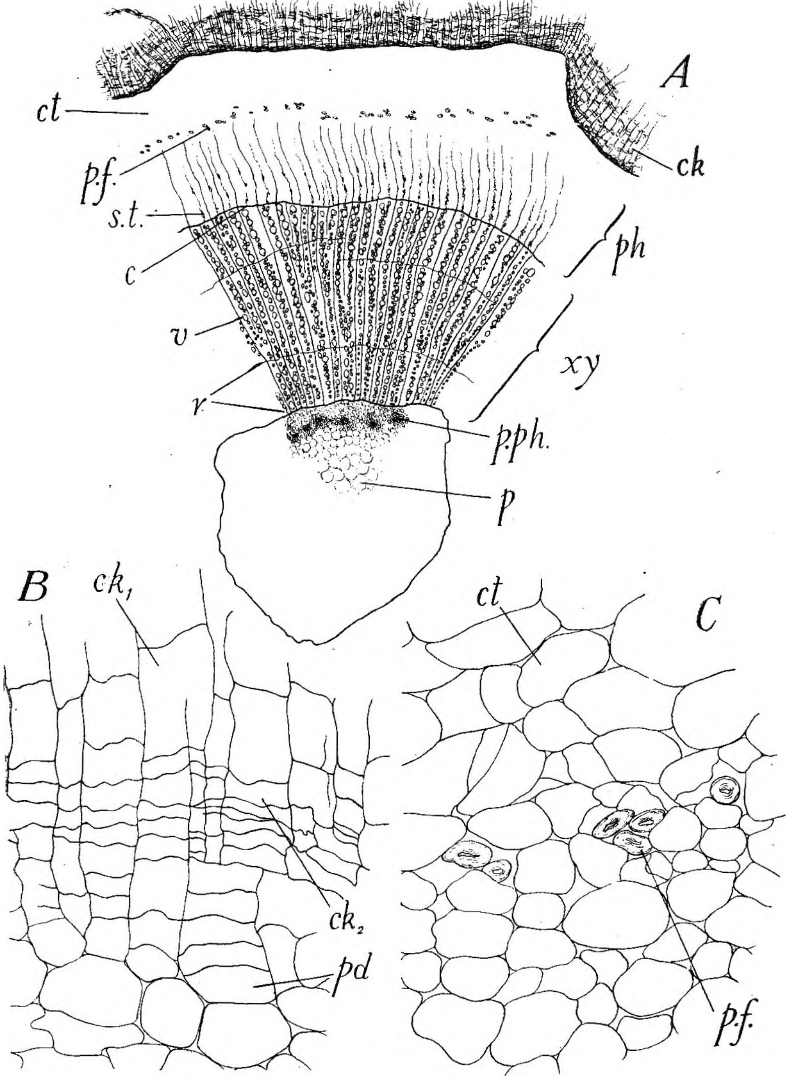

Fig. 7.—*Rauwolfia serpentina* Benth. Rhizome. A, diagrammatic transverse section ×25. B, transverse section of the tegumentary tissues ×200. C, transverse section of the pericyclic region; all drawings ×200. *c*, cambium; *ck*, cork; ck_1, wide cork cells; ck_2, narrow cork cells; *ct*, cortex; *p*, pith; *pd*, phelloderm; *ph*, phloem; *p.ph.*, peri-medullary phloem; *p.f.*, pericyclic fibres; *r*, growth ring; *s.t.*, sieve tissue; *xy*, xylem.

the root but, owing to the presence of some cells intermediate in radial width between those of the wide and the narrow cells, the layering is less distinct. The phelloderm in the rhizome is narrow and consists of about 3 to 6 layers only, as compared to about 12 layers present in

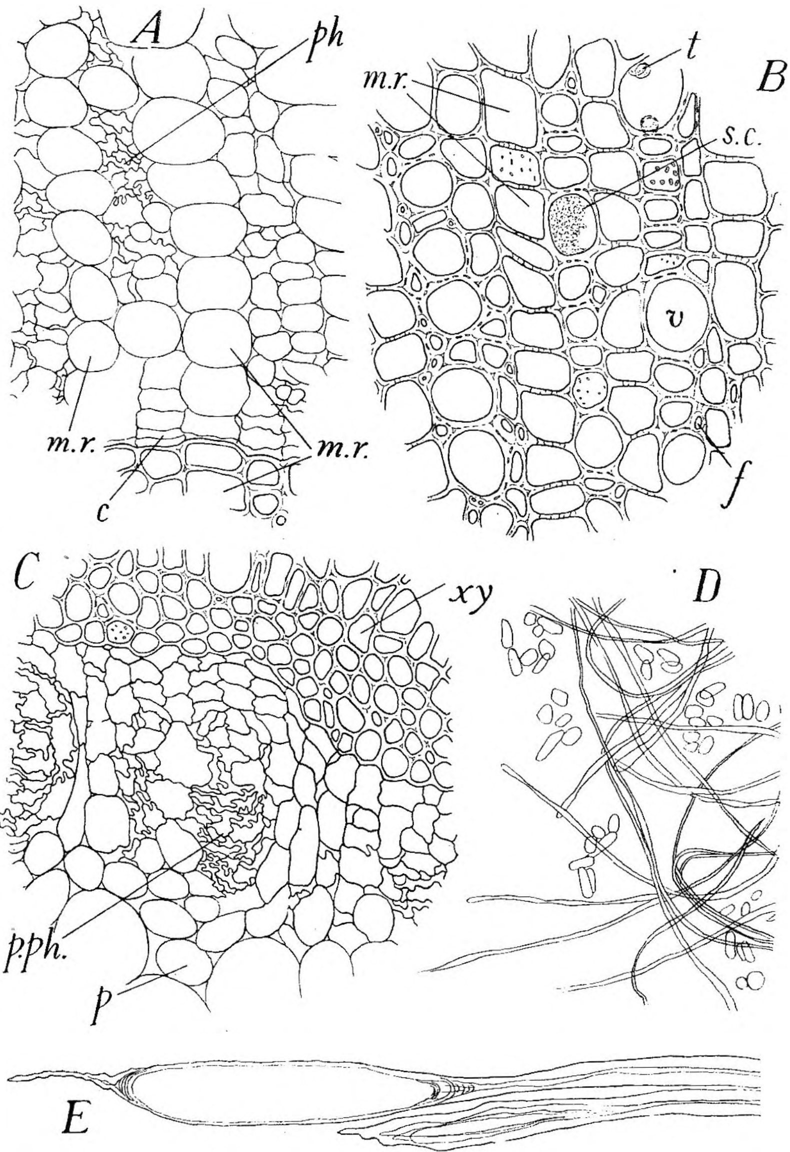

FIG. 8.—*Rauwolfia serpentina* Benth. Rhizome. A, transverse section of phloem adjacent to the cambial tissue ×200. B, transverse section of the wood ×200. C, peri-medullary phloem and pith ×200. D, pericyclic fibres and cells from potash preparation ×40. E, tip of pericyclic fibre showing local enlargement ×200. *c*, cambium cells; *f*, xylem fibre; *m.r.*, medullary ray; *p*, pith; *ph*, phloem; *p.ph.*, peri-medullary phloem; *s.c.*, secretion cell; *t*, tylose; *v*, vessel; *xy*, xylem.

the root. This is followed by a wide cortex and a pericycle (Fig. 7, C), the cell walls of which are very refractive and in which are present pericyclic fibres, either solitary or in groups of 2 to 4. The fibres (Figs. 8, D and E) have very thick walls and a correspondingly narrow lumen. Many of these fibres show a few scattered elongated ovoid enlargements especially near the ends, a feature which is typical of the Apocynaceæ. These fibres measure about 1·5 mm. in length and 7 to 20μ in diameter with local enlargements about 50μ in width. Secretion cells are more numerous in the rhizome than in the root and are found chiefly in the cortex and in medullary rays of the phloem. The medullary rays of the phloem (Fig. 8, A) are 1 to 3 cells wide, and the cells measure R = 18 to **28** to 49μ, L = 46 to **63** to 125μ and T = 18 to **35** to 77μ. The starch grains in the rhizome are smaller than those in the root, measuring 2 to **11** to 21μ. The medullary rays of the xylem (Fig. 8. B) are 1 to 4 cells wide, and the cells measure R = 7 to **32** to 53μ, L = 14 to **46** to 92μ and T = 7 to **21** to 49μ; the xylem parenchyma is more abundant than in the root (Fig. 8). The vessels closely resemble those of the root, measuring 105 to **308** to 490μ in length and 25 to **46** to 60μ in diameter. A peculiar feature of the rhizome, particularly in the older pieces, is the presence, in many of the vessels, of numerous tyloses

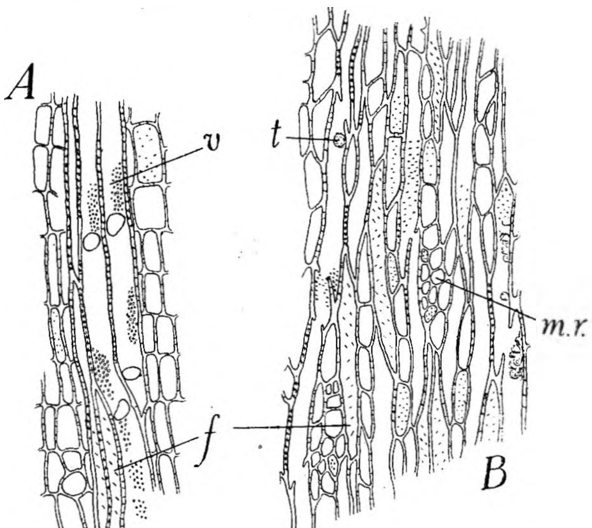

FIG. 9.—*Rauwolfia serpentina* Benth. Rhizome. A, radial longitudinal section of the wood. B, tangential longitudinal section of the wood, both drawings × 100. *f*, xylem fibre; *m.r.*, medullary ray; *t*, tylose; *v*, vessel.

(Figs. 8, B and 9, B) of varying sizes which sometimes almost block the lumen. In some of the vessels exhibiting tylosis, there are granular masses which stain bright red with phloroglucin and hydrochloric acid. Xylem fibres are comparatively abundant in the rhizome and are almost straight; they measure 193 to **560** to 753μ in length and 11 to **21** to 35μ in diameter.

The central pith (Figs. 7, A and 8, C) is small, about 0·75 to 1·5 mm. in diameter, and contains in its periphery about 20 small bundles of perimedullary phloem; the remainder of the pith consists of cellulosic parenchyma, the cells of which vary much in size and some of them are filled with a yellowish secretion similar to that in the cortex and phloem.

Laticiferous Tissue

As the presence of laticiferous tubes is usually regarded as an important characteristic of the Apocynaceæ, a careful search was made for these. No laticiferous tubes were found in the root, but occasional parenchymatous cells in the phloem were found to be filled with yellowish granular contents which stained brown with iodine (Fig. 3, D).

Examination of the rhizome also showed an absence of typical laticiferous tubes; there were, however, a number of secretion cells occurring in the cortex, in the phloem and in the pith, their granular contents staining yellow with iodine or sudan III. Occasionally the secretion cells are arranged in longitudinal rows of 2 to 4, but the transverse walls remain intact.

Powdered Rauwolfia

The salient features of the powdered drug are:—

1. Very numerous rounded and ovoid starch grains about 4 to **20** to 50μ in diameter, occasional grains being 2- to 4-compound. The starch is rapidly gelatinised in the cold by the action of 0·9 per cent. aqueous solution of potassium hydroxide, the action being more rapid than the similar action of the same solution on potato starch. Strong hydrochloric acid (sp. gr. 1·18) gelatinises the starch instantaneously in the cold.

2. Much lignified rectangular parenchyma having moderately thickened walls bearing simple pits; most of the cells contain numerous starch grains.

3. Fragments of xylem vessels about 36 to 54μ in diameter, with bordered pits and, associated with them, fragments of the characteristic xylem fibres.

4. Fragments of yellowish cork composed of polygonal-tabular cells of two types, broad and narrow.

5. Small quantities of cellulosic parenchyma, usually filled with starch grains, and occasional unlignified pericyclic fibres showing local swellings.

6. Occasional rounded rectangular secretion cells with yellowish granular contents; and scattered prismatic or irregularly triangular crystals of calcium oxalate.

Summary

1. Rauwolfia of Indian commerce consists of the dried rhizomes and roots, with occasional small pieces of attached aerial stem, of *Rauwolfia serpentina* Benth.

2. The important histological features are the cork, composed of alternating layers of broad and narrow cells, giving a somewhat spongy

and friable exterior to the drug; the narrow bark and the wide central mass of wood in the root or broad ring in the rhizome, showing about 3 to 8 growth rings; a tetrarch, or occasionally triarch, primary xylem in roots or a very small pith at the centre of rhizomes; in the periphery of the pith a ring of about 20 small groups of perimedullary phloem.

3. All the cells of the wood, including those of the medullary rays are lignified. The xylem vessels, fibres and parenchyma form narrow undulating radially arranged bands, separated by large medullary rays in the root and by smaller ones in the rhizome. The vessel segments are small and narrow and the xylem fibres are often irregularly shaped with long, usually much contorted, tapering ends; many of the vessels, especially in the rhizome, show tylosis and occasionally contain resinous masses. The phloem also occurs in undulating bands similar to those of the xylem; phloem fibres are absent.

4. In the rhizome, unlignified pericyclic fibres occur, either singly or in groups of 2 to 4; they show the local and sub-terminal elongated-oval enlargements characteristic of the Apocynaceæ.

5. All the parenchymatous tissues, except the cork, contain numerous starch grains, about 4 to **20** to 50μ in diameter. Cells containing a brownish yellow secretion occur in small numbers, either singly or in short longitudinal rows, in the bark, wood and pith, being most frequent in the cortex of pieces of rhizome. Typical laticiferous tubes are absent. Calcium oxalate occurs in numerous cells of the phloem and of the medullary rays of the bark; the crystals are either well-formed prisms or aggregates of irregular angular components.

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THE STRUCTURE OF γ-SUGARS

PART VI

THE SYNTHESIS OF A FURANOSE, A 6-METHYLKETOHEXOSE

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THE γ-sugars are unstable and have not yet been isolated in the free state. They occur in nature only in glycosidal combination, e.g., in sucrose, inulin, graminin and in sinistrin, on hydrolysis of which only the normal form of the sugar is isolated. Derivatives of many of the hexoses and pentoses are known to exist in the γ-form. The simplest derivatives of these labile sugars are the glycosides, which have been found to differ widely in their chemical properties from those of the normal sugars. Thus they are susceptible to hydrolysis by very dilute mineral acid and can be distilled without decomposition *in vacuo.* Furthermore, the γ-glycosides reduce permanganate solution in the cold, a property which has not yet been shown by any of the glycosides of normal sugars.

Haworth and collaborators are responsible for the accepted explanation of the difference in constitution, the γ-sugars being derived from furan, whilst the normal sugars are similarly related to pyran. In this connection it is of interest to note that Hersant and Linnell[1] obtained by synthesis a methylated hexose which could not possess a five-membered ring and which exhibited properties in agreement with those of the normal sugars. Fischer[2] first isolated γ-methyl-glucoside and Haworth and Law[3] established their occurrence in natural products by proving that the fructose residue in sucrose was a γ-form.

Evidence for the furanose structure of γ-fructose rests mainly on the degradation of tetramethyl-γ-fructose obtained on hydrolysis of heptamethylsucrose through trimethylfructofuronic acid to trimethyl-γ-arabinolactone and then to dimethoxysuccinic acid,[4,5,6]. Other structures[7,8] have been suggested, but most of them are based upon studies of the oxidation products of derivatives and these oxidations are far from being quantitative. Hartley and Linnell[9], however, on the basis of parachor studies of partially and fully methylated derivatives of γ-fructose, from the kinetic studies of the hydrolysis of sucrose and on account of the absence of mutarotation in both 3:4:6 trimethyl-γ-fructose and 6-methylfructose solutions, suggested for γ-fructose and its non-glycosidic derivatives an open chain keto-alcohol structure.

The determined parachor values of tetramethyl-γ-fructose and of tetramethyl-γ-methylfructoside were found to be significantly lower than those calculated for the structure assigned to γ-fructose by either Haworth[10] as a five-membered ring or by Hudson[11] as a four-membered ring structure. Again five- and six-membered oxygen ring compounds are not normally different in their stability (Linnell and Melhuish[12]).

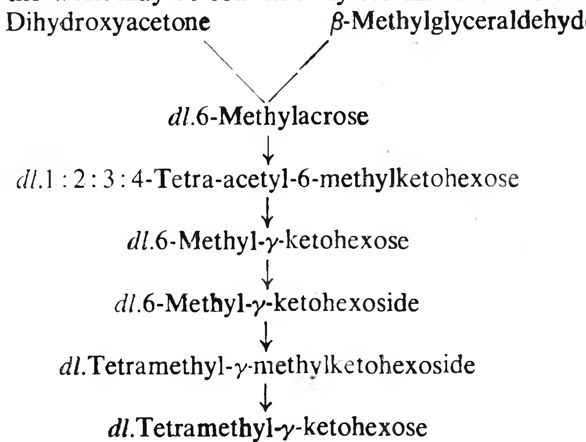
The stability of γ-fructose and the heat of activation of its conversion to normal fructose as obtained from the kinetic studies of sucrose lead to results which are not completely explained by the accepted furanose structure.

The half-life period of γ-fructose as has been shown by Hartley and Linnell[13] and others[14,15] to be too small to permit its isolation, and hence any structural enquiries concerning such compounds must rest on a study of their derivatives. The justification for such approach rests on a precise knowledge of the constitution of the derivative used. No derivative of a γ-sugar has yet been synthesised by an unambiguous route, although Hartley and Linnell[11] synthesised γ-fructose derivatives which could not exist in pyranose form[16].

It was, therefore, decided to attempt the synthesis of a monomethyl-ketohexose in which the position of the methoxy group would be established beyond doubt by the method used. Such a method would not only avoid the possibility of a ring change during methylation, but would also be free from any criticism which might be directed towards the drastic reactions involved in the oxidative degradation methods adopted by Haworth and his collaborators.

By a modification of Fischer's synthesis of acrose[17,18], using pure dihydroxyacetone and monomeric β-methylglyceric aldehyde, a mono-methyl-ketohexose was obtained which could not be other than a 6-monomethyl-ketohexose. No 6-monomethyl-ketohexose had been synthesised before.

The preparation of the starting materials for this synthesis have been previously reported[19] and the main series of reactions involved in the remainder of the work may be conveniently summarised as follows:



The condensation of dihydroxyacetone and glyceraldehyde was shown by Fischer[17] to take place in the presence of 1 per cent. of sodium hydroxide. Schmitz[20] (see also Jackson[21] and Neuberg[22]) obtained a relatively good yield of acrose using 0·1 per cent. barium hydroxide solution. Hersant and Linnell[1] effected condensation of dihydroxy-acetone and methyl-γ-glyceraldehyde by using 0·1 per cent. barium

hydroxide solution for a total period of 5 weeks, sufficient barium hydroxide being added from time to time to maintain the alkalinity of the reaction. In the present research it was decided to employ the latter method as being less likely to induce epimerisation or resinification.

Dihydroxyacetone and β-methylglyceraldehyde in equimolecular proportions were therefore condensed in 5 per cent. aqueous solution in the presence of 0·1 per cent. of barium hydroxide. After a few days the solution became yellow and sufficient barium hydroxide was added from time to time to maintain a well-marked alkalinity, the solution being kept in the dark for a period of six weeks.

Fischer[17] isolated *dl*-fructose from the condensation product through osazone formation, which he converted to osone and then to fructose by reduction. Hersant and Linnell[1] obtained *dl*-5-methylfructose by following the method of Fischer. Isolation was attempted according to Schmitz[20], who was able to isolate a crystalline hexose directly. The solution containing the condensation product was therefore neutralised and evaporated to dryness *in vacuo,* the residue being taken up in absolute alcohol, but on evaporation *in vacuo* no crystalline substances could be isolated. Addition of anhydrous ether to the alcoholic solution caused the separation of a white, amorphous substance which gave aggregates of needle crystals (m.pt. 99° to 101°C.) on treatment with phenylhydrazine acetate.

The number of products obtainable in this condensation must include derivations of *dl*-fructose, *dl*-sorbose, *dl*-tagatose and *dl*-psicose, but previous experience has shown[17] that *dl*-fructose predominates, the only other sugar isolated in small quantity being *dl*-sorbose. Since dihydroxyacetone in the alkaline medium may enolise, it is not impossible that derivatives of the eight *dl*-aldoses might be present in the product.

It is not surprising, therefore, that little success crowned the efforts to isolate the osazones directly from the alcoholic solution of the product. A small yield of a crystalline product melting at 130° to 132°C. was obtained, however, and this could be *dl*-methylsorbosazone, but the yield was very small. Recourse was, therefore, made to a different method of isolation through the acetylated product. The acetylation was attempted according to the method of Erwig and Koenigs[23], but the yield was too small. Modification of the process of Hudson and Braun[24] (by using dry material, increasing the amount of acetic anhydride, stirring for a longer time, and special treatment of the chloroform emulsion obtained on extraction) gave more promise. After removal of the chloroform the main product was a light yellow syrup together with a few crystals. Keeping over potassium hydroxide *in vacuo* for 14 days caused no alteration. The crystals were probably traces of acetylated aldoses and the syrup—the main product—represented a 69 per cent. yield and gave analytical figures for a tetra-acetylmethylhexose. Saponification indicated that the purity was about 99 per cent. This saponification required special care because the methylhexose resulting may be readily attacked by acids or alkalis. Erwig and Koenigs found that the

liberated fructose was slightly attacked by 3 hours' boiling with 0·1N sulphuric acid.

Hudson and Braun observed a similar action of acid on the liberated fructose and after various modifications discarded the method of acid hydrolysis. They found that if 0·1N sodium hydroxide was used for saponification of fructose penta-acetate by shaking at 0°C., the liberated fructose was not attacked by the amount of alkali present.

Determination of the saponification value of the tetra-acetylmethylhexose was then carried out by shaking with 0·1N sodium hydroxide at room temperature for 4½ hours and followed by titration with 0·1N sulphuric acid using phenolphthalein as indicator. After saponification concentration of the liquid *in vacuo* after neutralisation left a brown syrup which reduced Fehling's solution slowly in the cold, vigorously on warming and reduced alkaline permanganate solution in the cold.

Saponification of larger quantities of tetra-acetylmethylhexose was carried out under the same conditions and the liquid was neutralised with dilute acetic acid, filtered and evaporated to dryness *in vacuo*. The residue was then extracted with absolute alcohol, filtered from the sodium acetate, decolorised with charcoal and concentrated *in vacuo*. It was redissolved in alcohol and reconcentrated until sodium acetate was completely eliminated. A pale yellow syrup remained, which after keeping in a vacuum desiccator for a long time became a toffee-like mass. Methoxyl determination of the product gave 4 figures which were slightly high probably owing to the presence of a little 1:6-dimethylhexose formed by condensation of β-methylglyceraldehyde with monomethyldihydroxyacetone formed by the action of alkali on β-methylglyceraldehyde.

	Test	Result
1.	Molisch's test:—	Positive.
2.	Barfoed's reagent:—	Reduction on warming.
3.	Fehling's solution:—	Reduction slowly in the cold, vigorous on warming.
4.	Ammoniacal silver nitrate solution:—	Reduction in the cold, mirror on warming.
5.	Schiff's reagent:—	Magenta colour slowly.
6.	Alkaline potassium permanganate solution:—	Instantaneous reduction in the cold.
7.	Methyl alcoholic hydrochloric acid cold for 1 hour:—	Syrup did not reduce Fehling's solution, but reduced permanganate in the cold.
8.	Phenylhydrazine acetate solution:—	Needle crystalline osazone m.pt. 130° to 132°C.

9. After standing in a vacuum desiccator for 3 months, 6-methylketohexose was found unchanged and still reduced alkaline potassium permanganate in the cold.

Reduction of Fehling's solution and potassium permanganate in the cold, reaction with Schiff's reagent and formation of a methylglycoside

in the cold are held to be characteristics of γ-sugars and are not given by normal sugars under the specified conditions.

Glycoside formation was accomplished by dissolving a quantity of *dl*-γ-methylhexose in methyl alcohol containing 1 per cent. of dry hydrogen chloride and keeping the solution at room temperature for 2 hours. On isolation a yellow syrup remained which failed to reduce Fehling's solution even after warming, but reduced alkaline permanganate in the cold. Any glycoside obtained by this established method should be a γ-glycoside; its behaviour towards permanganate together with its syrupy consistency differentiates it from the crystalline normal glycopyranosides.

Further indication of the nature of this glycoside was given on attempting its hydrolysis with N/100 aqueous hydrochloric acid: the hydrolysis was complete in 1 hour, whereas γ-methylglucopyranoside was hardly affected under these conditions.

The mixture of 6-methylhexoses was methylated with dimethylsulphate and sodium hydroxide[25] followed by Purdie's[26] reagent. The product on isolation did not crystallise and gave analytical figures for a tetramethyl-methylhexoside. It did not reduce Fehling's solution, but reduced permanganate in the cold. On hydrolysis it gave a liquid product which reduced Fehling's solution and potassium permanganate in the cold.

The product of the condensation of β-methoxy-α-hydroxypropionaldehyde and dihydroxyacetone possessed properties which sharply differentiated it from a pyranose. The condensation could not yield an amylene-oxide structure owing to the blocking of the 6-hydroxyl group. It could possess a ketone structure or a 1:2, 2:3, 2:4 and 2:5-oxide rings of which the 2:5 is by far the most likely. The results of this synthetic experiment indicates that hexoses possessing other than the amylene-oxide structure exhibit properties far more reactive than the normal sugars. These differences are greater than would be expected between derivatives of furane and pyrane, but the results of these experiments are difficult to explain on any other basis.

The separation of the product of this synthesis into individual sugars by partition of the acetylated material by chromatographic methods will be communicated later.

EXPERIMENTAL

Condensation of β-methoxy-α-hydroxypropionaldehyde and dihydroxyacetone.—Freshly prepared β-methoxy-α-hydroxypropionaldehyde (20 g.) dissolved in 400 ml. of water was mixed with a solution of dihydroxyacetone (20 g.) in 400 ml. of water and 1 g. of barium hydroxide dissolved in a little hot water was added to the mixture. The solution was kept protected from light for 6 weeks, sufficient barium hydroxide being added from time to time to maintain a well-marked alkalinity. The solution became yellow in colour during the condensation.

Isolation of the products.—(a) An attempt to isolate by treatment of the above solution with phenylhydrazine-acetate mixture resulted in the

formation of a black oil from which a small quantity of pale yellow needles melting at 130° to 132°C. was obtained.

(b) The condensate was neutralised with dilute sulphuric acid and the filtered solution was evaporated *in vacuo* at 30° to 35°C. The light brown syrupy residue was dissolved in absolute alcohol and filtered. On removal of the alcohol no crystals appeared and the syrupy residue was redissolved in alcohol and diluted with ether when a voluminous, white, amorphous precipitate formed. After separation, washing with ether and drying over sulphuric acid a hygroscopic creamy product was obtained which reduced Fehling's solution on warming and ammoniacal silver nitrate solution in the cold.

The alcohol-ether filtrate was evaporated at ordinary temperature giving a light brown syrup which reduced Fehling's solution and ammoniacal silver nitrate solution.

6-*Methylketohexose.*—The solution (800 ml.) containing the condensation products was carefully neutralised and the clear filtrate was evaporated under reduced pressure at 30° to 35°C., a little alcohol being added towards the end of the process. A viscous yellow syrup (35 g.) was obtained which gave all the tests characteristic of a γ-sugar. In all some 200 g. was prepared.

6-*Methyltetra-acetylketohexose.*—The dried 6-methylketohexose (25 g.) was slowly added to a mixture of 150 ml. of acetic anhydride and 6.25 ml. of sulphuric acid, the mixture being strongly cooled. On stirring in the cold for about 2 hours complete solution was affected. The mixture was poured into 600 ml. of ice-cold water, neutralised with sodium bicarbonate, filtered and the residue, consisting of the acetylated sugar, was dissolved in chloroform. The filtrate was repeatedly extracted with chloroform, the chloroform solutions were washed with water, dried, treated with activated charcoal, filtered and evaporated under reduced pressure. The syrupy residue was kept over potassium hydroxide *in vacuo,* when signs of crystallisation appeared but the bulk of the material remained as a yellow syrupy liquid. Yield 32 g. (69 per cent.) Found C, 49·07; H, 7·03 per cent.; $C_{15}H_{22}O_{10}$ requires C, 49·72; H, 7·08 per cent. 0·5086 g. of syrup required 55·70 ml. of N/10 sodium hydroxide for complete saponification; theory requires 56·10 ml. of N/10 sodium hydroxide.

Purified 6-*methylketohexose.*—The tetra-acetate (75 g.) was hydrolysed with N/10 sodium hydroxide and after isolation in the usual way a yellow, very viscous syrup was obtained which reduced Fehling's solution slowly in the cold but vigorously on warming, reduced potassium permanganate solution in the cold and formed a glycoside on treatment with methyl alcoholic hydrogen chloride at normal temperature for 1 hour. These properties are characteristic of a γ-sugar. A determination of methoxyl using a modified Pregl method gave OCH_3, 16·78 per cent.: $C_6H_{11}O_5(OCH_3)$ requires 15·97 per cent. The slightly high figure was probably due to the presence of a small quantity of dimethyl derivative (*vide supra*).

6-Methyl-γ-methylketohexoside.—A solution of 6-methylketohexose (1 g.) in 15 ml. of pure methyl alcohol containing 1 per cent. of dry hydrogen chloride was kept at room temperature for 2 hours. The solution was neutralised with silver carbonate, filtered and the solvent removed by distillation. The residue, a brown syrup, failed to reduce Fehling's solution even after warming, but it reduced potassium permanganate solution in the cold. On hydrolysis the power to reduce Fehling's solution was restored.

Hydrolysis of 6-methyl-γ-methylhexoside.—A solution of 6-methyl-γ-methylhexoside (0·69 g.) in 12 ml. of N/100 hydrochloric acid was placed in a water-bath at 95°C. At intervals 0·2 ml. of the solution was removed and added to 1 ml. of Fehling's solution diluted with 2 ml. of water. The solution was replaced in the water-bath and the amount of reduction was observed at the end of 5 minutes. The results were compared with those of methylglucopyranoside treated similarly.

Time in Minutes after Beginning Heating at 95°C.	Reduction Observed	
	6-methyl-γ-methyl-ketohexoside	methylglucopyranoside
5	Distinct reduction	Very slight reduction
10	,, ,,	,, ,, ,,
15	Strong reduction	Slight reduction
30	Rapid ,,	,, ,,
60	Complete ,,	,, ,,

Tetramethyl-γ-methylketohexoside.—The 6-methylketohexose was methylated with dimethyl sulphate and sodium hydroxide by the normal method, at first at 30°C. and then at 70°C. After isolation the process was repeated and the product was worked up in the usual manner. The product was then further methylated, using methyl iodide and silver oxide. On isolation 12 g. of a yellow syrup was obtained which showed no reduction of Fehling's solution, but still reduced potassium permanganate solution in the cold. Found OCH_3, 60·19 per cent.; $C_6H_7O(OCH_3)_5$ requires OCH_3, 62 per cent.

Tetramethyl-γ-ketohexose.—Hydrolysis of the tetramethyl-γ-methylketohexoside (1 g.) was effected with 1 per cent. aqueous hydrochloric acid by heating under reflux for half an hour. After neutralisation and isolation in the usual manner a syrup remained which reduced Fehling's solution and potassium permanganate solution in the cold.

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A CHLOROHYDROXY-TRIPHENYLMETHANE DYE

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Ehrlich and Bechhold[1] first showed conclusively that the introduction of a halogen atom into the aromatic nucleus produced an increase in the bactericidal activity of phenols. Karpow[2] had already demonstrated that of the three isomeric chlorophenols the para-compound was the most active. These results received further confirmation in later work carried out by Kurodo[3] although Klarmann[4] showed that the activities of the three chlorophenols increased in the order *ortho-*, *para-*, *meta-*. An increase in the molecular weight of both simple phenols and halogenated phenols by the introduction of alkyl, aryl and aryl-alkyl groups into the molecule causes a marked increase in activity. Klarmann, Schternov and Gates[5] tested a series of alkylchlorophenols against 6 different micro-organisms and showed that whereas a peak of activity against *Eberthella typhi* was obtained in those compounds with 5 carbon side chains the peak of activity against *Staphylococcus aureus* and *Streptococcus hæmolyticus* was only attained when the side chain contained 7 or 8 carbon atoms. Furthermore *o*-alkyl-*p*-chlorophenols are more active than *o*-chloro-*p*-alkylphenols. In a corresponding series of benzylhalophenols Klarmann, Gates and Schternov[6] showed that a halogen which is *ortho* to a hydroxyl group increases the phenol coefficient less than when in the *para*-position, though the general level of activity in these diphenylmethane derivatives is lower than that for the corresponding alkylhalophenols. 5-Chloro-2-hydroxydiphenylmethane has approximately half the activity of 2-*n*-amyl-4-chlorophenol toward *Staph. aureus* and *Strep. hæmolyticus*. Contrary to the greater activity against both Gram-negative and Gram-positive organisms of bromophenols over chlorophenols in the simpler series, the monobromo derivatives of 2- and 4- hydroxydiphenylmethanes are less effective against Gram-negative organisms yet more so against Gram-positive types than the corresponding chloro compounds.

Basic dyes of the triphenylmethane series such as malachite green (I, $R = CH_3$, $X = Cl$), brilliant green (I, $R = C_2H_5$, $X = HSO_4$) and crystal violet (II, $R = CH_3$, $X = Cl$) are well established as antibacterial agents, yet little attention has been paid to the possibilities of their chloro- and hydroxy- derivatives as potential bactericides. New solid green 3B,

$$C_6H_5-C(=C_6H_4=\overset{+}{N}R_2\ X^-)-C_6H_4-NR_2$$

I

$$R_2N-C_6H_4-C(=C_6H_4=\overset{+}{N}R_2\ X^-)-C_6H_4-NR_2$$

II

which is 2-chloro-4′:4″-*bis*dimethylaminotriphenylmethyl chloride was found by Beckwith[7] to be active against typhoid in rabbits. No comparison of the activity of this compound with that of malachite green is recorded. Kligler[8] compared the activity of victoria green, 2:5-dichloro-4′:4″-*bis*dimethylaminotriphenylmethyl chloride with that of malachite green, and showed that the former was slightly more active than the latter. The effect of introducing an hydroxy-group into the molecule of malachite green structure was studied by Fairbrother and Renshaw[9]. 3-Hydroxy-4′:4″-*bis*dimethylaminotriphenylmethyl chloride was found to be too insoluble for testing, while the more soluble patent blue V, monosodium - 5 - hydroxy - 4′:4″-*bis*dimethylaminotriphenylmethyl-2:4-disulphonate possessed no antiseptic properties. Simon and Wood[10] had concluded earlier that all predominantly acidic triphenylmethane dyes are inactive and these findings were confirmed by Fairbrother and Renshaw[9]. But, in view of the presence of two sulphonic acid groups in the molecule of patent blue V, no conclusion can be drawn as to the effect of introducing the hydroxy group into the triphenylmethane molecule.

The compound III, 2-chloro-5-hydroxy-4′:4″-*bis*dimethylamino-triphenylmethyl sulphate, embodies the salient properties of two types of antibacterial substances. One of the aromatic rings introduces the

Cl, HO, C, $N(CH_3)_2$, $\overset{+}{=}N(CH_3)_2$ HSO_4^-

III

parachlorophenol groupings into the molecule, while an arylalkyl side chain is duplicated in the main triphenylmethane structure. Such a compound might be expected to combine the selective antibacterial action of the triphenylmethane dyes with the more general bacterial toxicity associated with phenolic substances and provide the basis for a new and powerful series of bactericides.

For the synthesis of III, 2-chloro-5-hydroxytoluene was chosen as a convenient starting point on account of its ready availability. The required orientation of chloro- and phenolic groups with respect to the alkyl side chain was thus already established. The oxidation of the alkyl side chain to the aldehyde was accomplished by chlorination to introduce two chlorine atoms, followed by hydrolysis. In the first instance the phenolic group was protected by acetylation and the acetyl derivative chlorinated in bright light at 110°C. to give the theoretical increase in weight for the introduction of two chlorine atoms. On cooling the product solidified and after re-crystallisation was identified as 2:4:6-trichloro-3-acetylcresol, m.pt. 35°C. It seemed possible that side chain chlorination could be achieved with a derivative of higher boiling-point and this expectation was realised with benzoyl-2-chloro-5-hydroxy-

toluene. This compound was chlorinated, in bright light, at an initial temperature of 140°C. which was slowly increased to 180°C. during the course of the reaction. 4-Chloro-3-dichloromethylphenyl benzoate (IV)

$CHCl_2$ Cl $OCOC_6H_5$

IV

CHO Cl $OCOC_6H_5$

V

was obtained as a pale yellow, viscous oil after fractional distillation *in vacuo*. This substance was easily converted into the corresponding aldehyde, 2-chloro-5-benzoylhydroxy-benzaldehyde (V) using the method of Hammick[11], boiling with alcoholic silver nitrate solution for 20 minutes. After concentration V crystallises from the filtrate as a white microcrystalline solid which readily forms a 2:4-dinitrophenylhydrazone and a semicarbazone. Its identity was confirmed by titration using hydroxylamine hydrochloride.

$—N(CH_3)_2$ Cl —CH $OCOC_6H_5$ $—N(CH_3)_2$

VI

$—N(CH_3)_2$ Cl —CH HO $—N(CH_3)_2$

VII

$—N(CH_3)_2$ Cl —C HO OH $—N(CH_3)_2$

VIII

2-Chloro-5-benzoylhydroxy-4′:4″-*bis*dimethylaminotriphenylmethane (VI) was obtained in 70 per cent. yield by condensing the aldehyde (V) with dimethylaniline, using phosphorus oxychloride as the condensing

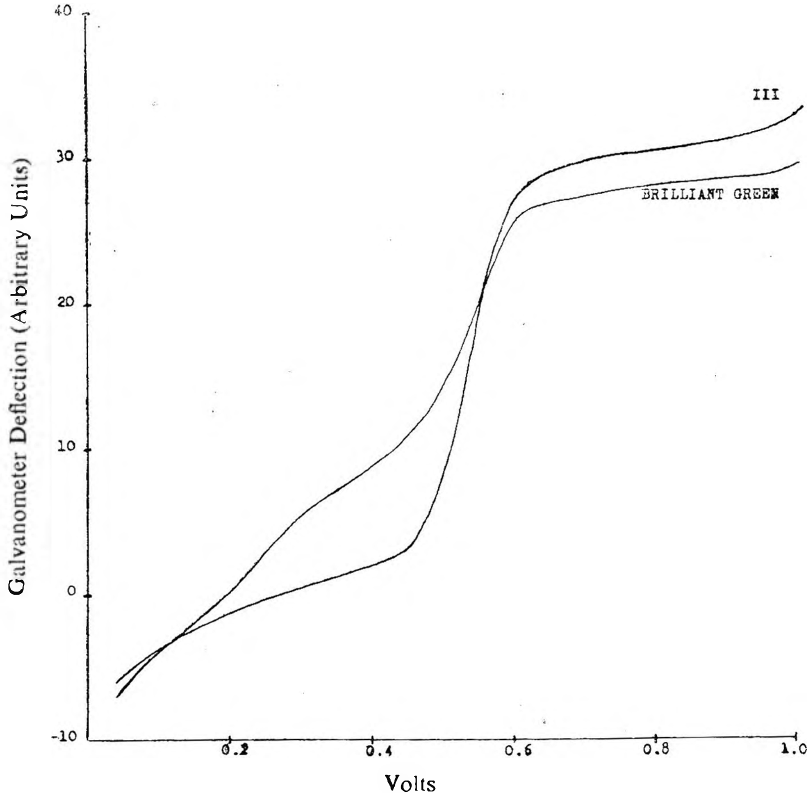
agent and *o*-dichlorobenzene as solvent, according to the conditions of Stryker[12]. The product twice recrystallised from alcohol (95 per cent.) was a white crystalline powder and readily formed a picrate which, on analysis and titration with standard sodium hydroxide solution, was shown to possess the formula $B_1[C_6H_2(NO_2)_3OH]_2$.

Debenzoylation of VI to 2-chloro-5-hydroxy-4′:4″-*bis*dimethylamino-triphenylmethane (VII) was achieved by refluxing with alcoholic solution of potassium hydroxide. The crude leucobase (VII) was separated in 98 per cent. yield in the form of dark green crystals. Purification was effected by chromatographic adsorption from benzene on a column of activated alumina, followed by recrystallisation from alcohol (95 per cent.) to give a white crystalline product in 90 per cent. yield. The picrate was obtained as a yellow crystalline solid and was shown by analysis and by titration against standard solution of sodium hydroxide to have the formula $B_1[C_6H_2(NO_2)_3OH]_2$.

Oxidation of VII to the carbinol base, 2-chloro-5-hydroxy-*bis*dimethylamino-triphenylmethylcarbinol (VIII) was carried out by the method of Minevitch[13] in 70 per cent. acetic acid at 0° to 5°C., using lead peroxide paste. The carbinol base (VIII) was obtained as a dark green scale product in 89 per cent. yield after purification by chromatographic adsorption on activated alumina from chloroform and elution from the column with the same solvent. It has no distinct melting-point and undergoes slow fusion with decomposition when heated for any length of time at temperatures over 100°C. It is insoluble in water, benzene, light petroleum (b.pt. 50° to 60°C.), ether and carbon tetrachloride; soluble in alcohol and very readily soluble in chloroform and acetone. The picrate of VIII was obtained as a dark yellowish green powder and was shown by analysis to possess the formula $B_1[C_6H_2(NO_2)_3OH]_2$.

The conversion of the carbinol base (VIII) to the dyestuff, 2-chloro-5-hydroxy-4′:4″-*bis*dimethylaminotriphenylmethyl sulphate (III) was accomplished by agitating a chloroform solution of VIII with water containing the calculated amount of sulphuric acid, and evaporating to dryness. The identity of the product was established by microanalysis, though direct titration on a semi-micro scale with titanous sulphate solution using the method defined for brilliant green in the British Pharmacopœia failed to give consistent results. Further confirmation of the identity of III was obtained by a study of its polarographic wave in comparison with that of a pure sample of brilliant green. The waves were plotted using a voltamescope on solutions buffered at approximately *p*H2 with hydrochloric acid and 0·1N potassium chloride, the latter substance serving also as the ground electrolyte. Gelatin at a concentration of 0·02 per cent. was used for " maximum " suppression and alcohol to facilitate solution of the dyestuffs. A blank was carried out to demonstrate the absence of interference of the ancillary substances present in the solution. Two typical waves are shown in Figure I. These are followed closely by a large hydrogen wave. The waves of the dyestuff (III) and of brilliant green are shown to be comparable. The half-wave potentials are

0·535V and 0·53V for III and brilliant green respectively, and are measured with respect to the pool mercury anode. The waves are completely cathodic, which may be taken as evidence of completeness of oxidation to the dye form.



The dye III is only sparingly soluble in water, insoluble in ether, benzene and light petroleum, but soluble in acetone, chloroform and alcohol. It is capable of dyeing both silk and wool directly, and cotton in the presence of suitable mordants. The green colours so obtained remain fast on exposure to ultra-violet light for short periods, but are not fast to the action of boiling soap solutions. Preliminary bacteriological tests in aqueous solutions showed that III inhibited the growth of *Streptococcus pyogenes* at a dilution of 1/80,000 and *Staphylococcus aureus* at more than 1/160,000. A 1/1000 solution did not inhibit the growth of *Escherichia coli* and *Pseudomonas pyocyanea.*

Conclusion

The chlorohydroxy-triphenylmethane dye III has been shown to possess a low order of antibacterial activity. The results of Fairbrother and Renshaw indicated that the presence of an acidic group in the molecule of a basic triphenylmethane dye, would decrease the activity. The intro-

duction of the phenolic group into the molecule of the dye III has demonstrated this effect and any enhancement of potency due to the presence of the chloro-group was insufficient to compensate for the reduction in activity due to the phenolic group.

Experimental

3-*Methyl*-4-*chlorophenyl benzoate*.—2-chloro-5-hydroxytoluene (71·2 g.) was dissolved in 5N sodium hydroxide solution (100 ml.). Benzoyl chloride (70·2 g.) was added slowly and the mixture shaken continuously for 10 minutes. A further addition of 5N sodium hydroxide solution (10 ml.) was made and the shaking repeated. The solid product was filtered from the solution, washed first with dilute sodium hydroxide solution, then with water, dried and recrystallised from alcohol (95 per cent.). Yield 110·5 g. (90 per cent.), m.pt. 86°C.

3-*Dichloromethyl*-4-*chlorophenyl benzoate (IV)*.—3-methyl-4-chlorophenyl benzoate (110·5 g.) was heated to 140°C. and a current of dry chlorine was slowly passed into the molten material for 9 hours. During the course of the chlorination the temperature was allowed to rise slowly to 180°C., and the apparatus was exposed to sunlight. The product, a dark brown viscous oil, was distilled *in vacuo*, and the second fraction, b.pt. 203° to 204°C./4 mm. Hg. was collected. Yield 94 g. (67 per cent.). μ. 1·6013. Found: C, 52·90; H, 2·94; Cl, 35·36. $C_{14}H_9O_2Cl_3$ requires C, 53·26; H, 2·88; Cl, 33·76 per cent.

2-*Chloro*-5-*benzoylhydroxybenzaldehyde* (V).—Silver nitrate (56 g.) was dissolved in distilled water (60 ml.), heated to 80°C. and added with continuous stirring to a solution of IV (51 g.) in boiling alcohol (95 per cent.) (250 ml.). The whole was refluxed for 20 minutes with continuous and vigorous stirring and then allowed to cool. Neutralisation was effected by the careful addition of the calculated amount of calcium carbonate and the precipitated silver chloride removed by filtration, washed with alcohol (95 per cent.) and dried. The filtrate was evaporated to dryness, the residue extracted with boiling absolute alcohol and filtered. On cooling V separated as a white crystalline solid, m.pt. 94° to 94·5°C. (Corr.). Yield (i) 34·1 g. A further yield of product was obtained by submitting the dried precipitated, silver chloride to continuous extraction with alcohol (95 per cent.), evaporating off the excess of alcohol and allowing to crystallise. Yield (ii) 1·5 g. Finally the combined mother liquors were concentrated and shaken with a saturated solution of sodium bisulphite. The aldehyde-bisulphite compound separated from the solution as a white solid which was filtered, washed with alcohol and then decomposed with sodium carbonate solution. The purified product was extracted with ether, the solution dried with anhydrous sodium sulphate, and the ether removed by evaporation. Yield (iii) 2·1 g. Total yield 37·7 g. (89·5 per cent.). Found: C, 63·19; H, 3·82; Cl, 14·62 per cent. Eq. wt., 263·1. $C_{14}H_9O_3$ requires C, 64·49; H, 3·48; Cl, 13·61 per cent. Eq. wt., 260·5. The aldehyde (V) forms a

2:4-dinitrophenylhydrazone, which crystallises from absolute alcohol in orange prisms, m.pt. 240° to 241°C. (Corr.). Found: C, 54·55; H, 3·05; N, 12·80; Cl, 8·76 per cent. $C_{20}H_{13}O_6N_4Cl$ requires C, 54·46; H, 2·97; N, 12·71; Cl, 8·05 per cent. The semicarbazone of V is a white micro-crystalline solid. It is practically insoluble in alcohol, but may be re-crystallised from dry acetone, m.pt. 225° to 226°C. (Corr.). Found: C, 56·31; H, 3·27; N, 12·8; Cl, 11·8 per cent. $C_{15}H_{12}O_3N_3Cl$ requires C, 56·68; H, 3·81; N, 13·22; Cl, 11·16 per cent.

2-*Chloro-5-benzoylhydroxy-4′:4″-bisdimethylaminotriphenylmethane (VI)*—V (13 g.) was refluxed at 100°C. for 3 hours with di-methylaniline (12·2 g.) and phosphorus oxychloride (7·7 g.), using *o*-dichlorobenzene (23 g.) as solvent. 5N sodium hydroxide solution (30 ml.) and water (30 ml.) were added and the *o*-dichlorobenzene re-moved by steam distillation. The crude product separated as a dark green sticky mass, which was then extracted with dilute hydrochloric acid (10 per cent.). The extract was filtered, diluted with distilled water (2 l.) and neutralised by the careful addition of strong ammonia solution. A pale green flocculent precipitate was obtained, which was filtered, washed dried and recrystallised from alcohol (95 per cent.), to give a white crystalline solid. Yield 17 g. (70·2 per cent.), m.pt. 150°C. (Corr.). Found: C, 71·74; H, 5·90; N, 5·56; Cl, 7·26 per cent. $C_{30}H_{29}O_2N_2Cl$ re-quires C, 74·30; H, 6·02; N, 5·77; Cl, 7·31 per cent. Picrate $B_1(C_6H_3O_7N_3)_2$, m.pt. 147·5° to 148°C. (Corr.). Found: C, 52·65; H, 3·59; N, 11·50; Cl, 2·98 per cent. Eq. Wt., 970·1. $C_{42}H_{35}O_{16}N_8Cl$ re-quires C, 53·47; H, 3·74; N, 11·86; Cl, 3·76 per cent. Eq. Wt., 942·5.

2-*Chloro-5-hydroxy-4′:4″-bisdimethylaminotriphenylmethane (VII)*—VI (15·7 g.) was refluxed for 4 hours with N/2 alcoholic potassium hydroxide (300 ml.). When cold the solution was just neutralised by the addition of N hydrochloric acid and the alcohol removed by distillation. The solution was made just alkaline by the addition of potassium car-bonate, and the solid product which separated was filtered from the solution, washed with water and dried. Yield of crude product 12·13 g. (98 per cent.). This dark green crystalline material was purified by chromatographic analysis, using a 1 per cent. solution in benzene on a column of activated alumina. Development of the chromatogram with a mixture of 10 parts of alcohol (95 per cent.) and 90 parts of benzene caused a separation into four distinct zones, which were coloured (a) dark green, (b) pink, (c) violet, (d) yellow in order from top to bottom of the column. These four fractions were collected separately by con-tinued elution of the column with the developing solvent. Fractions (a), (b) and (c) contained only traces of unidentified impurities. The solvent was removed from fraction (d) under reduced pressure, in a current of hydrogen to minimise oxidation and the pale green solid recrystallised from freshly distilled alcohol (95 per cent.) to give a pro-duct which was pure white. Yield 10·91 g., m.pt. 179° to 180° C. (Corr.). Found: C, 72·01; H, 6·37; N, 7·62; Cl, 9·84 per cent. $C_{23}H_{25}ON_2Cl$ re-quires C, 72·53; H, 6·61; N, 7·35; Cl, 9·31 per cent. Picrate

$B_1(C_6H_3O_7N_3)_2$, m.pt. 187° to 188°C. (Corr.), with initial softening at 185°C. Found: C, 49·83; H, 3·64; N, 13·5; Cl, 3·83 per cent. Eq. wt. 835·1. $C_{35}H_{31}O_{15}N_8Cl$ requires C, 50·07; H, 3·70; N, 13·36; Cl, 4·23 per cent. Eq. wt. 838·7.

2-*Chloro*-5-*hydroxy*-4′:4″-*bisdimethylaminotriphenylcarbinol* (*VIII*)—VII (5·95 g.) was dissolved in 70 per cent. acetic acid (50 ml.) and the solution cooled to between 0° and 5°C. The theoretical quantity of lead peroxide paste (prepared according to Gattermann[14]) suspended in 70 per cent. acetic acid (40 ml.) was added slowly to the above solution with continuous stirring over a period of about 10 minutes. The reaction was allowed to proceed at 0° to 5°C. for one hour with continuous stirring. Sufficient sodium sulphate solution to precipitate all the lead present was added and stirring continued for 20 minutes. The deep green solution was filtered to remove lead sulphate, made alkaline with sodium bicarbonate, and the precipitated carbinol base extracted with chloroform. The solvent was removed by distillation, and the dark green solid, after drying, continuously extracted with chloroform in a soxhlet apparatus. The chloroform solution was evaporated to 150 ml. and passed through a column of activated alumina. The dark green chromatogram was developed using a mixture of 90 parts of chloroform and 10 parts of alcohol (95 per cent.), when a gradual separation occurred into an upper dark green band and a lower yellow one. The latter was eluted from the column using the same solvent to give a yellow solution, which during the course of evaporation became pale green and finally left a dull green residue (0·15 g.) of unchanged leucobase. The contents of the dark green band on the column were eluted using a mixture of 80 parts of chloroform and 20 parts of alcohol (95 per cent.), and on evaporation of the solvent the product was obtained as a friable, dark, purplish-black solid. Yield 5·39 g. A second fraction (0·10 g.) was obtained by extruding the column, extracting continuously with chloroform for 2½ hours and evaporating the solvent. Total yield 88·6 per cent. Found: C, 68·55; H, 6·42; N, 7·09; Cl, 9·58 per cent. $C_{23}H_{25}O_2N_2Cl$ requires C, 69·56; H, 6·30; N, 7·06; Cl, 8·94 per cent. Picrate $B_1(C_6H_3O_7N_3)_2$. Found: C, 49·65; H, 3·94; N, 13·30; Cl, 4·26 per cent. $C_{35}H_{31}O_{16}N_8Cl$ requires C, 49·12; H, 3·65; N, 13·10; Cl, 4·15 per cent.

2-*Chloro*-5-*hydroxy*-4′:4″-*bisdimethylaminotriphenylmethyl sulphate* (*III*)—VIII (3 g.) was dissolved in chloroform (30 ml.). The calculated volume of N sulphuric acid solution and water (20 ml.) were added with continuous stirring, and the mixture maintained at 45° to 50°C. for 1 hour. The dye which is almost insoluble in water separates as a sticky mass which is obtained in fine scales on evaporation of the solvents and drying at 100°C. Found: C, 58·92; H, 5·68; N, 5·65; Cl, 7·15; S, 6·79 per cent. $C_{23}H_{25}O_5N_2Cl$ S requires C, 57·9; H, 5·68; N, 5·88; Cl, 7·44; S, 6·71 per cent.

Polarography. Polarographs of the dye (III) and of brilliant green were plotted under the same conditions using a voltamescope, and these are

illustrated in Figure 1. The composition of the two solutions used was as follows:—

Dye (VI), or brilliant green .... 5×10^{-4} M.
KCl .... 10^{-1} M.
Gelatin .... 0·02 per cent.
0·1N Hydrochloric acid.................................... 21 ml.
Alcohol (95 per cent.) .... 50 ml.
Distilled water to .... 100 ml.

Dissolved oxygen was removed from the solution by passing a stream of hydrogen for 10 minutes, and the polarographs were plotted under the following conditions: temperature 23·5°C., drop time of the mercury cathode 1·4 secs., height of the mercury head 71·5 cm.

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THE SPECTROPHOTOMETRIC DETERMINATION OF RUTIN AND QUERCETIN IN MIXTURES

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Rutin is a flavonol glycoside which is derived from quercetin by condensation of the sugar portion of the molecule with the phenolic hydroxyl group at position 3 of quercetin. On hydrolysis with dilute acid, rutin yields quercetin, rhamnose and glucose in equimolecular proportions.

Rutin has the following formula:

```
                                OH
  HO                           /
     \  /\    O    /=====\ /
      |    |/   \__/       \—OH
      |    |    |  \=======/
       \/ \  / \
       |   ||    O
       OH  O      \    H                        H
                   \   |                        |
                    \—C—————            —————C—————
                       |       |        |       |       |
                  H—C—OH       |        |  HO—C—H       |
                       |       |        |       |       |
                 HO—C—H        O        O  HO—C—H       O
                       |       |        |       |       |
                  H—C—OH       |        |   H—C—OH      |
                       |       |        |       |       |
                  H—C——————————         |   H—C—————————
                       |                |       |
                  H—C———————————————————       CH3
                       |
                       H
```

Tentative methods for the determination of rutin and quercetin have been suggested by Porter, Brice, Couch and Copley[1], and their methods have been modified by the Research Division of Penick and Co., New York.[2] The methods of Porter *et al.* are based on the determination of $E_{362 \cdot 5}$* and E_{375} for rutin and quercetin.

Penick and Co. state that if the ratio $\frac{E_{375}}{E_{362 \cdot 5}} = 0{\cdot}875 \pm 0{\cdot}004$ the quercetin content is reported as being less than 1 per cent., and the percentage of rutin is given by the relation $\frac{E_{362 \cdot 5} \times 100}{325{\cdot}5}$ where $325{\cdot}5 = E_{362 \cdot 5}$ for pure anhydrous rutin. It will later be shown that $\frac{E_{375}}{E_{362 \cdot 5}}$ is approximately 0·876 for rutin containing 1 per cent. of quercetin and that the contribution of 1 per cent. of quercetin to the gross absorption is approximately equivalent to that given by 2 per cent. of rutin. Thus in the case of rutin containing 1 per cent. of quercetin the rutin content given by the ratio $\frac{E_{362 \cdot 5} \times 100}{325{\cdot}5}$ will be 2 per cent. in excess of the true

*Throughout the paper E_x is used to indicate $E^{1 \text{ per cent}}_{1 \text{ cm.}}$ at wave length x mμ

value. If the ratio $\frac{E_{375}}{E_{362 \cdot 5}}$ is greater than 0·879, Penick and Co. calculate the rutin content from the formula.

$$\text{Rutin per cent.} = 1{\cdot}4722\ E_{362 \cdot 5} - 1{\cdot}3324\ E_{375}$$

$$\text{and Quercetin per cent} = -\ 0{\cdot}5406\ E_{362 \cdot 5} + 0{\cdot}6183\ E_{375}$$

Further, small differences, within the limits of experimental error in the values of $E_{362 \cdot 5}$ and E_{375} for rutin and quercetin will alter the formulæ with the result that the rutin content as given by the formula is only likely to be correct within ± 0·5 per cent. under optimum conditions.

Both Penick and Co. and Porter *et al.* previously dry the sample at 125°C. for 4 and 16 hours respectively in high vacuum before carrying out the assay. If the assay is to be applied to commercial samples such a procedure would necessitate a moisture determination carried out under the same conditions, and the time required to carry out an assay would be considerable. For this reason and the fact that no claims have been made to estimate less than 1 per cent. of quercetin in rutin-quercetin mixtures it was decided to undertake a systematic study of the problem.

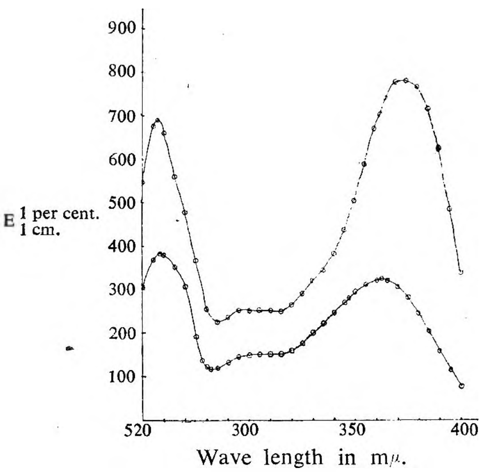

FIG. 1. Absorption spectra.
Upper graph—quercetin. Lower graph—rutin

Absorption Spectra of Rutin and Quercetin. When dissolved in alcohol (95 per cent.) containing 1 per cent. of 0·02N acetic acid both rutin and quercetin obey Beer's and Lambert's laws. As shown in Figure 1 rutin exhibits absorption maxima at 259 mμ and 362·5 mμ and quercetin exhibits maxima at 257 mμ and 375 mμ. The maxima at 362·5 mμ and 375 mμ are suitable for the determination of rutin and quercetin in mixtures.

Choice of Solvent. It was found that for rutin $E_{362 \cdot 5}$ increased with increasing alcohol concentration without marked change in the wave-

TABLE I

EFFECT OF INCREASING THE CONCENTRATION OF ALCOHOL ON WAVE LENGTH OF MAXIMUM ABSORPTION

Alcohol Concentration	$E_{362 \cdot 5}$	Alcohol Concentration	$E_{362 \cdot 5}$
50 per cent.	277	90 per cent.	292
60 ,, ...	281	95 per cent.	300
70 ,, ...	285	95+1 per cent. of 0·02N acetic acid	304
80 ,, ...	288		

length of maximum absorption. The results in Table I were obtained on a commercial sample.

The use of alcohol (95 per cent.) permits increased accuracy in the determination, since the solubility of rutin in acid alcohol (95 per cent.) is very much much greater than in alcohol (50 per cent.). Acetic acid (0·02N) is added to maintain the final dilution on the acid side of neutrality, since mention is made in the literature of the capacity of rutin to form metallic salts.[3,4]

Hydration of Rutin. If samples of rutin previously dried to constant weight at 110°C. are assayed, the maximum value of $E_{362 \cdot 5}$ which can be obtained for a sample which is completely soluble in acid alcohol (95 per cent.) is approximately 300. After drying samples at 110°C. in a vacuum below 1 mm. Hg. pressure over phosphorus pentoxide for 2 hours, and assaying the dried sample, it is found that the value of $E_{362 \cdot 5}$ increases to approximately 325. This corresponds to a loss of 7·7 per cent. of moisture on drying. Rutin is known to exist as the trihydrate and this contains approximately 8·1 per cent. of water of crystallisation. Thus, drying at 110°C. in a high vacuum for 2 hours removes all the water of crystallisation. From the above it seems likely that commercial samples of rutin will consist essentially of rutin trihydrate plus hygroscopic moisture. This has been confirmed for samples so far examined.

Preparation of Pure Rutin. A sample of crude rutin prepared by extraction from buckwheat was purified as follows:

A. The sample was dissolved in alcohol, the least possible quantity of alcohol being used, and the rutin was then reprecipitated by the addition of sufficient water to reduce the alcohol concentration below 10 per cent. After standing in a refrigerator overnight, the rutin was filtered and washed with water.

B. The purified rutin from section A was dissolved in the least possible quantity of alcohol (75 per cent.), warming to effect solution. The solution was cooled, and brown material, which was precipitated on cooling, was filtered off. The alcohol was then distilled off until the point of incipient crystallisation was reached. The solution was allowed to cool, the rutin filtered, washed with small amounts of alcohol (75 per cent.) and dried at 110°C.

C. The filtered rutin was then dissolved in boiling 99 per cent. *iso*propyl

TABLE II

SPECTROPHOTOMETRIC DATA FOR SAMPLES OF RUTIN AND QUERCETIN DRIED AT 110°C. IN A HIGH VACUUM

	E_{347}	$E_{362 \cdot 5}$	E_{375}
Rutin			
Original sample	279·4 , 277·8	321·0 , 321·8	280·0 , 280·7
Sample A	282·5 , 282·8	325·2 , 325·5	284·9 , 283·0
Sample B	281·4 , 282·6	324·9 , 325·2	282·4 , 283·2
Sample C	281·2 , 281·0	325·2 , 324·5	283·1 , 283·0
Quercetin			
Sample D	—	695 , 693	774 , 768
Sample E	460	705	786
Sample F	458	702	780

The values of E_{347}, $E_{362 \cdot 5}$ and E_{375} for samples C and F did not alter with subsequent treatment.

alcohol, the solution was concentrated, cooled to room temperature, filtered and poured into 10 volumes of hot water. When precipitation was complete the rutin was filtered off.

Preparation of Pure Quercetin. D. Crude quercetin was prepared by refluxing purified rutin with 1 per cent. hydrochloric acid (100 ml./g. of rutin). The quercetin was filtered off and washed with cold water. The crude quercetin was twice crystallised from alcohol (80 per cent.). Samples E and F were taken from the first and second recrystallisations respectively and sample F melted at 313°C. Samples A. B. C. D. E and F were dried at 110°C. in a high vacuum and examined spectrophotometrically with the results given in Table II.

Assay of Rutin and Quercetin in Mixtures. According to Vierordt,[7] two components of a mixture may be assayed by means of the following formulæ, if values of E for both substances and for the mixture are known for two selected wavelengths.

In the present case

$$\text{Percentage of rutin} = \left(\frac{c_0 b_1 - c_1 b_0}{a_0 b_1 - a_1 b_0}\right) \times 100$$

$$\text{Percentage of quercetin} = \left(\frac{c_0 a_1 - c_1 a_0}{b_0 a_1 - b_1 a_0}\right) \times 100$$

where a_0 = $E_{362\cdot5}$ pure anhydrous rutin.

a_1 = E_{375} pure anhydrous rutin.

b_0 = $E_{362\cdot5}$ pure quercetin.

b_1 = E_{375} pure quercetin.

c_0 = $E_{362\cdot5}$ mixture.

c_1 = E_{375} mixture.

The values of a_0, a_1, b_0 and b_1 have been determined experimentally and have been assigned the following values:—

$$a_0 = 325,\ a_1 = 283,\ b_0 = 702,\ b_1 = 780.$$

The equations then become

percentage of rutin = $1{\cdot}4224\ E_{362\cdot5} - 1{\cdot}2802\ E_{375}$

percentage of quercetin = $-\ 0{\cdot}5161\ E_{362\cdot5} + 0{\cdot}5927\ E_{375}$

The latest formulæ published by Penick and Co. are

percentage of rutin = $1{\cdot}4722\ E_{362\cdot5} - 1{\cdot}3324\ E_{375}$

percentage of quercetin = $-\ 0{\cdot}5406\ E_{362\cdot5} + 0{\cdot}6183\ E_{375}$

The results in Table III were given on mixtures of known composition, by the Penick formulæ, the formulæ derived above, and by a graphical method which will be described later in the paper.

From Table III it will be seen that in the case of the Penick formula, the percentage of quercetin is correct within the limits of experimental error to be expected in such a determination, whilst the rutin results with one exception are about 1 per cent. high. In the case of the derived formula, the percentage of rutin is approximately correct, whilst the quercetin results are about 0·5 per cent. high.

TABLE III

COMPARISON OF THE RESULTS OBTAINED BY THREE METHODS FOR MIXTURES OF KNOWN COMPOSITION

Theoretical		Penick Formula		Derived Formula		Graph	
Rutin per cent.	Quercetin per cent.	Rutin per cent.	Quercetin per cent.	Rutin per cent.	Quercetin per cent.	Rutin per cent.	Quercetin per cent.
99·03	0·97	101·20	0·72	100·00	1·40	99·25	0·75
98·60	1·40	99·67	1·58	98·40	2·20	98·60	1·40
98·08	1·92	98·36	1·61	99·90	1·98	98·05	1·95
97·52	2·48	98·55	2·62	97·08	3·20	97·30	2·70
97·14	2·86	98·37	3·00	97·23	3·60	96·40	3·60
96·59	3·41	97·68	3·47	96·58	4·00	96·30	3·70
96·23	3·77	97·52	3·79	96·43	4·36	96·00	4·00
95·69	4·31	96·54	4·33	95·59	4·88	95·60	4·40
95·53	4·67	96·66	4·54	95·62	5·08	95·30	4·70

The mixtures were made from purified rutin and quercetin previously dried at 110° C. for 2 hours in a high vacuum.

Since the ratio $\frac{E_{347}}{E_{375}}$ is a measure of the quercetin content, it was decided to attempt to use the ratio to measure the quercetin content and hence the rutin content by difference or calculation. Table IV has been constructed from the data for rutin and quercetin.

TABLE IV

MEASUREMENT OF RUTIN CONTENT BY THE RATIO $\frac{E_{347}}{E_{375}}$

Rutin per cent.	Quercetin per cent.	$\frac{E_{347}}{E_{375}}$	$\frac{E_{375}}{E_{362 \cdot 5}}$
100	0	0·9936	0·871
99	1	0·9828	0·876
98	2	0·9722	0·881
97	3	0·9618	0·886
96	4	0·9520	0·891
95	5	0·9419	0·895
94	6	0·9330	0·900

It will be seen that the ratio $\frac{E_{347}}{E_{375}}$ changes more rapidly with the increased amounts of quercetin than the ratio $\frac{E_{375}}{E_{362 \cdot 5}}$

The ratio $\frac{E_{347}}{E_{375}}$ has been determined for the mixtures in Table III, and the percentage of quercetin read off from the graph plotted from the data in Table IV. It will be seen that in nearly every case the percentages of rutin and quercetin agree with the theoretical values ± 0·2 per cent. This method is not applicable to the rapid assay of commercial samples, as it would necessitate drying to constant weight in high vacuum at 110°C. However, as the graph will indicate the relative amounts of rutin and quercetin, by means of a formula, samples may be assayed without previous drying.

The following method is proposed for the routine assay of a rutin-quercetin mixture.

Weigh 50 mg. and dissolve in ethyl alcohol (95 per cent.), using 50-ml. graduated flasks. Dilute suitably to give a density reading of 0·4 to 0·6 when the wave length scale of the Beckman spectrophotometer is set at 362·5 mμ. Add 0·5 ml. of 0·02N acetic acid to the last dilution and compare the optical density of this solution with that of a solution of ethyl alcohol (95 per cent.), containing a similar amount of acetic acid, using 1 cm. cells, a tungsten lamp and a Corning No. 9863 Red-Purple Corex A filter.

Calculate E_{347}, $E_{362 \cdot 5}$, E_{375} and the ratio $\frac{E_{347}}{E_{375}}$.

From Table IV or a prepared graph read off the composition of the mixture corresponding to the ratio.

If x = percentage of quercetin read on graph, then rutin: quercetin $= 100 - x : x$ or quercetin $= \left(\frac{x}{100 - x}\right)$ rutin.

Let the actual rutin content of sample be y per cent. anhydrous rutin, then quercetin content will be $\left(\frac{x}{100 - x}\right)$ y.

For a mixture $E_{362 \cdot 5} = 3 \cdot 25\, y + 7\, y \left(\frac{x}{100 - x}\right)$

Here x and $E_{362 \cdot 5}$ are known, so y, the anhydrous rutin content of the sample may be calculated.

From this the quercetin is given by $\left(\frac{x}{100 - x}\right)$ y

percentage of anhydrous rutin x 1·088 = percentage of rutin trihydrate.

It is important to ascertain that the maximum absorption does not lie on the ultra-violet side of 362·5 mμ. If the maximum is at a shorter wave length than 362·5 mμ, then the determination will be rendered inaccurate by the presence of other absorbing substances. None of the methods which have been proposed for the assay of rutin is directly applicable if there are considerable amounts of chlorophyll and red pigment present. Chlorophyll and red pigment may be tested for by the extraction of the sample with ether. If chlorophyll and/or red pigment are present, the ether will become coloured. Pure rutin is insoluble in ether. The above method may be applied after the chlorophyll and red pigment have been extracted. Porter *et al*[2] have given a method

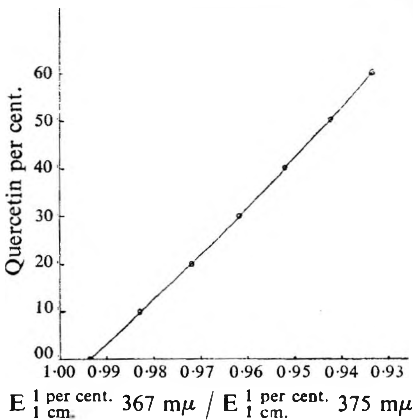

FIG. 2. Percentage of quercetin in mixtures of anhydrous rutin and quercetin.

for the quantitative determination of chlorophyll and red pigment in rutin.

Assay of Rutin in Tablets

Determine the mean weight of 20 tablets. Powder and mix well. Weigh 50 mg. and dissolve in 80 ml. of ethyl alcohol (95 per cent.), warming to effect solution. Filter and make up to volume with ethyl alcohol (95 per cent.) in a 100-ml. graduated flask. Dilute suitably for the spectrophotometer, adding 1 per cent. v/v of 0·02N acetic acid to the final dilution. Then proceed as instructed for the assay of rutin and quercetin.

$$\text{Mean weight per tablet in mg.} \times \frac{\text{percentage of anhydrous rutin}}{100}$$

$= \text{mg. of anhydrous rutin per tablet.}$

The above assay has been found satisfactory for tablets containing as excipients lactose, starch and gum acacia.

The spectrophotometric measurements in this investigation were carried out on a Beckman Quartz Spectrophotometer calibrated on the mercury lines of wave length 4047Å, 3650Å, 3341Å, and 3132Å.

Summary

1. The ultra-violet absorptions of rutin and quercetin have been investigated.
2. A rapid method has been developed for the accurate determination of the minor constituent of binary mixtures of rutin and quercetin, and a formula derived for the correction of the gross absorption for the absorption due to the minor constituent.
3. A comparison has been made between American methods of assay and the method in use in this laboratory.

The author wishes to express his thanks to the Directors of Allen and Hanburys, Ltd., for permission to publish this paper, to Dr. N. Evers for helpful criticism, and to Mr. W. Smith for providing laboratory facilities.

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THE COLORIMETRIC DETERMINATION OF ANEURINE BY AUERBACH'S METHOD

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Auerbach's method[1] for the colorimetric determination of aneurine in pharmaceutical preparations, like that adopted in the United States Pharmacopœia XII, is based on that of Melnick and Field[2] but is simpler and more rapid than either. Auerbach accelerated the colour development by heating at 60°C. for from 3 to 15 minutes, and for colourless simple solutions of aneurine be diluted with isopropyl alcohol instead of extracing the pigment with xylene, claiming an accuracy of ±3 per cent. Recently Elvidge[3] reported that the method was not reproducible and quoted errors of up to 13 per cent. in determinations without the use of standards; however, even using standards he obtained low and erratic results on tablets, the maximum deviations from the mean being +8 per cent. and −5 per cent. On the other hand Brown *et al.*[4] reported good agreement between the Melnick and Field colorimetric method and the thiochrome method and pointed out that the former was the more reliable whereas the latter, being more sensitive, was better for samples of low potency. The high sensitivity of the thiochrome method was acknowledged also by Adamson and Handisyde[5] who reported that in ordinary routine work with this method a greater precision than ±5 per cent. could not be relied upon. The present paper describes experiments carried out to establish a modified Auerbach's method which was used for control purposes before the thiochrome method became official in the British Pharmacopœia.

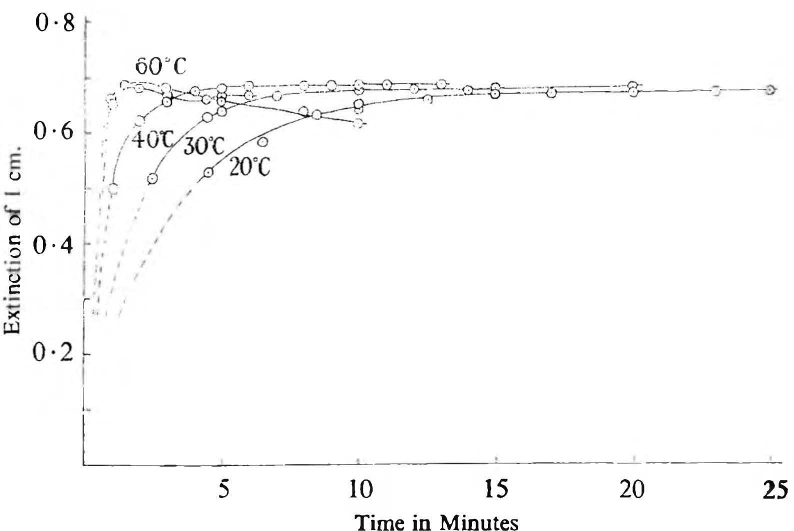

Fig. 1. Rate of Colour Development at Different Temperatures.

MODIFICATIONS TO THE ORIGINAL METHOD

The original method requires only small volumes of solution and reagents, for example 1 ml. and 2 ml., and an initial modification to increase precision, was to use 5 times the original amounts. However, difficulties in securing uniform heating of the larger volumes were encountered and hence consideration was given to determining conditions under which rapid coupling occurred at room temperature. Figure 1 shows the effect of temperature on the rate of development of colour, 20 to 25 minutes being required for complete coupling at room temperature.

Auerbach specifies the use of 20 to 25 per cent. ethyl alcohol or methyl alcohol as the solvent during coupling, and experiments showed that lower results were obtained with methyl alcohol and isopropyl alcohol but that constant maximum values were obtained with 20 to 25 per cent. ethyl alcohol. For simple solutions Auerbach uses a less alkaline diazotate reagent than that used for an elixir and tablets whereas Allport[6] recommends the more alkaline reagent for both. Experiments showed that the degree of alkalinity was important, as indeed was expected because of the importance of *p*H in coupling reactions, and an optimum was chosen. Slightly low results were obtained when the reagent was used immediately after preparation but constant values resulted when the reagent was used between 2 and 3 minutes after adding the alkali. The rate of colour development increased with increase in concentration of the diazotate reagent and, using 0·06 per cent., maximum colour was obtained in 10 minutes. Acid added after coupling slightly decreased the colour intensity, but when added before coupling was complete it arrested the coupling reaction and this effect was used in determining colour development curves.

SOLUTIONS OF ANEURINE

Although according to Auerbach many substances affect the intensity and shade of the colour produced, it was found that traces of inorganic salts and a relatively large proportion of dextrose in the preparations examined made no difference. The reproducibility and precision of the final method were tested by carrying out determinations on different days with freshly prepared reagents (Table I).

TABLE I

REPRODUCIBILITY OF PROPOSED METHOD

Day	Aneurine Solution	Diazotate Reagent	Extinction Value
1	1	A	1·042 1·040 1·044 1·040
2	2	B	1·030 1·033
		C	1·040 1·045
3	2	D	1·041 1·042
		E	1·038 1·041

From these results it was concluded that the modified method, unlike Auerbach's original method, was sufficiently reproducible to permit the use of a calibration curve. The proposed method for simple solutions is described below and calibration curves obtained with International Standard Vitamin B_1 and with a sample of aneurine hydrochloride B.P. are shown in Figure 2.

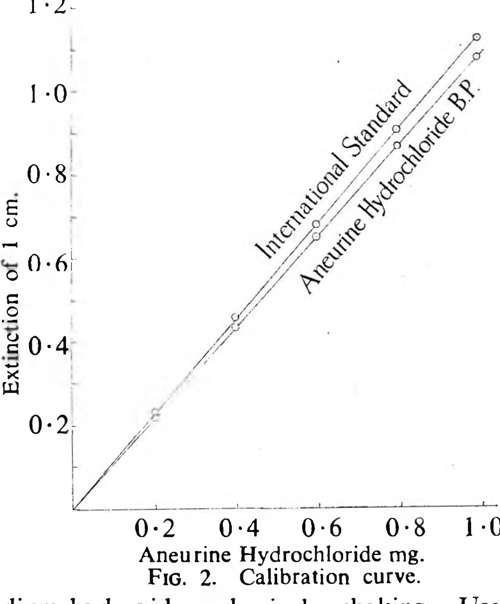

FIG. 2. Calibration curve.

Reagents. (1) *p*-Aminoacetophenone Solution, 0·06 per cent. in 0·2N hydrochloric acid. (2) Sodium nitrite Solution, 0·2 per cent. (freshly prepared). (3) Diazotate reagent, cool 10 ml. of *p*-aminoacetophenone solution to 5°C., add 3 ml. of sodium nitrite solution and mix. After 3 minutes add 3 ml. of 2N sodium hydroxide and mix by shaking. Use between 2 and 5 minutes after preparation.

Method. Prepare a dilution of the sample to contain about 0·1 mg. of aneurine hydrochloride /ml. and having an acid concentration equivalent to 0·01N. Transfer 5 ml. to a 50 ml. graduated flask, add 10 ml. of ethyl alcohol (50 per cent. v/v), mix, add 5 ml. of diazotate reagent and again mix. Place in a water-bath at 20°C. for 12 minutes and then dilute to 50 ml. with isopropyl alcohol. Determine the extinction in a 1 cm. cell using an Ilford 604 filter, subtract the value of a reagent blank and read off the amount of aneurine from a calibration curve.

TABLETS OF ANEURINE

Auerbach extracted aneurine from powdered tablets with alcohol (50 per cent.) by heating at 60°C. for 10 minutes. Using this method very low results were obtained (Table II) and this may account for Elvidge's results on tablets which, despite the use of standards, were on an average 20 per cent. low.

TABLE II
EXTRACTION OF ANEURINE FROM TABLETS

Method		Aneurine Hydrochloride found per cent.
Auerbach		1·66, 1·52, 1·54
	hours	
Alcohol (50 per cent.) at room temperature	19	1·88, 1·83
	46	2·05, 2·00
	146	1·81, 1·76

Modifications (Table II) gave higher results, but none was considered entirely satisfactory. The United States Pharmacopœia XII "dissolves" tablets in 0·01N hydrochloric acid whilst Wokes[7] heats for 10 minutes with a mixture of 15 ml. concentrated hydrochloric acid and 25 ml. of water. For other materials various methods have been used, for example, boiling with 1 per cent. hydrochloric acid[8] or for 10 minutes with 0·005N hydrochloric acid[9] and boiling for 1 hour with 0·4N sulphuric acid.[10] Recovery experiments made with the particular tablets under examination showed that the following method was satisfactory.

Method. Finely powder 20 tablets and weigh accurately an amount of powder expected to contain about 10 mg. of aneurine into a 100-ml. conical flask. Add exactly 5 ml. of dilute hydrochloric acid and 10 ml. of water, heat to boiling and boil gently for 4 minutes. Cool, add (x − 1) ml. of N sodium hydroxide, transfer to a 100 ml. graduated flask and dilute to a 100 ml. with water. (The value of x is the number of ml. of N sodium hydroxide required to neutralise the hydrochloric acid in a control determination, after cooling). Use 5 ml. of this solution for a determination as described for simple solutions.

SUMMARY

1. Auerbach's method for the colorimetric determination of aneurine has been modified. The modified method is of greater precision, is reproducible and permits the use of a calibration curve.
2. The inapplicability of Auerbach's method to certain tablets has been shown and a more satisfactory method is described.

Thanks are due to Dr. E. F. Hersant for helpful comments, to Mr. H. W. Johnson for preliminary experiments on tablets, and to the Directors of May and Baker, Ltd., for permission to publish this paper.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Colchicine, Polarographic Determination of. F. Santavy. (*Pharm. Acta Helvet.*, 1948, **23**, 380.) The method, applied to the seeds, is as follows. 5 g. of the powdered seeds is macerated for 3 hours at 75°C. with 96·5 g. of water. Water is added to make the weight up to its original value, followed by 3·5 ml. of a saturated solution of lead acetate. After filtration, 60 ml. of the filtrate is treated with 0·25 g. of trisodium phosphate, and again filtered. To 2 ml. of this solution is added 2 ml. of phosphate buffer solution (pH 7 to 8), and, after the removal of oxygen, the polarographic curve is determined. The quantity of colchicine is obtained from a standardisation curve with pure colchicine. On account of the presence of other reducible substances, the values are about 6 per cent. high. For the same reason the method is not suitable for other parts of the plant. For tincture of colchicum, 20 g. of the tincture is evaporated on the water bath to one fourth of its volume and, after cooling, diluted with water to 17 g. The determination is then continued as before with the addition of 1 g. of lead acetate solution and 2 ml. of a saturated solution of sodium phosphate. G. M.

Digitoxin, Effect of Various Alkalis on the Sensitivity of the Baljet Reaction. F. K. Bell and J. C. Krantz, Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 297.) The Baljet test, used by the U.S.P. XIII as a colorimetric control for digitoxin, depends upon the red colour produced when a methyl alcoholic solution of trinitrophenol containing sodium hydroxide is added to a methyl alcoholic solution of digitoxin. The effect of replacing the alkali with ammonium hydroxide, lithium hydroxide, tetramethylammonium hydroxide and tetraethylammonium hydroxide was investigated. With ammonium hydroxide the colour was not produced, and lithium hydroxide made no significant difference to the test. The quaternary ammonium bases, on the other hand, gave a deeper, more intense and more stable colour, which also reached its maximum intensity more rapidly. Using these results, the following procedure was developed for preparing the reaction mixture. To 2 ml. of a 0·02 per cent. solution of digitoxin in methyl alcohol, add 0·1 ml. of a 5 per cent. solution of trinitrophenol in methyl alcohol, mix, and add 2 ml. of a 10 per cent. aqueous solution of tetraethylammonium hydroxide. The maximum colour develops in 10 minutes and remains constant, within the limits of experimental error, for about 30 minutes. The modified test is twice as sensitive as that of the U.S.P., and appreciably more sensitive than the alternative Keller-Kiliani test of the U.S.P. G. R. K.

Linoleic Acid in Edible Fats, Determination of. W. J. Stainsby. (*Analyst*, 1948, **73**, 429.) The calculation of the composition of a fat containing saturated acids, oleic, and linoleic acids involves the use of 3 simultaneous equations; the total acid equation, another involving the use of iodine values of oleic and linoleic acids, and a third involving the quantitative titration of the acidic glycerides produced by oxidation of the fat. In the third determination the fat is oxidised in anhydrous acetone with potassium permanganate followed by titration of the acidic glycerides

after the removal of the steam-volatile acid products. Results obtained with several hydrogenated cottonseed oils and with 3 samples of sesame, sunflower-seed and palm oils compared very favourably with those obtained by the thiocyanogen method, and by the spectrophotometric method involving alkali isomerisation to a conjugated acid which is subsequently estimated from its ultra-violet absorption spectrum. The method can be extended with little loss of accuracy to determine the total unsaturated acids of oils containing more than 2 unsaturated acids. In the case of fats generally with a higher acid value than that allowed by the British Pharmacopœia, errors arise and such fats should be neutralised before the determination is carried out. R. E. S.

Methyl Alcohol, Quantitative Colorimetric Microdetermination of, with Chromotropic Acid Reagent. R. N. Boos. (*Anal. chem.*, 1948, **20**, 964.) The reaction of formaldehyde when heated with chromotropic acid (1:8-dihydroxynaphthalene-3:6-disulphonic acid) in the presence of sulphuric acid to give an intense violet-red colour is used as the basis for the determination of methyl alcohol. A known weight of organic material under test is mixed with water (4 ml.), distilled, and 3 ml. of the distillate collected. One ml. of this solution (diluted to contain 20 to 100 μg. of methyl alcohol per ml.) is oxidised for 10 minutes with 3 drops of dilute phosphoric acid solution (10 ml. of 50 per cent. acid diluted to 100 ml. with water) and 5 drops of potassium permanganate solution (5 per cent.); decolorisation of excess of potassium permanganate is effected by the addition drop by drop of saturated sodium bisulphite solution. Four drops of a 2 per cent. aqueous solution of chromotropic acid is added, the mixture heated at 60°C. for 15 minutes, cooled in an ice-bath, allowed to reach room temperature and then diluted to 10 ml. The intensity of colour of the solution is measured in a suitable colorimeter, the peak light absorption occurring at 5800Å. A blank determination is necessary each day as the chromotropic acid solution darkens with time. The reaction is specific and the following do not interfere: acetaldehyde, propionaldehyde, butyraldehyde, *iso*butyraldehyde, *iso*valeraldehyde, crotonaldehyde, chloral hydrate, glyoxal, benzaldehyde and phthalaldehyde. Glyceraldehyde gives a yellow colour. Good agreement was shown between the method proposed and the Zeisel method, and the method can also be used for the determination of methoxyl groups in methyl esters with a relative error of less than 2 per cent. R. E. S.

FIXED OILS, FATS AND WAXES

New Zealand Fish Oils. A. P. Oliver and F. B. Shorland. (*Biochem. J.*, 1948, **43**, 18.) The fats from 8 specimens of school or snapper shark (*Galeorhinus Australis*) selected at random were studied separately. The livers varied in oil content from 23·1 to 60·7 per cent. and contained from 66·8 to 93·0 per cent. of the total oil reserves of the fish. Tables are given which contain weights of organs and size of each specimen; proportions of tissues and distribution of fats; analytical determinations of vitamin A, unsaponifiable matter, saponification equivalent, and iodine value of liver, body, and head fats. Ester fractionation analyses of 4 of the liver oils, and of the phosphatide and glyceride fractions of the combined head and and body lipids showed that the liver fatty acids contained more palmitic acid and C_{18} unsaturated acids, but less stearic acid than the head and body lipids. The wider differences in content of C_{18}, C_{20} and C_{22}

unsaturated acids of the liver oils amounting to as much as 6·3 units per cent. were thought to be outside experimental error, although this was not conclusive in view of previous variations in accuracy. The composition of the liver fats did not appear to be influenced by the extent to which the liver was used for fat storage, in contrast to the results of Rapson obtained from a study of teleostean species. R. E. S.

Oils and Fats, Stability of. E. Sandell. (*Farm Revy*, 1948, **47**, 699, 715.) A low peroxide content of oils and fats is not a guarantee of stability, as in the preparation of the material it is possible that natural antoxidants may have been removed without an appreciable amount of oxidation occurring. Further, traces of metals may have a great influence. Lard has a much lower stability if fish oils have been used in the animal feeding-stuffs. Finally, a strongly oxidised fat may be refined to a low peroxide content, but its stability remains poor. In order to decide on the keeping properties of a sample of oil or fat it is thus necessary to apply special stability tests. In these tests the oxidation is accelerated by raising the temperature, increasing the surface exposed to air, by light, and by traces of metals, of which copper is the most active, while manganese, iron, and chromium also have a marked action. The latter method is of little practical value, since natural antoxidants (synergists) in the oils are probably effective by reason of forming complexes with traces of metals. Accelerated stability tests do not always predict accurately the behaviour of oils and fats on storage, since the reactions may take a different course under different conditions, but they form a useful guide. When testing fatty pharmaceutical preparations, with or without antoxidants, the stability tests should be carried out under conditions approximating as closely as possible to those encountered in actual use. Results obtained by the addition of an antoxidant to a pure fat cannot be extended to a galenical preparation made with that fat. G. M.

Rape Seed Oil, Component Acids of. M. N. Baliga and T. P. Hilditch. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 258.) The component acids of four rape seed oils—Indian (Toria, Guzerat), Polish (Danzig), and Argentine (Plate)—and of ravison and Jamba rape seed oil have been examined by crystallisation from ether at $-40°$ C. under suitable conditions, previous to ester-fractionation. In this way it was possible to determine approximately the 3 unsaturated and 5 saturated minor component acids as well as the 4 major ones, viz., erucic, oleic, linoleic and linolenic acids. The average fatty acid composition of the 4 rape oils is: palmitic 2·5, saturated C_{18}, C_{20}, C_{22}, C_{24} (together) 5, hexadecenoic 2, oleic 15, linoleic 13·5, linolenic 8, eicosenoic 5, erucic 48, docosadienoic 1 per cent. (wt.). Ravison oil fatty acids contain less erucic (39 per cent.) and more linoleic (21 per cent.). Jamba rape oil fatty acids contain less erucic (37·5 per cent.) and apparently larger proportions of oleic (*c.* 20 per cent.) and eicosenoic (*c.* 11 per cent.) acids. The procedure for the examination of the component acids of these cruciferous seed oils, admittedly difficult to resolve, is given in detail. H. F.

PLANT ANALYSIS

Pyrethrum Flowers, Analysis of. W. Mitchell, F. H. Tresadern and S. A. Wood. (*Analyst*, 1948, **73**, 484.) A systematic study has been made of the Seil method, depending on the fact that chrysanthemum

dicarboxylic acid is not volatile in steam in contrast to the monocarboxylic acid, and of the Wilcoxon-Holaday method, depending on the fact that only the monocarboxylic acid is readily soluble in light petroleum. Pure chrysanthemum acids were used in the study and the behaviour, recovery and stability of the acids under varying conditions are reported. The Seil method was found to give low results for pyrethrin I and slightly high results for pyrethrin II, the inaccuracies being due to a temperature effect and not to mineral acid. It is suggested that the apparent loss of chrysanthemum monocarboxylic acid is due to hydration and that the resultant hydroxy-acid is partly responsible for the slightly high figures for pyrethrin II. A modified Seil method gave accurate total pyrethrum figures when compared with the Wilcoxon-Holaday method. The latter method could give accurate results for pyrethrin I and for pyrethrin II if a small modification was used. The methods were applied to pyrethrum extracts confirming the results. The presence of extraneous volatile acids was confirmed but found not to interfere with the accuracy of the results by either method. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Vitamin B_{12}, A Cobalt Complex. E. L. Rickes, N. G. Brink, F. R. Koniuszy, T. R. Wood and K. Folkers. (*Science*, 1948, **108**, 134.) Vitamin B_{12} appears to be a cobalt co-ordination complex which, having six groups about the cobalt atom, could involve one or more organic moieties. The presence of cobalt is significant in view of the many biological studies which have shown it to be an essential trace element in nutrition. The cobaltous ion (1 μg./ml.) was without activity for *L. lactis* as contrasted with the high potency of B_{12} (0·000013 μg./ml., half maximum growth). Spectrographic examination of B_{12} showed the presence of phosphorus; nitrogen was present but tests for sulphur were negative. Microbiological assay of an aqueous solution at B_{12} (74μg./0·5 ml.) showed that autoclaving for 15 minutes at 121°C., did not change the activity within the experimental error of $11{\cdot}4 \times 10^6 \pm 0{\cdot}6 \times 10^6$ μ/mg. Vitamin B_{12} in 0·015N sodium hydroxide solution (0·2 μg./ml.) was inactivated (microbiological assay) at room temperature as follows: 20 per cent. (0·67 hr.), 45 per cent. (6 hr.), 90 per cent. (23 hr.), 95 per cent. (95 hr.); it was inactivated in 0·01N hydrochloric acid solution (10 μg./ml.) as follows: 18 per cent. (3 hr.), 75 per cent. (23 hr.), 89 per cent. (95 hr.). R. E. S.

BIOCHEMICAL ANALYSIS

Myanesin in Body Fluids and Tissues, Determination of. E. Titus, S. Ulick and A. P. Richardson. (*J. Pharmacol.*, 1948, **93**, 129.) Two procedures are described. The more useful of these, which is generally applicable to plasma and urine, depends on the fact that under proper conditions phenolic ethers can be made to couple with the more reactive diazonium compounds. A less sensitive method for plasma determinations, involves periodate oxidation of the glycerin side chain to formaldehyde, which may then be determined colorimetrically with chromotropic acid; it gives results in agreement with the coupling procedure. Determination of

plasma levels after intravenous injections into normal dogs of doses of 50 and 100 mg./kg. shows that the compound rapidly disappears from the blood stream. The total amount of myanesin excreted in the urine was in no case more than 2 per cent. of the original dose; it would appear that the drug is distributed throughout the body water. S. L. W.

Stilbœstrol, Hexœstrol and their Glucuronides in Urine, Colorimetric Estimation of. F. H. Malpress. (*Biochem. J.*, 1948, **43,** 132.) Methods of preliminary extraction are described which enable estimations of these œstrogens and their glucuronides in urine to be made, using the nitration method of Malpress. Detailed methods of extraction are given for free stilbœstrol and hexœstrol and for their monoglucuronides in cow's urine; modifications necessary for similar extractions from human urine are also described. It was found that hydrolysis of the urine resulted in destruction of added glucuronide and it was therefore essential to prepare extracts of urines before hydrolysing; traces of peroxide if present in ether used for extraction also reduced the recoveries of the œstrogens. Using the processes described, recoveries ranging from 66 to 79 (mean 70) per cent. were obtained for known amounts of stilbœstrol added to cow's urine, and from 52 to 64 (mean 60) per cent. for known amounts of stilbœstrol glucuronide; recoveries from human urine gave mean values of 80 and 63 per cent. for the free and conjugated forms respectively. The comparatively low recoveries of the glucuronide were due to the decomposition during hydrolysis. With hexœstrol recoveries of 70 per cent. of free œstrogen and 74 per cent. of hexœstrol glucuronide were obtained from cow's urine; for human urine the recovery was 85 per cent. for both free and combined forms. Volumes of urine used for estimation should contain from 0·5 to 2·0 mg. of œstrogen. Blank values for the free œstrogen process using cow's urine fell normally within the range 0·05 to 0·2 mg. of œstrogen/100 ml. of urine, although occasionally these values were greatly exceeded, blanks of 2 mg. being obtained. Blank measurements for the corresponding conjugated-œstrogen method were invariably low and of the order of 0·05 to 0·2 mg. of œstrogen/100 ml. of urine. The values given by the simplified modification applicable to human urine were less than 0·05 mg. of œstrogen for the free process, and less than 0·15 mg. of œstrogen for the conjugated form, from 100 ml. of urine. R. E. S.

Streptomycin in Tissues and Urine, Chemical Determination of. V. C. Jelinek and G. E. Boxer. (*J. biol. Chem.*, 1948, **175,** 367.) The previously reported method of estimation of streptomycin by determining the fluorescence of its acridyl hydrazone has been extended to permit the estimation of streptomycin in body tissues and in urine. The various methods of determining streptomycin are compared and analytical details of the isolation and estimation of streptomycin in lung, brain, heart, liver and spleen tissue and in urine are given. The recoveries of known amounts of streptomycin added to urine and tissues are recorded; they varied considerably. Human urine containing from 2 to 50 µg./ml. gave results of 95 ± 6 per cent.; dog liver gave recoveries of 103 ± 14 per cent.; rabbit brain gave recoveries of 99 ± 6 per cent. The lower limit of sensitivity was 2 µg./ml. of urine and 2 µg./ml. of tissue. The method was found to be of value in the determination of streptomycin in urine and tissues following parenteral administration. R. E. S.

Suramin in Plasma, Estimation of. J. C. Gage, F. L. Rose and M. Scott. (*Biochem. J.*, 1948, **42,** 574.) A method for estimating suramin

in aqueous solution or in serum and plasma is described. The procedure depends on the colour change (deep red to pale yellow) observed when suramin is added to 2-*p*-dimethylaminostyryl-6-acetamidoquinoline methochloride. The corresponding change in absorption is from λ_{max} 495 mμ to λ_{max} 450 mμ for the two colour bands, with the greatest difference between the two curves at 505 mμ. Plasma proteins do not interfere with the suramin reaction and the concentration of the drug in plasma may be determined by comparing the optical density of the dye solution (at 505 mμ), to which has been added diluted plasma (in sufficient 0·9 per cent. sodium chloride solution to prevent globulin precipitation), with that of the dye solution without suramin. Satisfactory results were also obtained using an absorptiometer with an Ilford 603 blue-green filter. The procedure yields similar results to those obtained using the hydrolysis method followed by diazotisation and coupling with methyl-α-naphthylamine. The hydrolysis products of suramin have been investigated and the specificity of the method is discussed.

R. E. S.

CHEMOTHERAPY

Dienœstrol and Hexœstrol, Tetra-alkyl Substituted Analogues of. J. B. Niederl and P. Weiss. (*J. Amer. chem. Soc.*, 1948, **70**, 2894.) By conversion of the phenols, *p*-xylenol, thymol and carvacrol into the corresponding 2:5-dialkyl-4-hydroxypropiophenone, tetra-alkyl analogues of dienœstrol and hexœstrol were prepared. The tetra-alkylated hexœstrols derived from thymol and carvacrol showed only feeble œstrogenic activity when injected subcutaneously in oily solution into ovariectomized rats. The compound derived from *p*-xylenol, 3:4-*bis*(2′:5′-dimethyl-4′-hydroxyphenyl)-hexane, in contrast, gave positive œstrus response in all the rats at 50 and at 5 μg. dose levels and in most of the rats at 2 and 1 μg. dose levels thus comparing favourably with its dimethyl analogue, 3:4-*bis*-(5′-methyl-4′-hydroxy-phenyl)-hexane prepared from *o*-cresol.

F. H.

Sulphones : Studies in the Chemotherapy of Tuberculosis. E. Hoggarth and A. Martin. (*Brit. J. Pharmacol.*, 1948, **3**, 146.) The testing of a large number of sulphones and related sulphonates and sulphonamides against *M. tuberculosis in vitro* is recorded. On the basis of high *in vitro* activity ten new compounds were selected for therapeutic tests on mice. Therapeutic activity was found with 4:4 diaminodiphenylsulphone and with 2:4′ diamino-5-thiazylphenylsulphone, but no activity was observed with any of the others. Therapeutic tests in mice show that high *in vitro* activity does not necessarily lead to activity *in vivo*.

S. L. W.

Thiohydantoins and Thioimidazoles. M. Jackman, M. Klenk, B. Fishburn, B. F. Tullar and S. Archer. (*J. Amer. chem. Soc.*, 1948, **70**, 2884.) As it had previously been shown that 2-thiohydantoin possessed half and 2-thioimidazole one and a half times the anti-thyroid activity of 2-thiouracil, and that enhancement of activity occurs on substitution of 2-thiouracil, a series of 5-alkyl-2-thiohydantoins and 4-alkyl-2-thioimidazoles has been prepared and examined. It was found that substitution in the 5-position did not result in any significant increase in the anti-thyroid activity of 2-thiohydantoin. In the 2-thioimidazole series, substitution in the 4-position increased the activity, 4-*n*-propyl-2-thioimidazole, the most active compound prepared, being about three times as active as 2-thioimidazole and about five times as active as 2-thiouracil.

F. H.

PHARMACY

DISPENSING

Oils and Fats, Sterilisation of. J. Kessler. (*Pharm. Acta Helvet.*, 1948, **23**, 387.) Oils and fats cannot be sterilised either in free steam or in the autoclave. Dry heating (90 minutes at 160°C.) is satisfactory.

G. M.

Sterilisation Technique, Efficiency of. O. Bang and A. T. Dalsgaard. (*Arch. Pharm. Chemi.*, 1948, **55**, 699.) For testing the efficacy of various methods of sterilisation, the authors used garden soil. This material required at least 20 minutes at 120°C. to produce complete sterility. The results of the tests showed that in 61 per cent. w/w alcohol, heating at 100°C. for at least 1 hour in a sealed container was necessary for complete sterilisation. When suspended in oil, the dry heating required was at least 10 hours at 140°C., 3 hours at 160°C., or 1 hour at 180°C. The alcohol method may be applied to the sterilisation of procaine hydrochloride and of boric acid in powder, also to laminaria. In the latter case the material is kept under 61 per cent. alcohol for 24 hours to extract soluble salts, then transferred to tubes, covered with the diluted alcohol, and closed with cotton wool and a loosely screwed-on lid. After 1 hour in flowing steam, the alcohol is removed and the tubes are dried at 105°C. G. M.

PHARMACOLOGY AND THERAPEUTICS

Conessine, *iso*Conessine and *neo*Conessine, Pharmacological Properties of. R. P. Stephenson. (*Brit. J. Pharmacol.*, 1948, **3**, 237.) Conessine, an alkaloid obtained from the bark and seeds of *Holarrhena antidysenterica*, and its isomers, *iso*conessine and *neo*conessine (prepared by treating conessine with sulphuric acid) possess properties very similar to those of quinine and quinidine; in doses, however, in which quinine was active as an antimalarial, conessine and its isomers showed no similar activity. When tested by intracutaneous injection into guinea-pigs connessine and its isomers were shown to possess marked local anæsthetic potency, conessine being about twice as active as cocaine, *iso*conessine about 50 per cent. stronger than cocaine, and *neo*conessine being about equal to cocaine. The relative local anæsthetic potencies of conessine and its isomers and of cocaine, quinidine and procaine are very similar to their related activities in depressing the action of acetylcholine on the frog rectus muscle. This is a further addition to the evidence that the action of acetylcholine is concerned with the sensation of pain. Conessine also resembles quinidine in diminishing the action of acetylcholine on the isolated intestine, the rabbit auricle, the frog rectus and on denervated mammalian muscle, and in lengthening the refractory period of cardiac tissue, and the effect of vagal stimulation on the heart in the anæsthetised rabbit is temporarily abolished by conessine as by quinidine.

S. L. W.

Dimercaprol (B.A.L.), Effect of Environmental Temperature on. F. F. McDonald. (*Brit. J. Pharmacol.*, 1948, **3**, 116.) Variations of 15 to 85 per cent. in mortality occurred in groups of rats used as standard controls in assaying samples of dimercaprol. So wide a difference in response was greater than would be expected by chance and some other external factor was suspected of contributing to the toxic effects of dimer-

caprol. Temperature being the greatest variant to which the rats were subjected, experiments were conducted to see if this affected the mortality of rats caused by a standard dose of dimercaprol. Rats injected intramuscularly with 140 mg./kg. of Oxford Standard B.A.L. were kept in thermostatically controlled chambers at temperatures varying from 39°F. to 84°F. for 72 hours after injection. The results showed a quite remarkable effect of temperature on toxicity, the mortality rate varying from 20 to 100 per cent., with a minimum mortality at 63°F. Rats used for assays should therefore be kept at an even temperature or in a thermostatically controlled room if possible, and no estimation of the relative toxicity of samples should be made without reference to the results obtained from a dose of a standard preparation given at the same time. If propylene glycol is used as a vehicle for injection, the required amount should be distilled off on the same day.

S. L. W.

Dimercaprol. (B.A.L.) and its Glucoside, Effects of, in Acute Lead Poisoning. M. Weatherall. (*Brit. J. Pharmacol.*, 1948, **3**, 137.) In mice poisoned by repeated intraperitoneal injections of lead acetate the mortality was reduced slightly by dimercaprol and significantly by the glucoside, but it was difficult to produce lead poisoning suitable for experimental study in these animals. In suitable concentrations dimercaprol prevented the action of lead acetate on rabbit red blood cells *in vitro*. If it was added half or one hour after the lead acetate the effect was small and consisted chiefly in preventing the full effect of the lead, not of significantly reversing the established change in fragility. Mixtures of dimercaprol and plasma in certain proportions, and plasma from rabbits injected with dimercaprol, protected washed erythrocytes from the effect of lead acetate less than did equal amounts of dimercaprol or plasma alone. In rabbits poisoned by a single dose of lead acetate given by stomach tube dimercaprol and the glucoside each significantly decreased the subsequent anæmia and increased the coproporphyrinuria. The mortality was apparently unaffected by dimercaprol but was reduced by the glucoside, though the number of rabbits was too small for the difference in mortality to be significant. The action in lead poisoning appears to be one of inactivating lead ions not yet taken up by cells, rather than of actually de-leading cells or altering the cell lead so as to prevent its fragility effect. There is certainly no reversal of poisoning comparable to that seen with arsenicals. Clearly the drug prevents the acute hæmolytic anæmia as long as it is available in the circulation, but on the other hand certain features of lead poisoning are enhanced, notably the coproporphyrin excretion and possibly the speed of the reticulocyte response. The available evidence does not warrant its use in clinical plumbism, but it would perhaps be premature to reject all dithiols as useless or dangerous. S. L. W.

Iron, Intravenous, in the treatment of Anæmia. H. G. B. Slack and J. F. Wilkinson. (*Lancet*, 1949, **256**, 11.) A stable iron-sucrose preparation suitable for intravenous administration was prepared as follows. Dissolve anhydrous ferric chloride 5.8 g. in distilled water 50 ml. on a water-bath at 95°C, add sucrose 28 g. and heat until dissolved; dissolve anhydrous sodium carbonate 1.8 g. and sodium hydroxide 5 g. in 25 ml. of water each; add the carbonate solution to the ferric chloride solution with stirring; add the sodium hydroxide solution, stir for 15 minutes, filter into rubber-capped vials or into ampoules and autoclave for 20 minutes. After autoclaving, a clear, dark brown solution is obtained, with a pH of about 10.5, which remains stable at room temperature for at least 12 months. The solution contains

2 per cent. of iron and 200 mg. is therefore contained in 10 ml. The scheme of dosage adopted in the treatment of 60 cases of iron-deficiency anæmia was 25 mg. on the first day, 50 mg. on the second day, 100 mg. on the third, and 200 mg. on the fourth and subsequent days, the injections being given into the antecubital veins at the rate of about 2 ml. per minute; the treatment was usually complete in 10 out-patient visits. Reactions, if any, were mild. In almost all cases the hæmatological and clinical responses were often as dramatic as those obtained when patients with pernicious anæmia in relapse receive adequate doses of a potent intramuscular liver extract, a reticulocyte peak of 10 to 18 per cent. developing within 7 to 10 days from the beginning of treatment. The calculated iron deficit given in this manner is utilised almost quantitatively and does not appear to require the addition of trace elements, ascorbic acid or folic acid. With a further 60 patients treated with a commercial iron-sucrose preparation there was no detectable difference in tolerance or response. S. L. W.

Paludrine, Activation of. F. Hawking and W. L. M. Perry. (*Brit. J. Pharmacol.*, 1948, **3**, 320.) Experiments with exo-erythrocytic forms of *Plasmodium gallinaceum* grown on tissue culture showed that paludrine in concentrations of 2 mg./l. exerts no apparent antimalarial action on the parasites *in vitro;* similarly, a concentration of paludrine of 20 mg./l. has no action *in vitro* on the endo-erythrocytic form of *P. cynomolgi;* these concentrations are higher than those commonly reached in the blood during human therapy. If, however, paludrine has been previously exposed to the action of body cells, either by injecting it into a monkey or fowl and collecting the serum, or by incubating it with minced rat liver, it exerts marked antimalarial action, preventing the development of the parasites. These results suggest that paludrine itself is not active against plasmodia, but that it undergoes some chemical modification by the body or by liver which converts it into a compound with plasmodicidal activities. S. L. W.

Phthioic Acid and Synthetic Analogues, Pathogenic Effect of. J. Ungar, C. E. Coulthard and N. Dickinson. (*Brit. J. exp. Path.*, 1948, **29**, 322.) A number of synthetic acids closely related chemically to phthioic acid were tested by intraperitoneal injection into the guinea-pigs. The doses given ranged from 10 to 200 mg. and the animals were examined at various intervals of time after the injection. One of the more active substances, 3:12:15-trimethyldocosanoic acid, produced waxy deposits in various abdominal organs within 8 days of the injection of 25 mg., and white rounded nodules within 14 days. Intradermal injection produced erythema within 24 hours and nodule formation within 3 days. The changes were similar to those produced by an irritant foreign body of lipoid nature and often similar to those due to tubercular lesions. The pathogenic properties of the acids tested do not appear to be related to chemical structure. H. T. B.

Posterior Pituitary Extract, Standardisation. Modification of Dale and Laidlaw Method. P. Holton. (*Brit. J. Pharmacol.*, 1948, **3**, 328.) The Dale and Laidlaw method suffered from three defects, namely, that suitable guinea-pigs were relatively scarce, that the assay often required many hours, and that the error was about 20 per cent. Schild's null hypothesis (*J. Physiol.*, 1942, **101**, 115) was applied to an assay employing a modification of the Dale and Laidlaw method. A rat's uterus was used as the test preparation, since rats are cheaper and more easily obtained. One assay is described, and the

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PHARMACOPŒIAS AND FORMULARIES

THE BRITISH PHARMACOPŒIA 1948
SOME OBSERVATIONS ON THE TESTS FOR PURITY

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IN the official tests for degree of purity, and the methods of assay for chemicals, the new British Pharmacopœia shows remarkable progress when compared to the previous one (1932). Throughout the book the formulation is more precise, the tests are more comprehensive, and frequently more rational; and many new methods have been introduced.

Determination of melting-points.—The technique—the capillary method—is that generally described in modern pharmacopœias, and is completely identical with the method used in the Pharmacopœia of 1932. The melting temperatures may be regarded as corrected temperatures consistent with the technique employed. On the other hand the definition, viz.: The temperature at which liquefaction of the substance occurs; this is indicated by the formation of a definite meniscus, may be subject to criticism, because it is expressed by a single temperature and not by a range between two temperatures. In general, commercial products are rarely of such purity that their melting-points are sharply defined; usually the melting-range extends over one or two degrees. The United States Pharmacopœia uses the term melting-range or melting-temperature, which is thus defined "The temperature at which the column of the sample (in the capillary tube) is observed to collapse definitely against the side of the tube at any point is defined as the beginning of melting, and the temperature at which the sample becomes liquid throughout is defined as the end of the melting." Many other Pharmacopœias (e.g. Danish, Swiss, Swedish) give a similar definition.

Determination of boiling-points.—Both the apparatus used in the determination of the boiling-point and the procedure are described in detail, as in the Pharmacopœia of 1932. The method is the conventional one, as employed in, e.g. the test for oils, and it is well known that this gives reproducible results, although the use of a cylindrical flask as described respectively in the Swiss and the new Danish Pharmacopœias is more rational.[1,2] It is a disadvantage that the procedure demands a large quantity of the substance, 100 ml. In most cases the procedure is employed to decide the character of mixtures of compounds as, for instance, creosote and cresol. It would have been an advantage if the technique in the determination of substances with low boiling-point as e.g cyclopropane (– 34.5°C.) and ethyl chloride (about + 12·5°C.) had been described in detail.

The determination of the boiling-point in the manner described can scarcely be regarded as a practical test for identity and such a test might be useful in many cases, e.g. amphetamine. A number of methods requiring small quantities only and giving reproducible results are available for that purpose; the method described by Siwoloboff,[3] and modifications,[4] may be mentioned.

Limit test for chlorides.—In practically every relevant instance the limit test for chloride is used; although vague expressions such as "no opalescence occurs immediately" have not been completely eliminated. The limit test is carried out in Nessler glasses using 50 ml. of the solution to be tested; to this is added 1 ml. of nitric acid and a solution of silver nitrate (N/10); then the mixture is stirred with a glass rod, and the observation of the reaction

is made 5 minutes later. The opalescence produced is compared to a standard opalescence, equivalent to 0·355 mg. Cl^-/50 ml. (0·071 mg. Cl^-/ml.).

The limit test for chlorides has been carefully investigated by Thörn[5] and by Reimers and Gottlieb[6]. The results of these investigations may be summarised as follows. The opalescence produced by minute quantities of chloride is in inverse ratio to the speed at which the reagent and the test solution are mixed together. Thus rapid mixing does not lead to the maximum sensibility, but gives the most reproducible results. If the reagent is added without shaking, it will give the maximum of sensibility, but with poor reproducibility. For instance the opalescence produced when the solution of silver nitrate is added to the solution to be tested and the liquid shaken after 1 minute, is about 3 times more intense than that produced after rapid mixing. The procedure as set out in the B.P. gives good reproducible results.

Limit test for sulphates.—The test for sulphates is a simple one. Dissolve the substance in water and transfer to a Nessler glass; add hydrochloric acid; dilute to 50 ml. with water, and add 1 ml. of solution of barium chloride. Stir immediately with a glass rod and set aside for 5 minutes. The turbidity produced is then compared to a standard turbidity equivalent to 2·4 mg. of SO_4/50 ml. (0·048 mg./ml.). The conditions here are not quite so simple as in the limit test for chlorides. Thörn[7] and Reimers and Gottlieb[8] have proved that to obtain good reproducibility, it is necessary for the solution to contain a minute quantity of scarcely visible crystals of barium sulphate. Actually the solubility of barium sulphate increases with the decrease in size of the particles owing to the greater surface energy of the small particles. The precipitation of barium sulphate from very dilute sulphate solutions must commence with the formation of microcrystals, the solubility of which may be as much as 1000 times greater than that of large barium sulphate crystals. The precipitation will be markedly inhibited unless the test is carried out with a reagent containing barium sulphate to induce the precipitation in the test sample. The Danish Pharmacopœia describes the use ot such a seeding reagent—the same quantities of sulphate in the test and in the standard turbidity—the concentration of which is chosen so that the precipitation is rapidly completed. The application of the seeding reagent results in both an increased sensibility and an increased reproducibility. The simple method of the B.P. cannot be said to lead to exact reproducible results.

Limit test for iron.—The limit test for iron is now carried out with thioglycollic acid in a solution containing citric acid to produce complex compounds with other cations, and an excess of ammonia to make the solution alkaline. In the presence of iron a pinkish-violet colour is produced. This is a notable improvement on the thiocyanate method formerly employed. Woods and Mellon[9] state that the thiocyanate method in general is inferior to several other methods, especially those using *o*-phenanthroline, and αα-dipyridyl or thioglycollic acid.

Thiogycollic acid as a reagent for iron was first proposed by Andreasch[10] and has subsequently been investigated by others, of whom Swank and Mellon[11] state that the thioglycollic acid method is remarkably free from the influence of other common anions, many of which must be entirely absent in other colorimetric methods. The following ions, in concentrations of 500 mg./100 ml. of solution, had no effect on the colour:— fluoride, iodide, nitrate, orthophosphate, sulphate, chlorate, tartrate, oxalate, citrate, acetate. bromide, thiocyanate, sulphite, and chloride; 250 mg. of boron trioxide, present as tetraborate ion, also has no effect. Pyrophosphate ion, when present in an amount equivalent to 500 mg. of phosphorus pentoxide decreases

the colour intensity by about 8 per cent., but 200 to 300 mg. can be present without serious error. Cyanide ion interferes seriously and must be absent. The lack of interference by nearly all anions and the reproducibility and sensitivity of the colour reaction makes the method superior to various other colorimetric determinations of iron. The procedure is just as simple as the thiocyanate method. The standard colour is equivalent to 0·04 mg. Fe/50 ml. (0·0008 mg./ml.)

Reaction.—For the determination of the reaction of a solution various indicators are used. It is not quite clear what intervals in the *p*H scale are covered by the designations, strongly acid, weakly acid, neutral, weakly alkaline, and strongly alkaline. Many pharmacopœias use similar terms to describe defined intervals of the *p*H scale. In many cases it would be more decisive to determine the *p*H value, or the interval between two *p*H values as a means of characterising the degree of purity. As the B.P. gives a full description of the colorimetric determination of *p*H values and the standard buffer solutions for preparing solutions with *p*H 1·2 to 10·0 such an indication might easily have been inserted more generally and more consistently. It must be regarded as a disadvantage that no test for the sensitivity of litmus paper is given, because the commercial grades of litmus paper vary considerably in this respect. The U.S.P. and the new Danish Pharmacopœia have specified tests, both for sensitivity and for the content of buffer substances in the paper.

Limit tests for metals.—The limit tests for lead and arsenic do not differ much from the previous Pharmacopœia. The expression of the limits in parts per million might well be introduced more generally into other Pharmacopœias. For lead the diphenylthiocarbazone method is used in some cases. It must be regarded as an advance that the B.P. specifies the tests for various individual metals (e.g. copper, zinc) and only in a very few cases uses the general term "heavy metals," which is used in some other Pharmacopœias, e.g. in the Danish, Swedish, Swiss, and U.S.P.

The test for arsenic takes the form used in the British Empire and U.S., while the modern continental Pharmacopœias use the hypophosphite test, which is simpler in technique and equally accurate.

Readily carbonisable substances.—The deletion of the test for readily carbonisable substances is comprehensible in a country where supplies are generally of great purity. One thing is certain, that, if the test is to be of any value, it is essential to have a series of matching fluids at one's disposal as given in the U.S.P. and in the new Danish Pharmacopœia. In the latter, the matching fluids are used not only in this test but generally to determine the colour of many faintly tinted solutions, also for some colorimetric determinations, for instance morphine in codeine and papaverine and for the colorimetric determination of the concentration of adrenaline in solutions.

As an exception, the B.P. includes a test for carbonisable substances in liquid paraffin and similar products. In these cases the colour which is produced in sulphuric acid, after shaking with the paraffin, is measured by means of standardised coloured glasses in accordance with the system of colour measurement adopted at the National Physical Laboratory, Teddington. Such standardised glasses might advantageously be used instead of matching fluids to measure the colour in the test for readily carbonisable substances and also for other approximate colorimetric measurements.

The tests for identification and purity must be said to meet all reasonable demands, a comment equally applicable to the assays. The tests for purity are not numerous, but are adequate for practical purposes, to ensure com-

pounds of sufficient purity. As a large number of new substances have been included a few of the tests and assays will be mentioned.

Ultraviolet absorption.—The absorption of ultraviolet light as a test covering both purity and identity has been included for the following substances: ascorbic acid, ethisterone, calciferol, dienœstrol, œstrone, progesterone, and the following drugs containing vitamin A: halibut-liver oil, concentrated solution of vitamin A, concentrated solution of vitamins A and D, and cod-liver oil. The extinction coefficient is referred to a 1 per cent. w/v solution and indicated for a given wave-length. For the practical purpose to which it is here applied this indication is more suitable than the molecular extinction coefficient. In some cases it might have been of value to give the absorption not only in the maximum but also in the minimum of the extinction curve.

The identification of substances which are so expensive that only small amounts are available are carried out by melting-point determinations on the pure substances or simple derivatives of them, these tests likewise are satisfactory. Among the more modern tests for identification the cyanogen bromide test for nicotinic acid and nicotinamide may be mentioned. For the determination of iodine ion the titration using potassium iodate in presence of potassium cyanide is used; this is an easy and reliable method, which is used also for determination of iodide in iodoxyl after hydrogenation with zinc dust and glacial acetic acid and in iodophthaleim after destruction by heating with anhydrous sodium carbonate. For the determination of iodine in thyroid the powder is heated with sodium carbonate and the iodine ion oxidised to iodate and titrated in the usual way.

For the qualitative test for organically bound chlorine reduction by sodium and amyl alcohol and subsequent titration of the chloride ion formed has been substituted; a more rational method than the old one. This test is used for benzoic acid, benzaldehyde, mandelic acid and its calcium salt, and vinyl ether.

For the determination of the content of bismuth in bismuth salts the old method of ignition is used in several cases, but in some the determination of bismuth as bismuth phosphate is used. This latter procedure is an excellent method and might have been adopted more widely, especially for the salicylate and subgallate, in both of which cases the ignition is protracted.

The assay of organic compounds, which cannot be titrated, in many cases proves difficult In some cases the determination of the content of nitrogen solves the problem. This method might also advantageously have been used for barbitone. For hexabarbitone and the sodium salt the determination of the double bond in the same way as the iodine value would have been a good assay.

The determination of alkaloids in alkaloidal salts has been discussed by van Os[12]. Here it should be sufficient to mention that the principle of weighing or titration of the base is always used in the B.P. and this is the rational way, although other pharmacopœias use only the determination of the anion.

The titrations with titanous chloride for some chemicals (menaphthone, methylene blue, crystal violet) are good.

For the determination of theobromine the excellent method of methylating and weighing the caffeine formed is used. For methylthiouracil no really satisfactory test for identification has been given, as the assay mentioned is inadequate for identification.

The Pharmacopœia contains a large number of Appendices which seem to be comprehensive and very satisfactory. Appendix I gives a list of

materials and solutions employed in tests, these describe the usual reagents, with a complete description of tests for identification and purity.

The solutions of reagents are also listed. It is regrettable that the respective concentrations are not given in simple molarity, but always in per cent. This is an unpractical and old-fashioned way. At least the concentrations of commonly used acids, bases, and some salts ought to be given in simple molarity as is the case in e.g. the Danish, Netherlands, Swedish, and Swiss Pharmacopœias, but, strangely enough, not in the U.S.P.

Appendix V, qualitative reactions and tests for substances mentioned in the Pharmacopœia, gives briefly but exhaustively most of the common identity tests.

The form of the individual monographs is clear, practical and well arranged, and all in all, the new Pharmacopœia is a great improvement on the previous one.

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ABSTRACTS (*continued from page 342*)

results calculated by Schild's method. Eight satisfactory assays were performed and the mean percentage error was 2·16. Fiducial limits for the estimate of potency were calculated and were found to be about half the value for the original Dale and Laidlaw method. The mean experimental time for an assay was 3¼ hours. S. L. W.

Sulphaguanidine, Absorption of. M. R. Fabre, M. T. Régnier and M. E. Grasset. (*Ann. pharm. Franc.,* 1948, **6,** 205.) The method of investigation previously applied to the absorption of sulphanilamide has been extended to sulphaguanidine. After a feed rich in fats dogs were anæsthetised with somnifen and a sample of chyle was collected by catheter. A dose of 5 g. of sulphaguanidine was then given into the stomach. The chyle and blood was examined at intervals for the presence of the drug. The results show that sulphaguanidine is absorbed more slowly than sulphanilamide. In the blood, the compound was first detected after 20 minutes, reaching a maximum after 6 hours. The maximum concentration in the blood was 2·2 mg./100 ml., which compares with the figure of 10·5 mg./100 ml. previously obtained for sulphanilamide. In the chyle, the first signs were detected after 70 minutes, reaching a maximum of 1·9 mg./100 ml. at 5 hours. On post mortem examination, no sulphaguanidine was recovered from the organs, the main quantity being in the urine (13 mg./100 ml.) and fæces (1·20 g./100 g.) G. M.

BOOK REVIEWS

A MANUAL OF PHARMACOLOGY by Torald Sollman. 7th Edition. Pp. 1132 and Index. W. B. Saunders Company, London and Philadelphia, 1948, 57s. 6d.

In pharmacology the present decade is one of "anti-drugs." During recent years, pharmacologists and chemists have achieved astonishing results by the development of poisons which act selectively upon one species of living organism or upon one particular type of cell, enzyme or end-organ. The application of the principle of selective poisoning of species has given us new rodenticides, insecticides and weed-killers, the antibiotics, the anti-malarials, antrycide and other antimicrobials. In functional pharmacology, selective poisoning of certain types of cells, enzymes or end organs has given us antihistamine, antithyroid, anticholinesterase and curarising agents. In toxicology, application of an analogous principle has produced dimercaprol. The principle which has already been so successful in the above work is already being applied in cancer research, using the experience gained in these fields. It is perhaps a relief to realise that other types of work have also borne important fruit, such as pteroylglutamic acid, vitamin B_{12} and dextran.

Two editions of Sollman's Manual of Pharmacology have appeared during this remarkable decade. The edition of 1942 has already established itself, like its predecessors, as a book in which a busy doctor, teacher or research worker may find either the pharmacological information he requires, or references to original papers in which to search further. The clear arrangement of this book, its concentration on what is important and its comprehensive system of references make it extremely useful as a source of information. The question naturally arises as to whether the seventh edition is as successful as its predecessors.

Since the layout of the seventh edition closely follows that of the sixth, the question resolves itself into one of how far the new edition includes and assimilates the developments of the intervening years. It is astonishing to find, owing to the fast growth of pharmacology, that many of the substances now occupying the minds of research workers and clinicians, such as aureomycin, chloromycetin and polymyxin among antibiotics, vitamin B_{12} and its associated factors, C10 and antrycide, are necessarily omitted, owing to their discovery or development having come too late for inclusion in this edition. Researches published slightly earlier are, on the other hand, treated in the excellent way we have come to expect from Professor Sollman.

For the English reader it is worth mentioning that, as is perhaps natural in an American textbook, attention tends to be concentrated on drugs likely to be of interest to the American reader. For example, chloroquine is given fifteen times as much space as proguanil (paludrine). Moreover, the reader is generally referred to the American literature, which may be more accessible in that continent.

H. O. J. Collier.

NEW REMEDIES

The asterisk (*) after the name of an article indicates that the information given is derived from the makers' publications. Further information regarding these articles may be obtained by application to the Editor.

Alepsal* is a combination of phenobarbitone, caffeine and belladonna for use in the treatment of epilepsy. It is claimed that the drugs exert a synergistic effect and that this prolongs the action, enables the use of a smaller dosage of barbiturate, and permits of continuous treatment without risk of toxic reactions or depression of the mental and physical condition of the patient. Alepsal tablets are issued in three strengths: Normal, containing phenobarbitone 1.54 g., belladonna 0.31 g., and caffeine 0.38 g.; Medium, half these dosages; Weak, phenobarbitone 0.23 g., belladonna 0.046 g., and caffeine 0.057 g. The dosage for adults is 2 tablets of Normal strength twice daily, or 1 or 2 tablets of Medium strength for mild cases, with smaller doses of Weak strength for children. The tablets are issued in tubes of 20 (Normal), 30 (Medium) and 80 (Weak). S. L. W.

Avloprocil* is a proprietary brand of procaine-penicillin oily injection, containing 300,000 units of penicillin and 120 mg. of procaine base in each ml. It is claimed that following a single intramuscular injection of 1 ml. effective blood levels can be demonstrated for 18 to 24 hours. The indications are the same as for penicillin oil-and-beeswax preparations, and it is thus of value in all infective conditions where maintained levels of penicillin in the blood are required, and where frequent injections are undesirable or inconvenient. It is of particular value in the out-patient treatment of gonorrhoea by single injection. A single intramuscular injection of 1 ml. (300,000 units) is given every 24 hours, but for severe infections 2 ml. may be necessary. In the treatment of syphilis a course of 1 or 2 ml. daily for 7 or 8 days is given either alone or in conjunction with arsenic or bismuth or both. Under normal storage conditions it retains its potency for at least 12 months. It is issued in single vials containing 10 ml., or in boxes of 5 vials containing 10 ml. S. L. W.

Cresatin* is the acetic ester of metacresol, and has antiseptic, fungicidal and analgesic properties. Its low volatility and oily character cause it to adhere to tissue surfaces, thus providing prolonged action. It is used in infections of the nose, throat and ear, and has been found of value in the treatment of ringworm of the feet; it may also be used in dental practice in the treatment of infected root canals and sockets. In most conditions it may be used undiluted, except when applied in the form of a saturated absorbent cotton packing to the auditory canal when it should be diluted with 3 or 4 parts of warm olive oil. It is supplied in 1-oz. bottles.

S. L. W.

Crystodigin* is a brand of crystalline digitoxin, 1 mg. producing the same effect by mouth as 1 g. of digitalis. The indications are the same as those for digitalis, its greatest value being exhibited in congestive heart failure, auricular fibrillation, and auricular flutter. Rapid digitalisation may be produced by a single dose of 1·2 mg., the effect being established within 6 hours or less. The maintenance dose is 0·2 mg. daily, though individual adjustment of this dose may be necessary, and may vary from 0·1 to 0·3 mg. daily. For slower digitalisation, daily doses of 0·2 to 0·6 mg. for several days are administered. For patients who have recently received digitalis

[*Continued on page 350*

LETTERS TO THE EDITOR

Aluminium Oxide for Quantitative Chromatographic Analysis.

Sir,—In my article on The British Pharmacopœia, 1948, The Assay of Alkaloidal Salts, which appeared in No. 1 of your Journal, I stated that aluminium oxide for quantitative chromatographic analysis must be completely free from alkali and must give a good adsorption test. I wish now to add a more complete set of tests which I would recommend if the chromatographic method is adopted.

Aluminium oxide for quantitive chromatographic analysis should comply with the following tests:—

Neutrality : Shake 1 g. of aluminium oxide with 20 ml. of water and filter; the filtrate is neutral to litmus paper.

Adsorption: Weigh 5 g. into an Erlenmeyer flask, add 20 ml. of a 1 per cent. procaine hydrochloride solution in alcohol and set aside for 15 minutes occasionally swinging the flask. Filter through a filter of diameter 11 cm. To 10 ml. of the filtrate add 10 ml. of water and 5 drops of bromothymol blue solution and titrate to a green colour with 0·1 N hydrochloric acid; 1·90 to 2·40 ml. should be required.

Volume. Pour 10 g. into the glass tube used for the determination, keeping the tube vertical against a firm surface and allowing the aluminium oxide to fall in ten portions from a height of 1 cm. The height of the column in the glass tube should then be 12·5 to 14·5 cm. Attach the glass tube with the aluminium oxide to a suction flask and draw 10 ml. of alcohol (90 per cent.) at 390 to 410 mm. Hg. through the column. The time from the beginning of the suction until the last of the alcohol has been drawn through the column should be 2½ to 5 minutes. A further 30 ml. of alcohol (90 per cent.) is then passed through the column in the same manner; the filtrate thus obtained is used for the following tests.

Alkalinity and Acidity. To 10 ml. of the filtrate add 10 ml. of water and 5 drops of bromophenol blue solution, a yellow or green colour is produced.

Soluble Substances. Evaporate 10 ml. of the filtrate to dryness and dry at 105°C. to constant weight. The weight of the residue must not exceed 2 mg.

Department of Pharmaceutical Chemistry, D. van Os.
The University,
Groningen, Netherlands.

NEW REMEDIES (*continued from page 349*)

preparations, only the maintenance dose should be used. It is supplied in packages of 30, 100 and 500 tablets containing either 0·1 or 0·2 mg. S. L. W.

Estigyn* is 17-ethinyl œstradiol, an ethinyl derivative of the naturally-occurring œstrogenic steroid **α-œstradiol**. Weight for weight it is claimed to be the most active œstrogenic substance known and is active when given orally. It is non-toxic in the usual therapeutic doses and is well tolerated. It is indicated in all conditions calling for treatment with œstrogens, especially hypo-ovarianism, menopausal disorders, inhibition of lactation and prostatic carcinoma. The usual dose is 0·05 mg. 3 times daily, though this may be increased to 6 times daily for inhibition of lactation. It is supplied in bottles of 25, 100 and 500 tablets each containing 0·05 mg. S. L. W.

Ethiodan* is ethyl-*p*-iodophenylundecoate, a mobile liquid used as a contrast medium for myelography, sp. gr 1·264 at 20°C., iodine in organic combination approximately 30 per cent. It is more stable than iodised oil and

[*Continued on page 352*

NEW APPARATUS

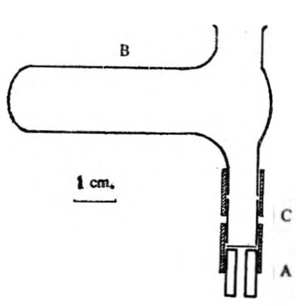
AN APPARATUS FOR SEMI-MICRO CRYSTALLISATION

By W. C. Evans and M. W. Partridge

From the University of Nottingham

Received March 16, 1949

Repeated recrystallisation of quantities of material of the order of 20 to 40 mg. often presents a difficult problem. Application of the usual micro-methods or of the conventional macro-methods, using small apparatus, frequently results in disappointing losses of material owing to premature crystallisation during filtration or to repeated transferences of small quantities of solutions or solids from vessel to vessel. We have found that an apparatus, consisting essentially of a crystallisation vessel attached to a form of the Schwinger filter, is very convenient for repeated and rapid crystallisations. The principle of the apparatus is somewhat similar to that of the Bergkampf[1] filter-beaker.



The apparatus, having the dimensions shown in the figure, is convenient for crystallisations from about 0·4 to 4 ml. of solvent. The surfaces, which meet inside the rubber sleeves, are ground flat. Cell C is tared together with the filtering unit A, and the disc of hardened filter paper secured by the lower rubber sleeve. The crude reaction product is collected, washed and dried in cell C, slight positive pressure being applied to B for rapid filtration. After weighing the product, the cell C and filtering unit are again attached to the crystallising vessel B and the thick-walled capillary tube is replaced by a plug of glass rod of the same dimensions. Most of the solid is tapped down into B and dissolved in hot solvent; by tilting the apparatus, the hot solution is run to and fro into C to dissolve solid adhering to the sides. Cell C is then removed and the filtering unit is attached directly to the crystallising vessel. The solution is reheated for a short time to warm the whole apparatus and then filtered, under slight positive pressure, into a micro-beaker by turning the apparatus through 90°. If crystallisation occurs during filtration, the crystals are easily dissolved in more hot solvent run to and fro from B and then filtered into the bulk of the solution.

The crystals which separate are collected in the same manner as the crude reaction product. Since filtration of the hot solution in further crystallisations is not normally necessary, the crystals are transferred to B as before and dissolved; crystallisation is then allowed to take place in B. The crystals are collected again in cell C. If some crystals adhere to the sides of B, they are redissolved in some of the mother liquor, allowed to crystallise and collected with the main bulk in cell C. The crystals are then washed and dried.

The process can be repeated as often as necessary. After the first hot filtration, there are no losses owing to transference from vessel to vessel, and the material is accessible for weighing and melting-point determination between each recrystallisation.

In the first two trials with this apparatus, hyoscyamine picrate, obtained

from hyoscyamine sulphate solution produced in a partition chromatogram of *Atropa Belladonna*[2] was used. The results of crystallising two samples of the picrate from aqueous alcohol are shown in the Table. We have since obtained equally satisfactory results in many other crystallisations.

Recrystallisations	Weight recovered mg.		Melting-point °C. (uncorrected)	
	(a)	(b)	(a)	(b)
Crude precipitate	21	40	162—3	162—3
First crystallisation with filtration of hot solution ...	14·5	34	164—5	164—5
Second crystallisation without filtration of hot solution	11·5	32	164—5	164—5
Third crystallisation without filtration of hot solution ...	10·5	30	164—5	164—5
Fourth crystallisation without filtration of hot solution	9·5	27	164—5	164—5

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NEW REMEDIES (*continued from page 350*)

is less irritating; its low viscosity also makes it easier to handle, and a comparatively small gauge needle may be used. Ethiodan is specifically indicated for use in the radiological diagnosis and localisation of cord tumours, herniated nucleus pulposus, intraspinal protrusion of intervertebral discs, and any other conditions in which obstructions in the cerebrospinal canal or compression of the cord are suspected. Normally, 3 ml. is injected immediately below the level at which the obstruction is suspected. It is issued in boxes of 3 ampoules each containing 3 ml. S. L. W.

Ferosan Tablets* contain exsiccated ferrous sulphate 3 gr., copper sulphate 1/25 gr., and manganese sulphate 1/25 gr. Their use is indicated in all cases of hypochromic microcytic anæmia, including anæmia due to chronic or acute hæmorrhage, idiopathic hypochromic anæmia and anæmia of pregnancy or lactation. The adult dosage is 1 or 2 tablets 3 times daily after meals. Ferosan tablets are supplied in bottles of 100 tablets. S. L. W.

Priscol* is the hydrochloride of 2-benzyl-4:5-imidazoline; in colourless crystals, freely soluble in water; m.pt. 171 °C. Its principal action is to dilate the peripheral vessels. This effect is primarily on the arterioles and the smaller arteries, and its use is therefore followed by hyperæmia and acceleration of the blood flow in the capillaries. The improved circulation is usually accompanied by a fall in blood pressure. It is indicated particularly for the treatment of peripheral vascular disorders, by intravenous, intramuscular or intra-arterial injection; arthritic conditions are treated by peri-articular injections or the local use of an ointment. It may also be employed as a local application, combined with parenteral or oral therapy, for the treatment of slow-healing wounds and ulcers. It is claimed to be especially valuable in ophthalmic conditions where active hyperæmia is desired; for this purpose, it is employed either in the form of drops of a 10 per cent. solution or by subconjunctival injection. Priscol is supplied in bottles of 40 or 200 tablets containing 25 mg., in boxes of 10 ampoules containing 1 ml. (10 mg.), in bottles of 10 ml. of 10 per cent. solution, and in tubes containing 20 g. of 10 per cent. ointment. S. L. W.

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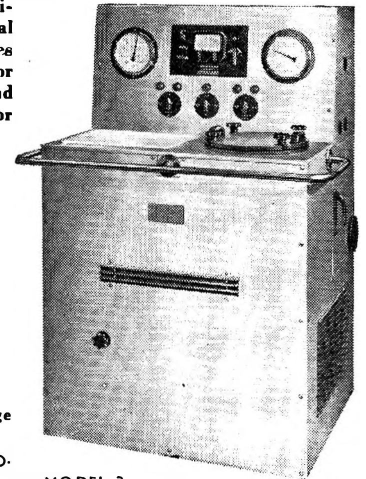

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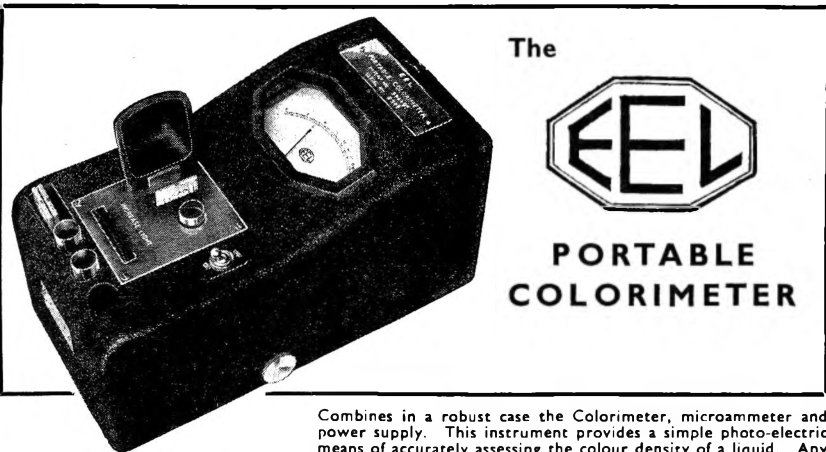

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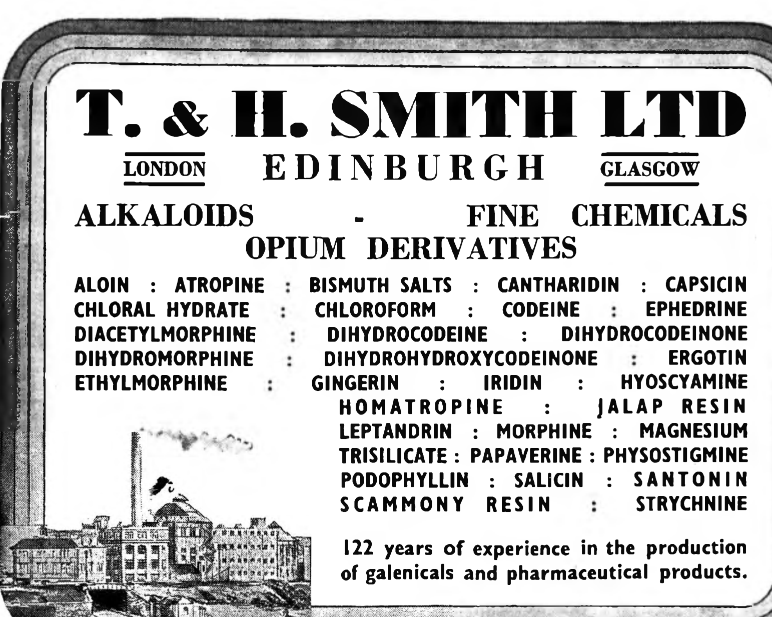

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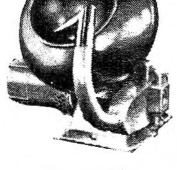

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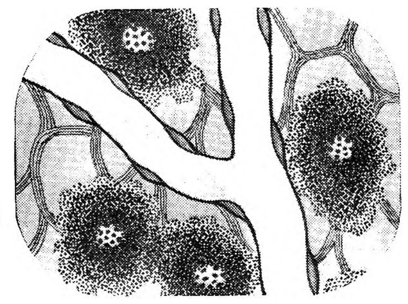

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