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REVIEW ARTICLE

THE APPLICATION OF SOME ANTHELMINTICS IN VETERINARY PRACTICE

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Helminthological Considerations

A comparison of the comprehensive account of the use of anthelmintics in veterinary practice by Chopra and Chandler[1] with the recent review of present-day treatments of parasitic infections by Foster,[2] illustrates that the past 25 years have witnessed some remarkable advances in the chemotherapy of the diseases of animals caused by helminths. Progress has been made in several directions which include not only the development of new anthelmintic drugs, but also advances in their application in the light of advancing knowledge of the epizootiology of some of the more important parasitic diseases.

Anthelmintics in veterinary practice are used in two important ways; namely, as adjuncts to the control of parasitic disease, and as curative agents when prophylaxis has not been practised or when it has failed. At the outset, however, it must be realised that anthelmintics do not represent the complete answer to the control of helminths of stock. They have their limitations, and though a few drugs are effective against the immature stages of some worms, most fail in this respect especially against larval stages which migrate within the body. Such a limitation is serious in the many cases where immature forms are more important pathogens than the sexually mature adults. Moreover, few anthelmintics are 100 per cent. efficient even against adult helminths. For many reasons, therefore, it will be clear that the aim in control should be to prevent infection by a combination of several methods. In the case of the important strongyloid nematode infections, emphasis must be placed on measures to reduce the chances of acquisition by the grazing host of the infective stages of the parasite through improvements in animal husbandry and grassland management. We must recognise, however, that with these parasites, the host rather than the pasture is the reservoir of infection and anthelmintics have a very important part to play in control, since their rational use represents an attack on the contamination phase of the life-cycle[3]. As prophylactic agents, therefore, the principle of anthelmintic usage comprises the elimination of significant burdens of harmful parasites and the prevention of their further accumulation to clinical or even sub-clinical levels. One method by which this may be achieved consists in the periodical dosing of animals constantly exposed to infection. In this way gross contamination of the grazing by helminth eggs and larvæ, which under present-day conditions of intensive stocking might otherwise lead to epizootics of severe disease, is avoided and susceptible young stock are thereby protected.

It is appropriate to refer at this stage to an entirely new method for the control of many of the important strongyloid nematodes of stock which has been developed within the last 10 years or so consequent upon the introduction of phenothiazine. Early in the development of the use of the drug, Shorb and Habermann[4] showed that although small sub-therapeutic doses of the drug in sheep had no immediate lethal effect on adult susceptible worms, they caused marked suppression of egg-laying by the female helminths and the few eggs that were produced were rendered non-viable. Taylor and Sanderson[5] also recorded inhibition of development of trichostrongylid eggs excreted by goats receiving small doses of phenothiazine. These observations opened a new field for prophylaxis because it became clear that if low-level administration could be made a continuous routine, without toxic effects, a very marked reduction in pasture contamination could be effected, and young susceptible animals grazing along with older stock could be given a considerable degree of protection. This method of administration has now been developed as a practical procedure for certain species of hosts. For example, where sheep have a sufficiently high salt requirement the administration of phenothiazine and salt mixtures, fed continuously, is widely practised in some countries in the control of certain strongyloid nematode infections. This principle of prophylaxis has also been developed for horses, in which the daily administration of small doses of phenothiazine causes a reduction to very low levels in the egg output of strongylid worms. This phenomenon, together with the failure of excreted eggs to develop into infective larvæ, has resulted in such a degree of protection for foals running with mares that it is now practically possible to rear these young animals nearly or completely worm-free as far as the strongylid worms are concerned.

So far, our discussion on anthelmintic usage in prophylaxis has been concerned with the strongyloid nematode infections of grazing animals, but it will be appreciated that in the remaining many and varied helminthiases the principles involved in control will vary considerably, and no hard and fast rules can be laid down since the epizootiological factors involved are not constant for all the parasites concerned. Limitation of space does not permit a detailed account of these manifold considerations, but one further example might be taken in order to outline some general principles. In selecting *Fasciola hepatica*, a very important parasite in ruminants, our purpose is to stress that reliance on anthelmintics alone can be regarded as no more than a contributory factor in the fundamental problem of control of fascioliasis. The helminth concerned is mollusc-transmitted and clearly the aim must be to prevent infection, preferably by eliminating the vector. But as long as snails abound, routine anthelmintic treatment will continue to provide a very valuable means of reducing the losses from chronic fascioliasis and, by eliminating the parasites from infected animals, such a routine will reduce pasture contamination with the eggs of the parasite. It must be recognised, however, that anthelmintics against *F. hepatica* have their limitations. In some areas the drugs prove too toxic for general use. Moreover, in epizootic years, when the snail populations increase markedly there is the possibility of severe losses from

acute fascioliasis caused by the mass migration of immature flukes through the liver substance. The known therapeutic agents are generally considered to be of little value against these immature forms. It will be clear that an attack upon the snail intermediate hosts, wherever possible, is a primary necessity in effecting a reduction in the incidence of fascioliasis. Effective molluscicidal development and application must accompany advances in chemotherapy.

The Administration of Anthelmintics

It is not possible within the scope of this review to discuss the administration of anthelmintics in detail, because the numerous species of animals involved have their own special requirements for particular drugs. The ruminants, however, do merit special attention in this respect since research during the past 20 years on the œsophageal groove reflex has constituted one of the important advances in anthelmintic development, particularly in sheep. It is well known that some anthelmintics, for example, carbon tetrachloride, phenothiazine, hexachlorethane and lead arsenate are effective no matter whether they are swallowed into the rumen or into the abomasum, but others, which include tetrachlorethylene, copper sulphate, soluble arsenical compounds and nicotine sulphate, are ineffective if swallowed into the rumen, and must be swallowed directly into the abomasum; an effect which can only be achieved by closure of the œsophageal groove. It follows, therefore, that closure of the groove by stimulating substances administered before, or in some cases together with, the anthelmintic agent increases the activity of the latter against worms in the abomasum and small intestine.

A considerable amount of research has been carried out in sheep. It is now well known that the oral administration of an aqueous solution of copper sulphate and certain other copper salts will stimulate closure of the groove in a high percentage of these animals, but it is important to recognise that this does not occur in all cases, and some instances of failure of anthelmintics known to be effective when swallowed directly into the abomasum are attributable to this fact[6].

Roberts[7] has discussed the position in cattle. Here there is a pressing need to test critically anthelmintics other than phenothiazine which, though effective against some helminths of cattle such as *Hæmonchus contortus*, *Œsophagostomum radiatum* and to some extent *Ostertagia ostertagi* and *Trichostrongylus axei*, leaves much to be desired in the treatment of certain other nematodes, such as *Cooperia* spp. and *Bunostomum phlebotomum*. But tests with anthelmintics, other than phenothiazine, in cattle have been impeded by a lack of knowledge concerning the stimuli causing closure of the groove in these animals. Copper sulphate, in contrast to its efficacy in sheep, has little effect on the bovine œsophageal groove. As long ago as 1930, however, Wester[8] showed that closure of the groove could be induced by the administration of various sodium salts including sodium bicarbonate. Riek[9] recently confirmed this observation and found that 60 ml. of a 10 per cent. aqueous solution of sodium bicarbonate was effective in closing the groove of

93 per cent. of 110 animals used. These developments should now enable critical tests to be made on those anthelmintics which are effective only when swallowed into the abomasum.

Anthelmintic Substances

The review which follows concerns some of the more important anthelmintics in current use in veterinary practice. In order to limit the extent of the article, particular reference will be made to the more recently introduced drugs. Reference to many of the older preparations must needs be omitted and, therefore, certain well-tried anthelmintics whose properties are well known are not discussed. These include copper sulphate, copper sulphate-sodium arsenite and copper sulphate-arsenic pentoxide mixtures used in the treatment of ovine hæmonchosis; copper sulphate-nicotine sulphate mixtures which are effective against *H. contortus* and, to a lesser extent, against *Ostertagia* and *Trichostrongylus* spp. and possibly against *Nematodirus* spp. and *Moniezia* infections in sheep; and sodium arsenite enemata against *Œ. columbianum*, the ovine nodular worm. Gordon[3 10] has given a concise account of the use of these anthelmintics in the control of sheep diseases. Copper sulphate and copper sulphate-nicotine mixtures are also used to a limited extent against trichostongylids in cattle, but further critical work is necessary to determine their efficacy in this host.

No detailed reference will be made to tetrachlorethylene, a drug which, if preceded by copper sulphate in order to close the œsophageal groove, is of value against *H. contortus*, *B. trigonocephalum*, and fairly effective against *Ostertagia* spp. and *Trichostrongylus* spp. in sheep. The efficacy of this drug against related helminth species in cattle requires further investigation. Tetrachlorethylene finds a wide use in the treatment of dogs and cats suffering from ascarid and hookworm infections against which it is highly efficient. Carbon tetrachloride, which is a highly effective agent against *Fasciola hepatica* in sheep, and also of value against *Ascaridia* in fowls, *Amidostomum* in geese and, to some extent, in the treatment of equine strongylidosis, has also been omitted.

Certain useful agents against ascarids, such as oil of chenopodium and carbon disulphide, are so well known that detailed discussion is unnecessary. Oil of chenopodium is still widely used against ascarids in pigs and, to a lesser extent, against roundworms in dogs, but for the latter hosts more satisfactory drugs are now available. Carbon disulphide, though possessing several disadvantages, remains the drug of choice for the control of ascariasis in horses and for the treatment of gastric myiasis caused by *Gastrophilus* larvæ.

Some reference will be made to diethylcarbamazine as an anthelmintic against ascarids in dogs and cats, but the filaricidal properties of this drug are not discussed in detail. Certain other agents used in canine filariasis including antimonials and arsenamide are beyond the scope of this article. Useful recent reviews on the use of these drugs in the treatment of *Dirofilaria immitis* infection of dogs have been prepared by Otto and Maren[11] and Otto[12].

As regards the treatment of cestode infections many of the older anthelmintics are now obsolete and they have been omitted. For example, extract of male fern is rarely used nowadays for dogs, its place being taken by more effective agents.. In the pages which follow, reference will be made to some of these newer preparations, but the arecoline-acetarsol compounds are not discussed because their high efficiency is well known and widely recognised.

Phenothiazine

Undoubtedly the most important development in anthelmintics has been the introduction of phenothiazine. This drug, first used in 1934[13] as an insecticide, was recognised in 1938 to have marked anthelmintic properties[14], and since then it has been investigated extensively and employed widely in the treatment and control of infections of animals with several species of nematodes. The literature on phenothiazine has become so extensive that it is beyond the scope of the present article to attempt to cover the whole of it. But several important reviews of the uses and properties of phenothiazine have appeared within the last 12 years[15,16], the most recent being that of Harwood[17].

Pharmacology and Toxicology. The fate of the drug in the vertebrate body has attracted considerable attention for several reasons. It is well known that phenothiazine is relatively costly to use, the dosage required is bulky and the preparation is therefore somewhat inconvenient to administer. With a view to reducing the cost and the bulk of the drug necessary to achieve success, attempts have been made to determine whether phenothiazine itself or some derivative of phenothiazine is necessary for anthelmintic activity, but the evidence still points to the former as the active agent. Another reason for research into the pharmacology of this drug is related to one of the earliest recognised effects of phenothiazine in the animal body, namely the red discolouration which appears when the urine and milk of treated animals are exposed to air. It is now well known that colourless leuco compounds (oxidation products of absorbed phenothiazine) are excreted in the urine, becoming converted into red dyes on exposure to the atmosphere. When phenothiazine is spilled on the coat or fleece of animals during administration a similar effect occurs, because phenothiazine undergoes oxidation readily, even outside the animal body, especially when moist. These constitute minor disadvantages of the drug. Research on oxidation products has been further stimulated by the recognition of a photosensitised keratitis which appears in some animals, especially in young cattle, when exposed to sunlight following treatment. This phenomenon can now be explained and reference will be made to it later. In general, phenothiazine is a well-tolerated drug, but toxic effects are occasionally seen, especially in horses and cattle, and this has led to considerable investigations on toxicology, but despite much research there are still many gaps in our knowledge.

The fate of the drug has been studied in several species of animals. Although the details of its metabolism show a variation between species,

it appears that when phenothiazine is administered by mouth some of it is absorbed as such from the alimentary tract (though in ruminants some of it may also be absorbed as oxidised derivatives), and the rest is eliminated with the fæces. The amount of phenothiazine which remains unabsorbed is a very important factor as regards anthelmintic activity. Davey and Innes[15] consider that there is little evidence that a derivative of phenothiazine is the anthelmintic. They believe that phenothiazine itself is the essential substance, and suggest that particles of the drug are taken in through the mouth of the nematode, so that for the dose to be effective a certain concentration of particles of phenothiazine has to be attained in the alimentary content. In support of this they express the view that the pronounced anthelmintic activity of the drug against helminths in the abomasum and large intestine of ruminants, as compared with the lower efficiency against worms in the small intestine, is related to the speed of passage of the drug through the alimentary tract. In connection with the uptake of the drug by the parasite they state that phenothiazine will not penetrate the cuticle, but Lazarus and Rogers[18,19] have investigated the mode of action of phenothiazine as an anthelmintic by using the drug labelled with Sulphur-35. They have shown that phenothiazine can enter the nematode through the cuticle and suggest that this may be the important route of entry. Using *Ascaridia galli*, the large roundworm of the fowl, they demonstrated that the uptake of phenothiazine by the worm was more rapid and much greater than that by the adjacent mucosa of the host's gut, a result which appears to indicate differential selection of the drug by the parasite. As regards the active agent, Harpur *et al.*[20] believe that the anthelmintic activity of the drug is attributable to the unabsorbed, but not necessarily unaltered fraction of the drug, and Esserman[21] concluded that phenothiazine and not its oxidation derivatives is the anthelmintic.

In sheep, Swales and Collier[22] found that roughly equal parts of the drug are excreted in the fæces and lost in the urine, about 40 per cent. being excreted through the kidneys. But the proportion of phenothiazine absorbed from the alimentary tract in relation to the quantity excreted in the fæces varies widely in individuals and in different animal species. It has been suggested that the rate of absorption of phenothiazine is influenced by the particle size of the drug. Collier *et al.*[23] showed that the micronised form is absorbed more readily than the ordinary commercial phenothiazine. On the other hand, Harpur *et al.*[20] found that increasing the particle size does not necessarily cause any decrease in the amount of phenothiazine which is absorbed. It should be pointed out that it is not yet clear what particle size will give most anthelmintic efficiency. It has been suggested that increasing the particle size may decrease the efficacy of the drug, but Guthrie and Harwood[24] found no difference in efficacy between micronised and coarsely ground phenothiazine against *Heterakis* and *Ascaridia* in chickens. It has also been shown that recent increases in particle size in commercial phenothiazine has not reduced the anthelmintic efficiency of the drug against *T. axei* in sheep[25].

Within recent years most work on the metabolism of phenothiazine after absorption has been carried out in ruminants, and although the position is still somewhat confused the recent work of Harpur *et al.*[20,26] has helped to clarify the situation. According to these investigations, the fate of phenothiazine in sheep is as follows. It was found that the drug is absorbed as such and is normally not oxidised to phenothiazone in the rumen of sheep, a finding in contrast to that of Swales and Collier[22] and Collier *et al.*[23], who came to the conclusion that phenothiazine undergoes rapid oxidation in the rumen to phenothiazone which allows increased solubility, ready absorption and rapid appearance of oxidation derivatives in the blood and urine of the treated animal. On the other hand, Clare[27] found phenothiazine sulphoxide in the rumen of sheep and cattle, but Harpur *et al.*[26] commenting on this finding, state that it seems possible that the extent of oxidation in the rumen is variable, and suggest that phenothiazine in the rumen content may undergo oxidation if it comes in contact with air in the presence of alkaline saliva during rumination. They believed that this would explain the occurrence of phenothiazine sulphoxide in the rumen.

After absorption much of the phenothiazine undergoes oxidation within the body, the derivatives being excreted mainly in the urine and bile, and to some extent in the milk of lactating animals. Clare and his colleagues[27,28], working in New Zealand, have been particularly interested in the oxidation products of phenothiazine within the body of cattle, especially since photosensitised keratitis is apt to occur under certain conditions particularly in young cattle[29]. Their results, summarised by Whitten[30], show that in cattle and sheep phenothiazine sulphoxide, which they regard as a common oxidation product of phenothiazine in the alimentary tract, is absorbed and carried by the portal blood to the liver, where it is normally converted into leucophenothiazone; this derivative is then excreted in the bile and urine. In young calves, the liver may fail to convert all of the sulphoxide; excess passes into the systemic circulation and some reaches the aqueous humour of the eye. Phenothiazine sulphoxide is the only known derivative of phenothiazine which is excreted in the lachrymal fluid and constitutes the photosensitising agent. Keratitis may occur if the animal is exposed to direct sunlight 12 to 36 hours after dosing. Wavelengths of 320 to 360 mμ are the active ones. By keeping the animals indoors and protected from sunlight on the day following treatment this hazard can be avoided. In sheep, the conversion of absorbed phenothiazine sulphoxide is more efficient, and as the sulphoxide does not normally appear in the systemic circulation, keratitis is not usually seen after treatment except possibly following the use of very large doses, but Gordon and Green[31] have reported photosensitisation in lambs in Tasmania after 15 g. doses; their cases showed skin lesions on the muzzle and ears as well as keratitis in some animals. Enzie and Whitmore[32] have described photosensitised keratitis in goats after normal therapeutic doses, and photosensitisation of the skin and cornea have been reported on several occasions in pigs. Clare[33] has given a succinct account of these phenomena.

As has been indicated, considerable attention has been given to the oxidation derivatives which appear in the urine, and Harwood[17] has given a summary of the present status of knowledge. In addition to phenothiazine itself, the products which appear in the urine vary in composition and relative proportion in different animal species, but as already indicated the colourless leuco compounds, leucophenothiazone and leucothionol, free or conjugated, are excreted. On exposure to the air these form the fast red dyes, phenothiazone and thionol. Coloured derivatives of phenothiazine also occur in the milk of lactating animals after treatment. Although they have not been investigated very extensively, they do not appear to be harmful when ingested along with milk; however, they render the milk æsthetically undesirable for man, and in view of the variation in susceptibility of man to phenothiazine, such milk should not be used for human consumption[34]. It may be used for stock feeding, however, as long as colouration persists. In addition to excretion in the urine and milk, oxidation products of phenothiazine are also excreted in the bile and derivatives have been identified in several animal species.

As regards the possible harmful effects of phenothiazine on the treated animal we have already stated that the drug is generally well-tolerated, but in some species there are toxic hazards and sometimes there is individual susceptibility even within an animal species. Davey and Innes[15], in their review, have considered toxicity at length because, as they remark, it is this factor which determines whether the substance can be regarded as a good anthelmintic. Harwood[17] cites many references to reports of toxicity in mammals. Having regard to the widespread use of phenothiazine in veterinary medicine, however, toxic effects, apart from photosensitised keratitis, have been observed on comparatively few occasions. Nevertheless, the occasional occurrence of poisoning, especially in horses and cattle, cannot be disregarded and care must be exercised in the treatment of debilitated and anæmic animals. Edwards[16] has suggested that it would be prudent also to refrain from administering phenothiazine to very young animals.

It is pertinent to consider the reactions of the various animals to the toxic effects of phenothiazine. Since the introduction of the drug large numbers of horses have been treated and, as long as the dose is kept low, toxic effects are not often seen. Nevertheless, intoxication in the form of marked hæmolytic anæmia has been reported several times, usually after large doses, but even standard therapeutic doses have on occasion produced toxic effects.

The cause of hæmolytic anæmia has not been fully explained but Harwood[17] believes that the lysolecithin theory of Collier and Allen[35] merits further study. These workers have shown that phenothiazine and some of its oxidation derivatives have no direct action on red cells, but *in vitro* they accelerate the lysis of horse erythrocytes by lysolecithin, a normal constituent of horse blood.

Cases of marked hæmolytic anæmia have also been recorded from some individual human beings, cattle and dogs following phenothiazine therapy. As phenothiazine is considered now to be too toxic for use in man and

there are no indications for its application in the treatment of dogs, we need only consider cattle. The toxicity of the drug for cattle is well recognised, but it is generally considered that provided a dosage rate of 0·2 g./lb. of body weight up to a maximum of 60 to 80 g. is not exceeded, toxic systemic effects should be minimal. This observation does not apply to the rather frequent occurrence in some countries, such as Australia and New Zealand, of photosensitised keratitis in calves to which reference has already been made.

Contrary to the position in cattle, sheep are highly resistant to intoxication by phenothiazine. Though Behrens[36] has recorded a reduction in the erythrocyte count and a decrease in hæmoglobin following the administration of phenothiazine, it is generally agreed that hæmolytic anæmia is not observed in sheep following treatment. The only important reservation with regard to sheep concerns its use in late pregnancy. As there are a few records of abortion in ewes following treatment administered during the last month of gestation, it is usually considered advisable that treatment of such animals should be avoided during that period. Goats appear to be nearly as resistant as sheep.

The use of phenothiazine in pigs has not revealed hæmolytic anæmia following treatment but young pigs do sometimes exhibit toxic effects in the form of ataxia and paralysis, although this is usually temporary. Edwards[16] considers that the drug has acquired an undeserved reputation for toxicity in pigs, probably because early experimental treatments were often made with excessive doses. He points out that very large numbers of pigs, infected with the nodular worm, *Œ. dentatum*, have been treated in America with few instances of poisoning.

Birds tolerate phenothiazine very well indeed, and there are few references to toxic effects, but it is interesting to note that Clapham[37] records reduced growth rates in pheasants kept continuously on mash containing 4 per cent. of phenothiazine, and keratitis occurred if the birds were exposed to sunlight.

Anthelmintic uses of phenothiazine: Harwood[17], whose excellent review has been of material help in preparing this section, describes the effectiveness of phenothiazine by taking the helminth parasites in systematic order. This method will be followed here.

There is no reliable evidence that phenothiazine is of any value against helminth parasites belonging to the phylum platyhelminthes. Its usefulness lies in its activity against certain nematodes. It is not effective against *Strongyloides* nor is it effective against helminths belonging to the order Trichinelloidea, and there is not much evidence for activity in spiruroid or filarioid nematode infections.

The chief value of the drug lies in its efficacy against certain worms belonging to the order Ascaroidea and many nematodes in the order Strongyloidea.

Its efficiency against worms in the order Ascaroidea shows considerable variation. It is highly effective in eliminating *Heterakis gallinæ*, the cæcal worm, from chickens and turkeys. And since *H. gallinæ* is of great importance in the epizootiology of histomoniasis ("blackhead") of

turkeys, fowl and other birds, the protozoan parasite being transmitted in large measure from bird to bird through the eggs of the nematode, the use of phenothiazine as a measure of prophylaxis of blackhead by eliminating *Heterakis* has been advocated[15]. Reports are conflicting as to the value of this measure even though successful reduction of *Heterakis* can be achieved by frequently repeated treatment. Wehr and Olivier[38] showed that prolonged feeding of phenothiazine to birds did not control histomoniasis. Their work indicated that phenothiazine given to turkeys in a concentration of up to 2 per cent. in the mash, for 4 to 6 weeks, did not prevent the *Heterakis* infection, although it did result in the subsequent expulsion of the worms before or soon after they reached maturity. But this effect would be too late to afford protection against histomoniasis.

Against *A. galli*, the large roundworm of fowls, the action of phenothiazine is much less marked, but Harwood and Guthrie[39] have shown that in combination with nicotine-bentonite, a synergistic effect was produced which resulted in the removal of a very high percentage of *Ascaridia*. Similar results were obtained by Jaquette and Wehr[40], and Harwood and Stunz[41] found phenothiazine and nicotine-bentonite effective against *Ascaridia* and *Heterakis* in turkeys. Against oxyurids the drug is variable in action. Although it is effective against some of the oxyurids of rodents and against *Enterobius vermicularis* of man, it is not of much value in *Oxyuris equi* infection of horses. Phenothiazine has a limited value as far as the important roundworms in the family Ascaridæ are concerned, but this does not merit discussion since more effective anthelmintics are available for these infections.

The chief value of phenothiazine in veterinary practice lies in its efficacy against many of the strongyloid nematodes. In horses and other equidæ, phenothiazine has a marked anthelmintic value against worms belonging to the family Strongylidæ, helminths responsible for serious losses especially in young horses. Within this family the drug is very efficient in doses of 30 g. for adult horses, against *Trichonema* spp. and other small strongyles. Gibson[42] has shown that it is especially effective against the adult worms, but its efficacy against immature worms does not appear to be so high. He considers, however, that its efficiency against the large strongyles (*Strongylus* spp.) is low. In the early days of work on phenothiazine the drug was thought to have a marked anthelmintic value against these large strongyles, but Gibson's work and the studies of Poynter[43] appear to indicate that the conclusions of earlier workers were sometimes too optimistic; it seems that a very large dose may be highly efficient but there is a danger of toxicity. Nevertheless, standard therapeutic doses of phenothiazine have some anthelmintic value against the adult worms belonging to this genus and, at present, phenothiazine probably represents the drug of choice, although it has been suggested that glycarsamide may have a high therapeutic value,[44] but further investigation is necessary.

The use of periodical full therapeutic doses of phenothiazine given 2 or 3 times a year remains the current method of control of strongyle worms of horses in many countries. It is generally recognised as being imperfect,

however, largely because it does not eliminate infection entirely, and in the intervals between treatments, eggs are produced by those helminths which have escaped anthelmintic action; infective larvæ are produced on the pasture, and when susceptible horses ingest these the developing forms of some species undergo their usual body migration where they may cause serious lesions. Successful control should aim at preventing damage by larvæ. This can only be achieved by grazing clean pastures, but until recently it has been difficult to apply this concept in practice. During the past few years much attention has been given to prophylaxis by means of continuous low level phenothiazine administration, the principle being to reduce pasture contamination by depression of egg production by the worms in the host and inhibition of larval development of any excreted eggs. Foster and Habermann[45] gave 5 g. of phenothiazine weekly in the feed of horses and noted a reduction in the egg-production of strongyle worms. Todd[46] in America has summarised his work in the further development of this method which involves the daily administration of small doses. He describes its practical applications and recommends the administration of 2 g. doses of phenothiazine daily for the first 21 days of each month. There are several reports in the literature which confirm the results of this method, but in adopting this regimen fears have arisen that long-continued dosage might have adverse effect on the horse through a possible cumulative action of the drug. This has not been substantiated[47]. Another doubt arose concerning the possible development of phenothiazine-resistant strains of helminths through continued sub-therapeutic dosage, but there is no evidence that this occurs. A warning has been sounded, however, by Gibson[48] that the production of near worm-free foals, such as this method allows, may not be entirely desirable since such an animal if exposed to heavy infection, when older, may suffer severely from strongylidosis, as a result of its having been deprived of an earlier opportunity to acquire a degree of infection which would stimulate resistance. This hypothesis depends, of course, upon the assumption that resistance against helminths is dependent not only upon age but also upon previous infection.

It is often questioned if prolonged low level administration will eliminate the worms themselves from existing infections in horses. Todd[46] believes that they are gradually eliminated over a period of time, but Drudge *et al.*[49], following upon a long-term study, came to the conclusion that the method was relatively ineffective in removing strongyles from the intestinal tract; moreover, it was relatively ineffective in preventing the development of worms from ingested infective larvæ. The important effect of low level administration lies in the sterilising action on the female worm, the inhibition of development of excreted eggs, and the consequent reduction in pasture contamination.

Phenothiazine has a marked action on certain other helminths in the family Strongylidæ. It is highly effective against the œsophagostomes in all animal hosts, regardless of species, and it has proved a most valuable anthelmintic in the treatment of nodular worm, *Œ. columbianum*, in sheep, a serious condition and the cause of severe losses in some countries. Its

value against the large-mouthed bowel worm, *Chabertia ovina*, is not clear and reports are conflicting. The drug is of no value in the treatment of *Stephanurus dentatus* infection, the kidney worm of pigs, but Threlkeld and Johnson[50] have suggested the possibility of using phenothiazine to control *Stephanurus* by scattering the drug on infected fæces to prevent the development of the larvæ. Against *Syngamus* in birds, therapeutic treatment with phenothiazine given orally to rid the birds of adult worms has not been successful, but Clapham[37] has obtained successful prophylaxis in pheasants by feeding 4 per cent. of phenothiazine in mash continuously which ensures destruction of the infective larvæ before they migrate from the alimentary canal.

The hookworms (family Ancylostomidæ) are not, in general, successfully controlled by phenothiazine with the exception of *B. trigonocephalum* in sheep, in which a high percentage of the worms can be removed by appropriate dosage. Results in the treatment of hookworm of cattle, *B. phlebotomum*, are disappointing. Recent work by Riek[51] indicates that phenothiazine given to calves at dose rates of 0·1 to 0·3 g./lb. of body weight failed to show any marked efficiency against this species. Some slight efficiency was recorded with 0·2 g./lb. of body weight, but it is of interest that an increase to 0·3 g./lb. of body weight was not more efficient against this worm. A possible use for phenothiazine in controlling *Bunostomum* in cattle has been suggested by the work of Mayhew[52] who has shown that, although the daily administration of small doses of 1·5 g. of phenothiazine in the grain ration did not affect egg production, it prevented the development of infective larvæ in the fæces; in this way some degree of control may be expected but more data are necessary.

In addition to its value in strongylidosis of equines and œsophagostomiasis in ruminants, phenothiazine represents the drug of choice for the treatment and control of many of the trichostrongylid infections in ruminants.

We will consider firstly the value of full therapeutic doses. In sheep, phenothiazine has a very high value as an anthelmintic but, with the exception of *H. contortus*, is effective largely against adult worms. *H. contortus* is very susceptible, and doses from 6 to 10 g. give nearly 100 per cent. efficiency[53]. The drug is effective against both the mature and the immature worms, which represents a marked superiority over other agents. Against *Ostertagia* the drug is not so efficient but doses of 20 g. have been recorded as eliminating 90 per cent. of the worms. Further work is necessary, however, to determine the anthelmintic efficacy of phenothiazine against this genus. *T. axei*, the smallest of the trichostrongylid worms in the abomasum of ruminants, is more resistant than *Hæmonchus*, but Gibson[54] has shown that doses of 20 g. will give 96 per cent. efficiency against the worms, and 40 g. doses give 100 per cent. efficiency. The drug has a variable efficiency against trichostrongylids in the small intestine. It is practically inefficient against *Nematodirus* spp. but efficiency can be obtained against *Trichostrongylus* spp. and *Cooperia* spp. provided that full doses of up to 40 g. are given to adult sheep[54]. It has been suggested that the failure of phenothiazine in some outbreaks of trichostrongylosis

is possibly due to development of phenothiazine resistance by the worms, but this has not been substantiated. Sinclair[56] was unable to produce it experimentally in *T. colubriformis* infections in sheep. He emphasises that the customary dose of 20 g. is too low. Gibson[54] has suggested that the true explanation of so-called resistance lies in failure to administer sufficiently large doses.

In goats phenothiazine has proved to be a useful anthelmintic, but Harwood[17] has pointed out that, because these animals seem to be favourable hosts for the intestinal species of *Trichostrongylus*, large therapeutic doses up to 37·5 g. should be used.

In cattle phenothiazine is useful against certain trichostrongylids, but its efficacy against some species in the bovine host, as compared with other ruminants, is of a comparatively low order. Riek[51] has provided a concise review of the literature on the value of the drug against nematodes in cattle. He finds that a dosage rate of 0·1 g./lb. of body weight is effective against *H. contortus* (and *Œ. radiatum*) but a dosage rate of 0·2 g./lb. of body weight is necessary for the removal of *Trichostrongylus* spp. The higher dose, however, has little effect against *Ostertagia* and is only slightly efficient against *Cooperia* spp. Cauthen[57] has also reviewed the literature with special reference to the use of phenothiazine against *O. ostertagi* and *T. axei*. Experimental work recorded by this author shows that doses of approximately 0·2 g. give about 75 per cent. efficiency against *O. ostertagi* and nearly 100 per cent. against *T. axei*. Most workers agree that the maximum dose which can be given reasonably safely consists of 0·2 g./lb. of body weight up to a maximum of 60 to 80 g. but even at this level, occasional toxic effects are noted in animals in poor condition. At this dosage rate, however, taking into consideration all the susceptible nematodes of cattle, it appears that a satisfactory degree of efficiency can be obtained against *H. contortus*, *T. axei* and *Œ. radiatum*, but its efficacy is low against *B. phlebotomum* and *Cooperia* spp. Further investigation is required to determine the value of phenothiazine in *Ostertagia* infections.

We have already referred to the development of phenothiazine and salt therapy for ruminants, and it is appropriate at this point to refer to this new concept in more detail.

Following on the demonstration of the value of small doses of phenothiazine in inhibiting larval development of certain strongyloid nematodes in the fæces of treated animals, Habermann and Shorb[58] developed the principle of low level continuous administration for sheep. They found that the daily ingestion of 0·5 g. per animal was necessary. The details of administration as recommended by various authors differ slightly, but most workers seem to be agreed that, early in spring, the animals should be treated with full therapeutic doses of the drug followed by the use of phenothiazine and salt mixtures at concentrations of 1 : 9 to 1 : 14 which are kept before the sheep at all times and protected from the weather. Continuous intake is necessary in order to ensure dependable prophylaxis. Successful results arise in part from the effect of phenothiazine in causing some reduction of egg output of worms present in the bowel (though with

some species this effect may be inconstant) but chiefly from the suppression of development of those eggs which are excreted in the fæces. If any evidence of failure appears full therapy must be instituted, and in any case a full treatment should be given in the early winter.

It has been suggested that third stage larvæ of trichostrongylid worms which are ingested during this method of prophylaxis fail to develop further within the host. But authorities differ and many believe that low level treatment does not necessarily prevent the establishment of infective larvæ within the host. Gibson[59] showed that when a daily dose of 1 g. of phenothiazine was given to lambs within a few minutes of a dose of infective *T. axei* larvæ, the treatment appeared to have some effect in reducing the number of larvæ which developed to maturity. When, however, there was an interval of several hours between the phenothiazine and the larvæ, the treatment had no effect on the establishment of the larvæ in the host. It did not prevent the development of parasitic gastritis in sheep continuously exposed to infection, when several hours elapsed between phenothiazine and larval administration. Gibson[59] suggests that the good results reported from the field must be due essentially to a reduction in the pasture larval count by phenothiazine inhibition of egg development in the fæces. Foster[60] has emphasised that the treatment must be started on the first day on which the sheep are turned onto spring pastures, at the beginning of the grazing season.

The actual worm burden of adult sheep does not appear to be much affected by low level administration, but opinions differ according to whether the method does actually reduce the burden of an infection which was in existence when the regime was started. Gibson[59] found that there was no significant reduction, but it is interesting to note that Page[61], working with lambs with pure infections of *H. contortus*, found that 0·5 g. of phenothiazine administered daily had a marked anthelmintic effect on lambs with heavy infection. It seems that there may be some species differences in this effect.

In Britain, Harbour *et al.*[62] and Pollard *et al.*[63] have reported the value of phenothiazine and salt mixtures in reducing pasture contamination, but the application of this method as a routine has not been adopted because the additional salt requirements of sheep in this country are too low to ensure the adequate ingestion of sufficient quantities of phenothiazine by this device. In America the method is widely used in the control of nodular worm and trichostrongylid infections. Foster[64] has given an account of an 11-year experiment in helminth control in a flock of sheep using this measure, and his results show that although helminths are not eradicated, they are adequately controlled and no clinical outbreaks occurred in his experimental animals except when the drug was withdrawn for a period of about 5 months. He found no evidence that phenothiazine-resistant strains developed.

Low-level administration of the drug has not yet been adopted as a routine procedure in cattle practice. Experimental evidence suggests promising results but its practical application needs further investigation. Much will depend on the status of the animals concerned in relation to

salt and mineral requirement before free-choice methods can be utilised. Foster[65] has reviewed recent experimental work with cattle.

HEXACHLORETHANE

This drug was investigated as long ago as 1925 when Hall and Cramm[66] tested it, without success, for anthelmintic activity against hookworms in dogs. It was first used in the treatment of fascioliasis in cattle in Germany by Thienel[67], and De Blieck and Baudet[68] and Hilz and Scheuble[69] also reported favourably on its efficacy. Its fasciolicidal properties were not widely recognised, however, until Olsen[70] carried out preliminary trials with the drug against *F. hepatica* in cattle in America. The results of more extensive trials by Olsen[71,72] established hexachlorethane as a relatively safe drug with a high degree of efficiency for the treatment of *F. hepatica* in cattle. This constitutes an important advance for it is well known that hitherto the treatment of fascioliasis in cattle had been very unsatisfactory. It is also recommended against *F. gigantica*[73].

Hexachlorethane is also used in fascioliasis of sheep[74], but its anthelmintic activity does not appear to be superior to that of carbon tetrachloride in this host unless the dose is increased from 15 g. to 30 g. for an adult sheep[75]. Southcott[76], however, found that the increased anthelmintic activity from the large dose was more than offset by the enhanced toxicity danger. In connection with the relative efficiency of these two drugs it is interesting to note that Chance and Mansour[77] who made a kymographic study of the action of certain drugs on the liver fluke, found that carbon tetrachloride and hexachlorethane at low concentrations acted as stimulants, but at high concentrations they were lethal to the worms, and hexachlorethane was believed to have a more pronounced effect than the other drugs tested. Hexachlorethane is also of value for the treatment of fascioliasis in goats[78].

In the ruminant the drug is absorbed from the bowel, passes by the portal blood to the liver, and is excreted in the bile where it exerts its effects on mature or nearly mature flukes. Kaplan and Sakellarious[79] claimed, however, that the drug is effective against immature flukes, but most workers believe that the drug is effective only against mature or nearly mature flukes in the bile ducts and immature migrating flukes are unaffected. But even against adult *F. hepatica*, there is a reservation about the efficacy of the drug. It is now well recognised that where marked cirrhosis exists, hexachlorethane may not be effective against flukes lying in grossly thickened bile ducts[78,80], excretion of the drug being inadequate through the damaged tissues so that some worms escape the anthelmintic effect. In general, though the toxicity of hexachlorethane appears to be low (Olsen[74], Lapage *et al.*[81] and Stauffer *et al.*[80] found hexachlorethane to be well tolerated) nevertheless, toxic manifestations do occur occasionally in ruminants, especially in those on a high protein diet. Intoxication is evidenced by incoordination of gait, drowziness and lack of appetite which generally passes off, but some deaths have been recorded. Southcott[76] reported toxicity in sheep after treatment with doses of 15 to 30 g.; some animals recovered after the parenteral

administration of calcium borogluconate, but there were some deaths amongst animals receiving 30 g. doses. It is interesting that his experimental sheep tolerated carbon tetrachloride without ill-effect. It would seem, therefore, that caution should be exercised before the mass treatment of animals is undertaken since herd idiosyncracies may exist. Care must also be taken when debilitated animals, particularly those with severe liver damage, are treated.

Hexachlorethane has also been investigated for activity against other helminths. Kaplan and Sakellarious[79] claimed that the drug was effective against *Dicrocoelium dendriticum* in sheep, but this does not appear to have been confirmed. Olsen[82] showed that hexachlorethane was effective against adult rumen flukes in cattle and sheep, but Gordon (unpublished) points out that the evaluation of hexachlorethane against immature *Paramphistomum* spp. in the duodenum of cattle is a pressing need, because this stage of the parasite is probably of much greater importance than the adult rumen-inhabiting worms.

Olsen[83] reviewed the literature on the use of hexachlorethane against the nematode parasites of cattle. The drug is useful in the treatment of *H. contortus* infection and, to a lesser extent, against *T. axei*, but it appears to be ineffective against *Ostertagia* spp. and *B. phlebotomum*. Roberts *et al.*[84] found the drug to be of little value in cattle against species of nematodes other than *H. contortus*. On the other hand, Daubney[85] removed nearly 100 per cent. of hookworms from a sheep with hexachlorethane. Olsen and Wade[86] investigated its efficacy against certain nematodes in sheep and goats, and found the drug to be highly effective against *H. contortus* but its efficiency was variable against *Ostertagia* spp. It was ineffective against intestinal trichostrongyles, nodular worms and whipworms. Kingsbury[87], however, has reported that the drug is of some value in the treatment of *Nematodirus* infections in lambs.

Sodium Fluoride

The introduction of sodium fluoride for the treatment of *Ascaris lumbricoides* infection of pigs[88] has simplied the anthelmintic treatment of these animals. It is highly effective, removing over 90 per cent. of ascarids and surpasses oil of chenopodium in efficiency[89]. The drug removes both mature and immature worms. It is also fairly effective against some of the stomach-worms of pigs, for example, *Ascarops* and *Physocephalus*; some reports also indicate therapeutic activity against *Hyostrongylus*[90].

Most authorities agree that the drug should be administered in dry ground feed, either as 1 per cent. of the amount of feed which the pig will consume in 1 day, or the dosage may be computed according to body weight at a dosage rate of 0·1 to 0·15 g. of sodium fluoride/lb. of body weight, this quantity being mixed with the amount of feed normally consumed by the pig in 1 day.

The recommendations as regards dosage usually apply to the commercial grade of sodium fluoride which is 73 per cent. pure[91]. Luke and Gordon[92] have drawn attention to possible dangers in using preparations

with a higher sodium fluoride content such as the technical grade which has a purity of about 98 per cent., and they suggest that some deaths which have occurred after treatment may have resulted from using the purer product at the standard dosage rate.

To ensure safety, the aim should be to allow the pigs to ingest small quantities of the medicated feed over the whole day, and by mixing the drug with dry feed, which is thereby rendered somewhat unpalatable, excessive ingestion is avoided. If, however, sodium fluoride is given to pigs under a wet feeding system, the toxic hazard of the drug is increased because wet medicated feed is more rapidly consumed. It is, therefore, important that the drug should be used only for pigs kept on a dry feeding system. With slight overdosage, pigs generally vomit and the danger of excessive intake is thereby lessened, but with gross overdosage a rapidly fatal hæmorrhagic gastro-enteritis follows. When sodium fluoride is used properly the drug is safe for pigs. It has a distinct advantage over other anthelmintics for these animals because it can be given in medicated feed as a mass treatment. The medicated feed should be supplied to pigs in batches of even size, and it is usual to recommend that not more than eight pigs be included in each group, but to avoid excessive consumption by any 1 or 2 pigs in a particular group it is advisable to limit the number to a maximum of 4.

When Habermann *et al.*[88] tested sodium fluoride against helminths in pigs they also investigated the use of the drug in certain other hosts, and evidence of activity against *Parascaris equorum* in horses was obtained. Critical tests with the drug in horses, made by Todd *et al.*[93] showed that a dose of 2·5 g./100 lb. of body weight, administered in aqueous solution, gave a very high degree of efficiency against mature and immature ascarids, over 99 per cent. of the worms being expelled from the small intestine. The drug was fairly well tolerated and although moderate to severe diarrhœa occurred in some of the animals within 48 hours after treatment, no deaths occurred and recovery soon followed treatment. The authors considered that the efficacy of the drug more than offset the temporary gastro-intestinal disturbance. Indeed, the moderate diarrhœa usually encountered was regarded as salutary in the prevention of possible absorption of toxic materials from disintegrating helminths. The drug surpasses carbon disulphide, the present drug of choice in horses, both in efficiency, since it is effective against immature worms in the gut as well as against sexually mature worms, and in the ease of administration because it can be given without preliminary starvation. It cannot be recommended for therapeutic purposes, however, until further investigations have been made concerning the occasional severe diarrhœa and hæmolytic changes in the blood which sometimes follow its use.

Toluene

This drug was previously tested in dogs by Hall and Wigdor[94] in a limited study. Later Enzie[95] examined the anthelmintic action of toluene together with certain halogen substitution products in the dog. He found that although toluene itself constituted a very effective agent

against ascarids and hookworms, the introduction of halogens usually resulted in a reduction of efficiency especially against hookworms. Enzie and Colglazier[96] recorded the results of more extensive trials and showed that toluene at a dosage rate of 0·1 ml./lb. of body weight after 18 to 24 hours fast was well-tolerated and highly effective against ascarids and hookworm in dogs. The drug proved to be equally effective against these worms in cats. Comparative studies showed that it compared very favourably in efficacy and safety with *n*-butyl chloride. Toluene, being easier to administer, may replace *n*-butyl chloride as an anthelmintic for dogs and cats. The drug is sometimes given together with the cestode anthelmintic, dichlorophen, in the form of a proprietary preparation[97,98].

Several studies have been carried out on the use of toluene against ascarids and *Gastrophilus* larvæ in horses[99,100,101]. Critical tests have shown that the drug is well-tolerated and a high degree of efficacy has been obtained against ascarids, but the results are not uniformly good against bot-fly larvæ. *G. intestinalis* appears to be eliminated more readily than some other species and successful results have been recorded by Graham and Alford[102].

Toluene as an anthelmintic for horses is still in the experimental stage and further work is necessary before it can be recommended for use in routine therapy. It would appear, however, that the drug promises to be a more suitable agent against ascarids than carbon disulphide, the present drug of choice, since it can be administered more readily and fasting of the animal, an important consideration in young stock, is not essential. Moreover, severe gastritis which sometimes occurs under carbon disulphide is not a feature of toluene therapy.

n-BUTYL CHLORIDE

This substance which is widely used in canine practice, especially in America, was examined critically together with other chlorinated alkyl hydrocarbons by Wright and Schaffer[103] who found it to be a very effective ascaricide, removing 98·7 per cent. of ascarids. The drug was 84·3 per cent. effective against hookworms, but its efficacy against whipworms averagered only 18·2 per cent. It was ineffective against tapeworms. The drug was well tolerated. Harwood *et al.*[104] confirmed the high efficiency of the drug against ascarids and hookworm, and reported an efficiency of about 52 per cent. against *Trichuris vulpis*, the whipworm of dogs.

A limited amount of investigation concerning its value in horses has been recorded. Harwood *et al.*[105] tested the drug on 3 horses and found that a dose of 0·1 ml./lb. of body weight removed up to 100 per cent. of small strongyles (cylicostomes), and between 74 and 100 per cent. of large strongyles. Trum[106] found that this dose effectively removed small strongyles and *Oxyuris equi* from one animal.

DIETHYLCARBAMAZINE

This piperazine compound has been extensively tested in the treatment of *Dirofilaria immitis* infection in the dog, in which it is highly effective against microfilariæ, but indifferently active against adult worms.[11]

It is highly effective, however, against ascarids of dogs and cats. Hewitt *et al.*[107] tested the drug against these infections in dogs, and found that single oral doses of 50 mg./kg. were nearly 100 per cent. effective; 2 oral doses of 25 mg./kg. within a 24 hour period removing 100 per cent. of the worms. It was also effective when administered intraperitoneally. The drug was ineffective against hookworms, whipworms and tapeworms. Fasting before treatment and purgation after treatment were unnecessary and the drug was well-tolerated, although vomiting occurred in some animals. Harned *et al.*[108] have shown that dogs will tolerate doses many times those necessary for the removal of ascarids. Kanegis[109] used the anthelmintic for the treatment of cats and kittens in oral doses of 25 mg./lb. of body weight, and confirmed its high efficiency against ascarids. Other reports of successful trials in dogs and cats have been recorded[110].

It has been used, apparently successfully, against *Spirocerca lupi* in the dog[111], and in one case of canine strongyloidiasis[112].

Barium Antimonyl Tartrate

Beach and Stewart[113] described the preparation of this compound. It was first used therapeutically by Wehr *et al.*[114] who showed that, when inhaled as a dust, it was 98 per cent. effective against *S. trachea*, gapeworm infection in chickens. Later, Wehr and Olivier[115] described its use for pheasants and showed that it was as effective in these birds as in chickens. Moynihan and Musfeldt[116] confirmed these results. The drug is effective also against *Syngamus* infection in turkeys.

Arecoline Hydrobromide

Hall and Shillinger[117] and Ross[118] described experiments on the efficiency of arecoline against cestode infection in the alimentary tract of the dog. Batham[119] conducted a more extensive study and provided some interesting information concerning the action of arecoline hydrobromide against tapeworms in the dog. His work showed that the drug given orally was highly effective against *Echinococcus granulosus* and against the larger tænioid cestodes. When the drug was given by subcutaneous injection only a purgative action resulted but when the drug was administered orally both an anthelminitic action and purgation followed. From *in vitro* work with some of the larger tænioid cestodes, he concluded that the drug exerts its anthelmintic action by causing the muscles of the tapeworm to undergo prolonged relaxation so that the hold on the intestine is lost and the detached worms are then expelled by the purgative action of the drug. The high efficiency of arecoline hydrobromide against *E. granulosus* makes the drug a valuable anthelmintic in countries where anti-hydatid measures are vigorously pursued, but it should be appreciated that it may fail to eliminate the cestodes in a small proportion of cases.

Dichlorophen

This compound has been introduced for the treatment of *Tænia* and *Dipylidium* infections in dogs[120]. Its action upon these tapeworms is of particular interest because it causes disintegration of the worm

in situ within the bowel, and the proglottids are usually excreted in an unrecognisable form. The drug is generally well-tolerated by dogs, although vomiting sometimes follows its use. Reports as to its efficiency as a tæniacide are conflicting. Some authors have commented very favourably on its value[120,121], but the experience of others indicates that dichlorophen does not compare in efficiency with arecoline hydrobromide or the arecoline-acetarsol compounds. Dichlorophen appears to be of low efficiency against *E. granulosus*[122]; it therefore does not fulfil the requirements of a routine cestode anthelmintic for hydatid control in dogs.

Harries[123] obtained satisfactory results in the treatment of *Moniezia expansa*. infection in sheep with dichlorophen, but Enzie *et al.*[124] observed that the drug is not as reliable as other available agents for the removal of this tapeworm. Olsen[125] investigated the use of dichlorophen against *Thysanosoma actinioides* infection of the liver of lambs. Variable results were obtained, however, and there was no evidence that treatment was justified because the degree of efficiency of the drug was too low to make its use economical. Allen and Jackson[126] also noted little appreciable effect.

Lead Arsenate

This drug was first reported as a cestode anthelmintic by Harwood and Guthrie[127] who used it successfully to remove tapeworms from chickens, but found that it was too toxic for general use. McCulloch and McCoy[128] showed it to be effective for the treatment of *Moniezia* infection of sheep, and Radeleff[129] reported on its use in lambs, kids and calves, in all of which it proved to be a generally satisfactory agent. Calves sometimes showed evidence of colic, but this was relieved by the administration of castor oil one hour after dosing with the anthelmintic. Habermann and Carlson[130] and Ward and Scales[131] have confirmed the value of the drug against *M. expansa*.

As the tapeworm is generally considered to be virtually harmless to the host, some authorities doubt the necessity for the treatment of this infection in ruminants but Foster and Habermann[132] consider that its removal causes a decided improvement in the health of infected animals. In discussing the merits of the drug, they believe that the drug can be used safely, but they emphasise that our knowledge of the toxicity is incomplete and caution must be exercised especially in pregnant animals. Allen and Jongeling[133] recorded mild enteritis, loss of appetite and one death following treatment. Morgan *et al.*[134] found a dose of 1 g. to be 100 per cent. efficient against *M. expansa* in sheep, while 0·5 g. reduced its efficiency to 62 per cent. They consider that the optimum dose and the possibility of cumulative toxic effects merit further investigation. Lead arsenate is compatible with phenothiazine and the two drugs can be administered together[135]. Despite a high degree of efficiency against *Moniezia*, Habermann and Carlson[130] have shown that lead arsenate is ineffective against the fringed tapeworm of ruminants, *T. actinioides*, which lives in the bile ducts.

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BRITISH PHARMACEUTICAL CONFERENCE OXFORD, 1954

SCIENCE PAPERS AND DISCUSSIONS

(*continued from page 905*)

A METHOD FOR STUDYING PERCUTANEOUS ABSORPTION IN THE RAT

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While considerable progress has been made in the discovery and formulation of new ointment bases, much has still to be learnt of their effects on the skin. An ointment consists of two distinct parts—the base itself and the incorporated medicament. Absorption of the medicament through the skin may or may not be desirable, and it will be influenced by the nature of the base and by the chemical and physical properties of the drug itself.

Many different methods have been described for studying the absorption of drugs applied to the intact skin, but most of them are open to criticism. Their usefulness compared with clinical studies are also doubtful, but they provide valuable information in comparing the relative effectiveness of different ointment bases. *In vitro*[1] methods can be dismissed as being quite useless in providing reliable information on skin absorption. Early methods for studying skin absorption were based on the urinary excretion of some easily recognised substance such as potassium iodide or a salicylate.[2,3,4,5] They cannot be very sensitive and the results are influenced by many extraneous factors. The introduction of radioactive tracer substances, which can be readily detected even in minute quantities, provides a new line of approach with great potentialities.[3] Unfortunately few people have the special equipment and facilities for handling radioactive material. Histological studies[6,7] have given important information on skin penetration, but they do not give a measure of systemic absorption. They have shown the distribution of the materials within the skin structures, and have indicated that the most important route of absorption is through the sebaceous glands.

Pharmacologically active drugs, such as morphine and strychnine, have been applied to the skin of mice and their physiological effects observed.[8] We have devised such a method, based on the action of eserine in depressing the activity of the enzyme cholinesterase in the rat. The rat when injected with acetylcholine secretes opaque reddish brown tears. This phenomenon of chromodacryorrhœa was first described by Freud,[9] and

it was shown that the sensitivity of the rat is increased considerably by eserine.[10] The response has been used for an accurate quantitative assay of anticholinesterase drugs.[11] We have found it to be a convenient method for measuring percutaneous absorption and we are using it to study the absorption of eserine and its salts from various ointment bases. This paper describes the method and some of the results which we have obtained.

Materials

Male albino rats weighing 100 to 200 g. were used, since these have been reported to give the most satisfactory and consistent results. The standard acetylcholine solution was prepared by dissolving 100 mg. of acetylcholine chloride and 100 mg. of KH_2PO_4,$12H_2O$ in 100 ml. of a 0·8 g./100 ml. solution of sodium chloride. This solution has *p*H 4·1 to 4·3 and is stable for several weeks if kept in the refrigerator. Fresh dilutions of this standard solution were prepared in 0·9 per cent. sodium chloride solution each time the injections were made.

Method

The hair was removed over an area of approximately 25 sq. cm. of the skin of the back of the rat, using clippers to avoid trauma of the skin which is common after shaving or using depilatory pastes. The rats were placed into separate tins to prevent contamination by contact with each other. The initial sensitivity of the rats was determined by injecting subcutaneously a dose of 50 μg./100 g. of acetylcholine solution and any rats which produced red tears were regarded as too sensitive and not used in the experiment. A weighed amount of the ointment base (250 mg.), containing a known concentration of eserine, was applied to the whole area of denuded skin and rubbed well in for 30 seconds using the finger enclosed in a rubber finger stall. The rats were then kept under constant observation to prevent them licking their backs. In most experiments 24 rats were used, 3 groups of 4 for the test preparation and 3 groups of 4 for the standard. 3 concentrations of eserine were applied in the ointments, in the same ratio 0·5, 0·25 and 0·125 per cent. Eserine base dissolved in white soft paraffin was used as the standard for comparison in most of the experiments which we have so far carried out on the oily bases. The response to 50 μg. of acetylcholine was measured again 30, 60 and 120 minutes after the inunction. The red tear response was measured by inserting a small piece of filter paper into the corner of each eye and it was convenient to stick these pieces on to a sheet of graph paper before assessing the response obtained. The magnitude of the response was scored as follows[11]:— a very red and copious secretion, 4; a red but not copious secretion, 3; pink or a trace of pink, 2; a slight trace of pink, 1.

Experimental

Sensitivity to acetylcholine. Suitable doses of acetylcholine for the experiments were first determined. Burgen[11] reported 300 μg./100 g. of bodyweight to give a red tear response in normal rats and after eserine

the threshold fell to 35 μg. of acetylcholine or less. We found a dose of 50 μg. of acetylcholine to be most suitable for our experiments. This dose did not give a response in normal rats, except when they were unduly sensitive. If a red tear response was obtained at this dose level in the preliminary test, the rat was not used in the experiment. An occasional rat did not give a red tear response even with large doses of acetylcholine, so all new rats were first tested at a dose level of 300 μg./100 g. and discarded as being insensitive if no response was obtained.

Contamination effects. Absorption of eserine can occur by the oral route due to the rats licking their backs. This could only be prevented by keeping the rats under constant observation during the test. Covering the skin proved impracticable with large numbers of rats, and there was the objection that a rise in skin temperature would occur which would increase the rate of absorption. No really satisfactory way of restraining the rats has been found, and anæsthesia affected the results.

Frequency of injections. Acetylcholine should not be given more frequently than once in 30 minutes. Injections at 30, 60 and 120 minutes after applying the ointment were found to be most satisfactory.

Repeating the experiments. The inhibition of cholinesterase by eserine is reversible and the rats could be used again with weekly intervals between the tests.

Evaluation of the response. The lowering of the blood cholinesterase will be determined by 3 main factors—the concentration of eserine in the ointment base; the time over which the observations are made and the rate of absorption through the skin. A maximal effect is often obtained in 30 minutes with the highest concentration of eserine, but not with the lower concentrations. The responses for each rat were therefore summated over the 2-hour period and these totals used in the final calculations.

Calculation of the results. It was not expected that accurate quantitative assays could be made of all ointment bases. Different bases influence the rate and magnitude of absorption of eserine and the standard and test have not the same constitution. These differences confound the basic principles of biological assay[12] which state that the active principle must be the same in the standard and test and that neither should contain any substance which in any way modifies the behaviour of the active principle. However, some form of statistical evaluation of the results obtained was desirable, and the experiments were designed to enable this to be done.

Table I shows the results obtained in an experiment comparing arachis oil (test) with white soft paraffin (standard). These results have been analysed (Table II) by the usual procedure for a 6-point assay,[13] and the sums of squares between doses subdivided by the use of polynomial coefficients. We may conclude that:—(*a*) there is a significant regression between the red tear response and the log. concentration of eserine in the ointment; (*b*) there is no significant deviation from parallelism between the two separate response lines; and (*c*) the curvature of the combined regression line is not significant and the difference in curvature of the two response lines is not significant.

TABLE I

TOTAL RED TEAR RESPONSE OF RATS

Base	Standard			Test		
	White soft paraffin			Arachis oil		
Concentration of eserine (per cent.)	0·125	0·25	0·50	0·125	0·25	0·5
Response (total red tear secretion per rat)	5	6	8	8	12	10
	10	9	12	4	4	10
	1	6	4	4	4	12
	1	5	8	7	5	12
Dose totals	17	26	32	23	25	44

TABLE II

ANALYSIS OF VARIANCE OF THE DATA OF TABLE I

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	P
Between doses	5	107·7	—		
Difference between preparations	1	12	12	1·37	>0·2
Linear regression	1	81	81	9·27	<0·01
Departure from parallelism	1	2·25	2·25	0·26	>0·2
Curvature of combined curve	1	4·08	4·08	0·47	>0·2
Differences of curvatures	1	8·33	8·33	0·95	>0·2
Within doses (error)	18	157·3	8·74	—	—
Total	23	265			

The test therefore provides a valid assay and calculation of the relative potency of the two preparations gave a ratio of 1 to 1·5. The value of s —the standard deviation of a single observation—was 2·95 and the value for b—the slope of the regression line—was 30. The value for s/b or λ, which is the index of precision, was 0·1, which is high. Calculation gave fiducial limits of error ($P = 0{\cdot}05$) from 45 to 341 per cent. Thus a large number of animals would have to be used to obtain a high degree of precision. Repeating this experiment gave a ratio of standard to test of 1·45 which is in very good agreement with the first result. These experiments suggest that the absorption of eserine base is better from arachis oil than from white soft paraffin, a result which might be expected.

Unfortunately not all experiments have been as successful as these. One particular difficulty has been the occurrence of one or more rats in a group which did not give a red tear response after eserine. This occurred most frequently in the groups receiving the lowest concentration of eserine. One solution is to convert the results to quantal responses and to use the probit transformation, but this was not satisfactory due to a marked deviation from parallelism between the response lines. If the O responses occurred only in the lowest concentration groups these groups could be omitted and the calculations made for a 2 plus 2 dose assay. Another difficulty which sometimes occurred was a significant deviation from parallelism between the standard and test response lines. It occurred most frequently when the standard and test bases were very different from each other. Such tests did not give valid quantitative estimates and no quantitative conclusions could be made.

Results with other bases. Comparisons have been also made of the absorption of eserine base from solutions in white soft paraffin and in lard. Absorption was better from lard, the ratio being 1·5 to 1·0. A direct comparison of the absorption of eserine base from arachis oil and from castor oil showed better absorption to occur from castor oil, the ratio being 1·7 to 1. Investigations on the absorption of eserine and its salts from other ointment bases are in progress.

Conclusions

Experiments we have carried out so far have shown absorption of eserine base to be better from lard, arachis oil and castor oil than from white soft paraffin. Absorption was better from castor oil than from arachis oil.

Summary

1. A method is described for comparing the absorption of eserine from ointment bases.

2. It is based on the potentiating action of eserine on the red tear response of the rat to acetylcholine.

3. Absorption of eserine base was better from lard, arachis oil and castor oil than from white soft paraffin.

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DISCUSSION

The paper was presented by Dr. G. F. Somers.

The Chairman said that Dr. Somers's method was a promising one and would no doubt be applied to the newer ointment bases.

Dr. K. Bullock (Manchester) suggested the alternative method by the manometric Warburg estimation of blood cholinesterases. Also inhibition by eserine was reversible and eserine was slowly destroyed by the cholinesterase. Would it not have been better to have used one of the irreversible anticholinesterases such as the double quaternary compounds or some of the phosphorus compounds, although the latter were difficult to handle?

MR. D. N. GORE (Dorking) suggested the alternative approach of the use of labelled isotopes.

DR. J. G. DARE (Leeds) said that were Dr. Somers to look on his experiment as quantitative pharmacology as distinct from a biological assay his method was quite valid and beyond reproach.

MR. K. L. SMITH (Nottingham) said that he always used the figure b^2/s^2 as the index of precision. The author said that the value s/b was 0·1 "which was high"; did he mean that 0·1 was high or that the precision of the experiment was high? A figure of 0·1 was quite good for a biological assay and for an accuracy of plus or minus 10 per cent. only 100 animals would be needed. He thought that the experiment could properly be considered a valid biological assay as the authors were trying to see whether eserine was absorbed better from one ointment base than from another and theirs was the only method.

DR. SOMERS, in reply, said they intended to use the method for evaluation of the newer ointment bases but they had tried it out first on well-established bases. It would be applied not only to oily bases but also to emulsified bases containing water. Replying to Dr. Bullock, he said that Warburg manometers were expensive and there would be experimental difficulties with the method. Eserine had been used because its action was reversible and the rats could therefore be used again as, after about a fortnight, they redeveloped their cholinesterases. If irreversible compounds, such as "parathion," were used, the rats could not be used again. Radioactive methods offered a promising field, but many people were unable to acquire the special apparatus needed. Radioactive tracers could be used in animals and also clinically. Replying to Mr. Smith, he said that he had always used s/b, which he regarded as the established method. It would not be practicable to use 100 rats in one experiment.

THE APPLICATION OF INFRA-RED SPECTROSCOPY TO PHARMACEUTICAL ANALYSIS

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The problem of finding suitable or more satisfactory methods for the analysis of pharmaceutical preparations is an ever-growing one. The critical purity of many of the raw materials and intermediates, in addition to the examination of the finished pharmaceuticals, presents to the analyst a wide variety of problems. In order to be able to investigate such problems adequately it is essential to have available the necessary equipment for the appropriate and best method of analysis, irrespective of its specialised nature and the initial cost.

Light absorption methods in the ultra-violet and visible regions of the spectrum have proved to be of such great value for routine analytical techniques that no pharmaceutical laboratory is now complete without a spectrophotometer. The increasing complexity and multi-component nature of many medicinal preparations, however, and the need to analyse mixtures of isomers and of compounds which are difficult to separate chemically, are beginning to expose the limitations of the ultra-violet and visible regions. It is for such problems as these that the absorption in the infra-red region is potentially so useful.

The infra-red technique has for many years held an established position in most university and some industrial laboratories for investigating problems of a research nature, but in this country, apart possibly from the petroleum industry, its development for routine analytical work has in the past been proceeding extremely slowly. This is due partly to the fact that only recently has the necessary equipment become sufficiently reliable for routine industrial use on quantitative problems, and partly to the high initial cost of the equipment. The rapidly growing importance of infra-red spectroscopy as an analytical tool, however, is evidenced by the increasing number of industrial laboratories which are now beginning to apply this method of analysis to many of their problems. The field of pharmaceutical analysis, with its complex organic molecules, multi-component mixtures and structural isomers, provides abundant opportunities for the exploitation of this comparatively new technique. Our laboratory has been developing infra-red techniques for problems of a pharmaceutical nature for a little over a year. The wide variety of methods evolved for problems which, if at all, could only be investigated at length and with difficulty by other more established chemical or physical techniques, is sufficient justification for the initial expensive outlay on equipment.

The fact that every organic compound has its own characteristic infra-red spectrum is the basis of infra-red analysis, both qualitative and quantitative. Qualitative problems consist usually of a comparison of spectra, that of the sample with one or more reference spectra of

pure compounds. Such a comparison may be used to identify single compounds, mixtures, impurities present, and to establish or verify molecular structures. It is the usefulness of the technique in the quantitative analysis of mixtures of compounds which are difficult to separate chemically, however, which is rapidly making an infra-red spectrometer such an essential part of any pharmaceutical laboratory. These quantitative problems require an initial period of investigation to establish a procedure and working conditions which can be reproduced each time an analysis is required. Although all the quantitative methods used are based on Beer's Law relating absorbance and concentration, each problem presents its own difficulties and has to be treated entirely independently.

Sample Preparation

Before a spectrum may be recorded, the sample has to be prepared in a suitable manner. For a quick qualitative comparison of almost all solid samples the paraffin mull technique is by far the most popular. As little as 5 mg. of sample is ground with a repurified paraffin oil, and the paste obtained is squeezed evenly between two rock-salt plates. The ease and speed of sample preparation, the sharpness and definition of absorption bands due to the crystalline form of the sample, and apart from four absorption bands, the excellent transparency of the repurified paraffin oil over the 2μ to 15μ region are decided advantages over solution work.

When a solution spectrum is required, many difficulties have in most cases to be overcome. A solvent must be chosen which has little or no absorption in the region where the interest lies, and the sample must be sufficiently soluble in that solvent to give a satisfactory spectrum. If the solubility problem proves difficult, a thicker cell may be used, but as this also increases the solvent absorption, it may then be necessary to increase the amount of energy reaching the detector by widening the slits of the monochromator, with a consequent loss of resolution. Hence in the majority of cases, a compromise has to be reached between choice of solvent, cell thickness and slit width, and this may entail a considerable period of preliminary investigation.

An evaporated film on a rock-salt plate, or a silver chloride plate in the case of water-soluble materials, is sometimes a convenient way of preparing a sample, and a more recent development for solids is the pressed disc technique, where the sample is finely ground and mixed with potassium bromide or potassium chloride powder. When pressed under vacuum a solid and robust glass-like disc is produced[1,2].

Qualitative Analysis

Identification of Compounds. A positive test for the identity of a compound is the specificity of its infra-red spectrum[3]. The 8μ to 15μ region of the spectrum is commonly called the fingerprint region, and the absorption bands here may be considered as characteristic of each particular compound. For comparison purposes, it is necessary to have spectra of pure compounds, and a library of such reference spectra is an essential part of any infra-red laboratory.

For example a synthetic compound was received with a request for its identification as œstrone. Its spectrum was recorded in repurified paraffin oil over the 8μ to 15μ region and was compared with the spectrum of natural œstrone. It was found to be identical and the wavelengths of 14 characteristic absorption bands corresponded as shown in Table I.

TABLE I

ŒSTRONE ABSORPTION BANDS

Wavlength (μ)	
Natural œstrone	Synthetic œstrone
7·80	7·79
8·05	8·04
8·66	8·66
9·20	9·20
9·47	9·47
9·96	9·97
10·39	10·38
10·69	10·69
10·89	10·89
11·17	11·18
11·42	11·44
11·74	11·74
12·21	12·26
12·71	12·71

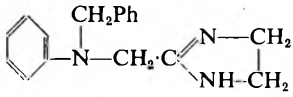
Other keto-steroids and alkaloidal plant extracts have similarly been identified by direct comparison of spectra.

Criteria of Purity. The library of spectra of pure substances may be used to establish the purity or otherwise of a particular unknown sample. The spectrum of a particular sample of *o*-chlorphenol was run over the 8μ to 15μ region and comparison with the spectrum of pure *o*-chlorphenol indicated additional absorptions at 6·67, 9·12, 9·89, 11·73 and 13·89μ. Reference to spectra of other chloro-substituted phenols showed that the 6·67, 9·12 and 9·89μ absorptions were due to *p*-chlorphenol and the 11·73 and 13·89μ absorptions were due to 2 : 4-dichlorphenol which were present as impurities.

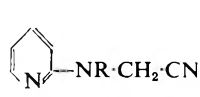
If the impurities in the sample are of too small a concentration to show when a normal spectrum is run, then the technique of recording the spectrum of the sample with the pure substance in the blank beam of a double-beam spectrometer, will in most cases show evidence of any impurities. Concentrations of impurities of as small as 0·05 per cent. of the major constituent have been found, observed and estimated quantitatively by this technique.

Molecular Structure. The immense volume of work which has been carried out in the past by spectroscopists on the correlation of infra-red spectra with molecular structure enables one to determine the presence or otherwise of a particular structural grouping within a molecule[4]. Stretching, bending and rocking vibrations are the most common of the many different modes of vibration which give rise to absorptions in the 2μ to 15μ region of the spectrum. A particular vibration of a specific molecular grouping has an absorption band at a characteristic wavelength, and for work of a research or investigational nature, the interpretation of a spectrum in terms of specific structural groups can often provide most valuable information.

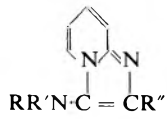
A particular research investigation was originated to prepare analogues of the antihistamine antazoline (I)[5]. The starting point was 2-pyridyl-aminoacetonitrile (II; R = H), which on benzylation in chloroform solution afforded two monobenzyl derivatives, m.pt. 83° to 84° C. and 100° C. respectively, and a dibenzyl derivative, m.pt. 144° C.



(I)



(II)



(III)

The infra-red spectrum of the benzyl derivative, m.pt. 83° to 84° C. showed an absorption band at 4·45μ in chloroform solution, due to the $-C\equiv N$ group, but this band was absent from the spectrum of the benzyl compound, m.pt. 100° C. This was in accordance with the chemical evidence that the monobenzyl compound of lower m.pt. was *N*-benzyl-*N*-2-pyridylaminoacetonitrile (II; R = Ph.CH_2), and that the other monobenzyl derivative, m.pt. 100° C. was 5-benzylaminopyridino(1′:2′-1:2)glyoxaline with the structure (III; R = R″ = H, R′ = Ph.CH_2). This latter possibility arose, as did the dibenzyl derivative, from the capacity of 2-pyridylaminoacetonitrile to react also in the tautomeric form (III; R = R′ = R″ = H). Absence of the $-C\equiv N$ group was likewise indicated for the acetyl derivative of 2-pyridylaminoacetonitrile, 5-acetamidopyridino(1′:2′-1:2)glyoxaline (III; R = R″ = H, R′ = Ac). The presence or absence of NH or NH_2 absorptions at about 2·9μ provided additional evidence that 2-pyridylaminoacetonitrile (II; R = H) can react also as 5-aminopyridino(1′:2′-1:2)glyoxaline (III; R = R′ = R″ = H).

Spectra correlations have also assisted in identifying unknown extracts and in distinguishing between different crystalline forms of the same substance. An investigation into the infra-red spectrum of cortisone acetate succeeded in identifying 5 different crystalline forms. The spectra of 7 samples, A, B, C, D, E, F, G, in chloroform solution were recorded over the 5μ to 15μ region. These solution spectra were identical, and in the double-bond region, the positions of the 4 carbonyl and the C = C stretching vibration wavelengths were in agreement with the results of the work of Jones *et al.* on the side chains of C — 21 steroids[6]. (Table II.)

TABLE II

ABSORPTION BANDS OF CORTISONE ACETATE IN THE 6μ REGION

Functional group	Wavelength (μ)	
	Chloroform solution	Paraffin mull
Acetate C=O	5·73	5·73
20-ketone	5·80	5·80
11-ketone	5·86	5·87
3-ketone	6·01	5·97
		6·05
4–5 C=C	6·19	6·20

However, by grinding the samples with repurified paraffin oil, mulls were obtained and the spectra recorded were then those of the samples in their crystalline forms. In the carbonyl region they all showed 5 C = O bands; the 3-ketone band appeared to have split, and all the bands were very much sharper than the corresponding solution bands. The relative intensities of the 5 bands, however, differed considerably from sample to sample, although F and C were similar, as were also E and B.

The main difference in these solid state spectra was in the relative intensities of the two components of the 3-ketone absorption at 6·05μ and 5·97μ respectively. In the spectra of E and B, these were of approximately equal intensity, in the spectra of F, C and D the 5·97μ absorption was the more intense, the 6·05μ absorption appearing as a strong shoulder on the curve, and in the spectra of G and A the 6·05μ absorption was the stronger of the two, more so for A than for the G sample. (Table III.) Associated with the relative increase of the 6·05μ absorption was a corresponding, although less pronounced, increase in the relative intensities of the 5·73μ and 5·80μ absorptions. All absorptions were measured relative to the 11-ketone absorption at 5·37μ, which was the strongest of the 5 C = O bands.

TABLE III

THE RELATIONSHIP BETWEEN DIFFERENT CRYSTAL MODIFICATIONS OF CORTISONE ACETATE AND THE RATIO OF THE INTENSITIES OF THE TWO COMPONENTS OF THE 3-KETONE ABSORPTION

Sample of cortisone acetate	Ratio of absorbances at 5·97μ and 6·05μ
F	1·61
C	1·59
D	1·44
E	1·01
B	1·01
G	0·87
A	0·62

The differences in relative intensities of the bands suggested strongly the presence of more than one crystal modification, these probably being present in different proportions in different samples. X-ray diffraction patterns provided additional evidence of this, as also did recrystallising experiments, which produced identical modifications and spectra from originally different samples.

Quantitative Analysis

Single Component Analysis. The determination of phenobarbitone provides an example of the simplest form of quantitative analysis. Tablets of an alkaloidal extract (2 mg.) and phenobarbitone (15 mg.) are examined for their phenobarbitone content. Phenobarbitone has two characteristic carbonyl absorption bands at 5·75μ and 5·84μ which occur in the form of a doublet. The procedure is to extract the phenobarbitone, and with it the alkaloidal extract, from the tablets and to compare the absorbance with that of a standard solution of phenobarbitone at 5·75μ. At the concentration used, the alkaloidal extract has no appreciable absorption at this wavelength, and a straight line calibration between absorbance and phenobarbitone concentration over the range 0 to 1 per cent. verifies Beer's law for this particular band. A direct comparison may therefore be made between the absorbances of the sample and the standard.

The standard is prepared by dissolving 75 mg. of phenobarbitone in 10 ml. of chloroform. The tablets are finely ground and the equivalent weight of 5 tablets put into a sintered funnel. 8 × 15 ml. chloroform extracts are drawn through the sinter by suction and taken to dryness. The residue is dissolved in 10 ml. of chloroform. The absorbances of both standard and sample solutions are then measured at 5·75μ, using a 0·15 mm. cell thickness and a slit width of 0·08 mm., and the phenobarbitone content of the sample solution thereby determined. The

average time for a complete phenobarbitone determination is less than one hour, and throughout the past 6 months production batches of these tablets have been examined satisfactorily by this method.

Further examples of this single component analysis are the determination of dieldrin[7] and aldrin[7], both insecticidal materials in agricultural and horticultural preparations. A colorimetric method has been described[8] for determining aldrin which is long and tedious, and which requires care and accurate control of critical conditions during the determination procedure. A hydrogen bromide method for determining dieldrin in formulations is similarly time-consuming, and both methods are susceptible to inteference from extraneous materials. The infra-red technique is specific for both dieldrin and for aldrin, entails a straightforward extraction procedure and a simple comparison determination.

Dieldrin is soluble in carbon disulphide and a record of its spectrum shows a band at 12·37μ suitable for an analysis. The dieldrin is extracted with ether from a sample of the preparation, the extract evaporated to dryness, and the residue dissolved in carbon disulphide. The absorbance of this solution is compared with that of a standard solution of dieldrin at 12·37μ, thereby giving the concentration of the solution and hence the amount of dieldrin in the sample. A similar method is used for aldrin, a characteristic band at 8·47μ being used in this case.

Multi-component Analysis. The simplest technique of multi-component analysis is a direct development of the comparison method described above for dieldrin and for phenobarbitone. Essentially it entails comparing the absorbances of an extract of the components with the absorbances of a multi-component standard. Each component has its characteristic key wavelength at which the other component(s) have little or no absorption. The determination of dieldrin and a commercial product of benzene hexachloride is a straightforward application of this method.

A weighed quantity of sample is shaken with ether, which extracts the dieldrin and the benzene hexachloride. After filtering and evaporating to dryness the residue is dissolved in carbon disulphide. The standard is similarly prepared from weighed amounts of dieldrin and benzene hexachloride. The concentrations of dieldrin and benzene hexachloride in the standard are approximately the same as those in the sample extract. Dieldrin has an absorption band at 9·93μ and benzene hexachloride one at 10·48μ, at each of which the other component has very little absorption. The spectra of both sample and standard solutions were recorded over the wavelength range covering these two key absorption bands using a 1·19 mm. cell and a slit width of 0·20 mm. A direct comparison of absorbances at 9·93μ for the dieldrin and at 10·48μ for the benzene hexachloride gave duplicate and triplicate results on two samples as shown in Table IV.

The alkaloids strychnine and brucine may be determined directly without the necessity of a separation procedure. In the carbonyl region, they both have a strong C = O absorption at 6·06μ, and strychnine has an additional though weaker band at 6·32μ[9]. Working curves were obtained for the absorbances of both alkaloids at 6·06μ, covering the concentration range 0 to 1 per cent., and with chloroform as solvent in a 0·13 mm. cell. They

were also obtained for strychnine as the major component and brucine as the minor component at 6·32μ, over the same concentration range 0 to 1 per cent., but using thicker cells, 1·19 mm. A base-line method[10,11] was used for measuring the absorbances at 6·32μ because of interference from the stronger absorptions at 6·06μ. At both wavelengths the curves obtained approximated very closely to straight lines, indicating the validity of Beer's law for the absorption bands used.

TABLE IV

RESULTS OF DIELDRIN AND BENZENE HEXACHLORIDE DETERMINATIONS

Sample	Dieldrin, per cent.	Benzene hexachloride, per cent.
1	12·69 12·55	8·85 8·84
2	12·55 12·50 12·48	8·39 8·65 8·63

Synthetic mixtures were then prepared and the absorbances of each measured at the two key wavelengths, 6·06μ and 6·32μ, using the appropriate cells. A first approximation was obtained for the strychnine content using the working curve for 6·32μ. Passing to the working curves for the 6·06μ absorptions, the absorbance due to this amount of strychnine was obtained and subtracted from the absorbance of the mixture at this wavelength. This gave the absorbance of the brucine and a first approximation of the brucine content was then obtained from its working curve at 6·06μ. This enabled a correction and a second approximation to be obtained of the strychnine content and so on to a second approximation of the brucine content. The results on 4 synthetic mixtures were as in Table V.

TABLE V

RESULTS OF STRYCHNINE AND BRUCINE DETERMINATIONS

Mixture number	Concentration as per cent. w/v in chloroform			
	Strychnine		Brucine	
	Taken	Found	Taken	Found
1	0·51	0·50	0·50	0·50
2	0·41	0·39	0·61	0·61
3	0·26	0·26	0·25	0·26
4	0·31	0·30	0·20	0·22

The increasing tendency to combine 2 or more drugs in medicinal preparations has complicated analytical methods and provided an excellent opening for infra-red techniques. The determination of aminophylline and phenobarbitone in tablets is an example of this. Various methods for determining the 2 compounds individually are reviewed in a paper by Bartilucci and Discher[12], and preliminary investigations into a potentiometric titration method for determining both compounds in a mixture are described. The results obtained were not considered satisfactory, although further work was in progress. The infra-red method, besides being specific for aminophylline and for phenobarbitone is simple and time-saving.

Aminophylline B.P. contains between 75·0 per cent. and 82·0 per cent. of anhydrous theophylline and between 12·3 per cent. and 13·3 per cent. of ethylenediamine, and it is the infra-red absorption of the theophylline, together with that of the phenobarbitone, which is made use of in the analysis. Theophylline has 2 characteristic absorption bands at 5·87μ and 6·03μ, and phenobarbitone two at 5·75μ and 5·84μ. The theophylline and the phenobarbitone are both extracted from the tablets with a 3 : 1 mixture

of chloroform and *iso*propanol, and are estimated by comparing the absorption of the extract with that of a standard. The standard is itself an extract from a mixture of phenobarbitone and that batch of aminophylline which was used in the tablet manufacture. Because of the relatively small concentration of phenobarbitone in the particular tablets for which this determination was designed, each substance has to be estimated separately. The spectra of the sample and standard extracts in chloroform solution are each run in cells of thickness 0·15 mm., against a blank of chloroform. Comparison of the spectra at 6·03μ gives an estimation of the aminophylline content, the phenobarbitone concentration being insufficient to interfere at this cell thickness and at this wavelength. The spectra of the two extracts are then run in thicker cells, 1·21 mm., against a standard blank of theophylline. This blank balances out the strong theophylline absorption, leaving the 5·75μ phenobarbitone band of sufficient intensity to permit a direct comparison. Because of the neighbouring strong theophylline absorption at 5·87μ, the light transmission at the other phenobarbitone wavelength of 5·84μ is too small to permit a further comparison check.

The equivalent weight of one tablet, finely-ground, is weighed into a sintered funnel and extracted with 8 quantities, each of 15 ml., of a 3 : 1 mixture of chloroform and *iso*propanol. The combined extracts are taken to dryness and the residue dissolved in 50 ml. of chloroform. The standard is prepared by shaking for 30 minutes 170 mg. of aminophylline and 8 mg. of phenobarbitone with 50 ml. of chloroform, and then filtering. To prepare the blank, 170 mg. of aminophylline is shaken with 50 ml. of chloroform for 30 minutes and filtered. The absorbances of both sample and standard solution are determined at 6·03μ using a 0·15 mm. cell with a slit width of 0·08 mm. Using a 1·21 mm. cell and having the theophylline blank in the blank beam, the absorbances of both sample and standard solutions are then determined at 5·75μ with the same slit conditions. A direct comparison of absorbances then gives the concentrations and hence the amounts of phenobarbitone and aminophylline in the tablets. A batch of tablets was examined as above with the following results:—

Aminophylline	173 mg. ≡ 2·67 grains/tab.
Phenobarbitone	7·7 mg. ≡ 0·12 grains/tab.
Total ..	2·79 grains/tab.

This is to be compared with a chemical assay result of 2·65 grains/tab. for the combined aminophylline and phenobarbitone content, in which the B.P. extraction procedure described for theophylline was used.

Major Component Compensation. This technique is most useful when one is more interested in estimating the amounts of constituents of the order of 1 per cent., such as impurities, than in estimating the major components. An example of this was the estimation of impurities present in a sample of γ-picoline, reputed to be about 98 per cent. pure. The method of Coulson and Hales[13] for the determination of β-picoline, γ-picoline,

2:6-lutidine and 2-ethylpyridine in mixtures appears to be quite satisfactory for mixtures containing the different components in amounts varying from 5 per cent, to 90 per cent., but the small absorptions of the impurities, when these are present in amounts less than 1 per cent., are completely masked by the intense absorption of the γ-picoline, and a different technique has to be used. As in the qualitative identification of impurities already referred to, a solution of pure γ-picoline is used in the blank cell of the double-beam spectrometer to balance out this intense absorption, thereby uncovering the comparatively weak absorptions of the impurities. This technique also makes it possible to use stronger concentrations of sample so that the absorption of the impurities are sufficiently intense to permit their accurate measurement.

Using highly purified samples of the impurities α-picoline, β-picoline, 2:6-lutidine and *o*-xylene, obtained from the Chemical Research Laboratory, Teddington, working curves for each were prepared in *cyclo*hexane solution at their respective characteristic wavelengths: α-picoline, 13·38μ; β-picoline, 14·09μ; 2:6-lutidine 12·99μ; *o*-xylene 13·49μ. Each absorption band is at a wavelength where there is little or no interference from the absorptions of the other 3 components, providing all are of the same order of concentration. The spectrum of a solution of the sample in *cyclo*hexane against a solution of pure γ-picoline is then recorded, and the absorbances and concentrations of the 4 impurities are measured and calculated. Two samples were examined in triplicate with the results as shown in Table VI.

TABLE VI

RESULTS OF DETERMINATIONS OF IMPURITIES IN γ-PICOLINE

Sample	Impurities, per cent.				
	α-picoline	β-picoline	2:6-lutidine	*o*-xylene	Total
1	0·45	0·05	0·37	0·29	1·16
	0·43	0·05	0·37	0·27	1·12
	0·41	0·05	0·36	0·28	1·10
2	0·56	0·04	0·38	0·29	1·27
	0·58	0·06	0·37	0·30	1·31
	0·57	0·10	0·41	0·30	1·38

Solutions of compounds in oils from which they cannot be easily extracted provide a further example of an application of this method. Injection of testosterone propionate is a 10 mg./ml. solution of testosterone propionate in arachis oil. An infra-red technique enables a determination of the testosterone propionate to be made with much greater certainty than is possible by the ultra-violet method, which invariably gives rise to inaccuracies due to the strong absorption of arachis oil in the region of the testosterone propionate maximum at about 230 mμ, and the consequent low intensity of transmitted light by the arachis oil solution. Testosterone propionate has a characteristic infra-red absorption band at 5·95μ, and the absorbance of the sample at this wavelength is compared with that of a standard solution of testosterone propionate in arachis oil. Arachis oil has

a C = O absorption at about 5·75μ, and the 5·95μ testosterone propionate absorption falls on the side of this band. At a concentration of 10 mg./ml. it occurs as a shoulder, but by putting arachis oil of the same batch as that used in the manufacture of the injection in the blank beam, the C = O absorption is balanced out, leaving the testosterone band resolved and of sufficient intensity to permit a direct quantitative measurement.

The standard is prepared by weighing 100 mg. of testosterone propionate and dissolving in 10 ml. of arachis oil. Gentle heating is required. The testosterone propionate and arachis oil should be of the same batch as those used in making up the injection. The absorbances of both standard and sample are then determined at 5·95μ, using a 0·15 mm. cell and having arachis oil in the blank beam. A constant slit width of 0·14 mm. is used.

To ensure that a direct comparison between the absorbance of standard and sample may be permitted, i.e., to verify that Beer's law is obeyed by this particular absorption band, standards of 5, 10 and 15 mg./ml. were made up and their absorbances obtained as above. A plot of absorbance against concentration gave a curve approximating very closely to a straight line. A sample was examined as above in triplicate with the following results:—10·2, 10·0, 10·1 mg./ml. testosterone propionate (theory 10·0 mg./ml.).

Summary

1. A general outline of infra-red techniques is presented for the purpose of indicating the potential range of applications in the routine examination of pharmaceutical materials.

2. Examples of qualitative investigations include the identification of compounds, the observation of impurities and spectra-structure correlations.

3. Quantitative analyses of single compounds and of multi-component mixtures are described which are impossible, more difficult or time-consuming to perform by chemical or other physical methods.

4. Advantages of the analytical techniques described are the ease of sample preparation, the straightforward nature of extraction procedures, and the fundamental fact that every organic compound has its own specific infra-red spectrum.

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DISCUSSION

The paper was presented by DR. D. C. GARRATT.

The CHAIRMAN asked the cost of the apparatus and was informed that it was between £3000 and £4000, which Dr. Garratt claimed was not expensive when one considered the resulting saving of labour.

DR. F. HARTLEY (London) said that Dr. Garratt had rightly pointed out that the capital cost ought not to be the paramount consideration; rather, it ought to be the saving which might ultimately be achieved. Unfortunately, there were always "teething" troubles with new equipment. In his reference to the examination of steroid hormones in oily solutions the only example given was a solution containing 10 mg./ml. He understood that the British instruments available normally required about 10 mg. of material for a successful examination. Micromethods were available in American instruments. Having regard to the low concentrations of testosterone which were often used did the authors consider that they could get an accuracy of $\pm$ 5 to 10 per cent. when dealing with solutions of a strength of 2 or 5 mg./ml.?

DR. W. MITCHELL (London) asked if the authors had any experience of the analysis of essential oils by infra-red spectroscopy. Could the method pick out the numerous individual components in an essential oil or did overlapping of bands tend to make analysis impossible? Could infra-red spectroscopy distinguish between *Mentha piperita* and *M. arvensis* oils; if so, could it detect the latter as an adulterant in the former?

DR. R. E. STUCKEY (London) asked for further details of the type of instrument used. When the instrument was properly adjusted did it continue to work satisfactorily for prolonged periods without readjustment? In the estimation of testosterone in arachis oil could the authors give the relative values for the absorption of the oil and the dissolved testosterone at different concentrations?

MR. S. G. E. STEVENS (London) said it was interesting to learn that the instrument was being used for quantitative work. Could the quantitative application of the infra-red spectrometer be used for complex preparations containing two or more compounds with closely related chemical structures?

MR. P. G. MARSHALL, in reply, said the steroid solution submitted for analysis contained approximately 10 mg./ml. and they based their analysis on concentrations of that order, but he did not see why similar orders of accuracy should not be obtained with lower concentrations by using thicker cells. Replying to Dr. Stuckey, he said that they had used a Hilger double beam instrument. The arachis oil absorption was larger than that of the testosterone propionate, but that was immaterial in that the absorption was balanced out. Tests on different concentrations in the arachis oil gave a good straight line result relating absorption to concentration; and the range was large enough to give an accuracy of $\pm$ 1 per cent. As for distinguishing between essential oils, the advantage of the infra-red spectrometer was that the absorption was specific for a particular material,

and with a mixture depending on the relative concentrations the different oils could be detected. Regarding complex mixtures, he said that whether the different materials present could be distinguished depended on the relative amounts present. Their instrument had been in operation for 18 months. For 3 or 4 months they had had trouble setting it up, but since then there had been no difficulty, and for the past year there had been no need to re-set any of the controls. Reproducibility of results from day to day was good, although for work of the highest accuracy it was advisable to use standards rather than to compare an absorption with another made the previous month.

DR. GARRATT said that work on essential oils of the kind suggested by Dr. Mitchell would take a considerable time.

THE LEUCOCYTE RESPONSE IN THE RABBIT TO PYROGEN FROM *PROTEUS VULGARIS*

PART II. NEUTROPHIL AND TEMPERATURE RESPONSES

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Received July 16, 1954

THE changes which occur among the neutrophils in the circulating blood of rabbits after the intravenous injection of pyrogen were considered worthy of investigation because there is evidence suggesting a relationship between these changes and the occurrence of fever as a result of a pyrogenic stimulus[1]. Several authors use[2], or have suggested the use[3] of, the alteration in the total number of circulating leucocytes as an index of pyrogenic activity. We have found this index unreliable in rabbits, and therefore decided to trace the changes occurring in the overall lobar configuration presented by the nuclei of the circulating rabbit neutrophils subsequent to the intravenous injection of pyrogen, and investigate its merits and potentialities as an index of activity. Previous reports of the effect of pyrogen on neutrophils are concerned with alterations in total numbers, some including comment upon the obvious addition of young neutrophils with unsegmented nuclei, others describing a "shift to the left," but none reports any attempt to measure this shift.

This report describes an endeavour to measure this "shift to the left" which occurs in rabbits after the intravenous injection of pyrogen, by calculating the percentage fall in the average number of lobes per neutrophil, and to compare this measurement as a possible index of pyrogenic activity with measurement of the average maximal rise of temperature in rabbits.

MATERIALS AND METHODS

The pyrogen preparation used was the dialysed freeze-dried sterile supernatant liquid from a culture of *Proteus vulgaris* grown in a simple glucose-ammonium-salt medium containing nicotinic acid (2×10^{-5}M). Its chemical simplicity and the fact that it can be prepared pyrogen-free commended this medium as an appropriate choice for *Proteus vulgaris*. The results of repeated tests before and after the investigation have satisfied us that this pyrogen preparation maintained its potency unaltered throughout the tests. The medium could not, in itself, be shown to have any detectable effect on temperature or white blood cells in the rabbit, nor indeed to produce any toxic effects after repeated intravenous injection under the conditions of the test.

In the investigation 4 dose-levels were used:—

	Dose	Log dose
A	0·02 ml./kg.	$\bar{2}$·3010
B	0·06324 „	$\bar{2}$·8010
C	0·1125 „	$\bar{1}$·0510
D	0·2 „	$\bar{1}$·3010

It will be seen that log B is equally spaced between log A and log D and that log C is equidistant from log B and log D. D was chosen as the highest dose because it elicited temperature and white blood-cell responses equivalent to about 75 per cent. of the maximal responses which, in our experience, can be elicited by this pyrogenic preparation under these conditions of experiment. The freeze-dried material was dissolved in apyrogenic saline immediately prior to injection. The dilution was such that each dose of original dialysed supernatant (e.g., 0·2 ml./kg., etc.) was contained in 2 ml., so that no matter what the dose, each animal received 2 ml./kg. of solution.

30 previously unused rabbits of both sexes were employed in the investigation and were divided into 6 groups of 5, the members within each group having similar normal values for the average number of lobes per neutrophil. Each animal received one injection per week over 12 weeks and 15 rabbits (3 groups) were used on each occasion; they were denied food during the 36 hours preceding the test. Environmental temperature was maintained at a reasonably constant level throughout.

The technique used in the investigation of temperature response was essentially that developed by Wylie and Todd[4], with certain minor alterations and refinements. This method where each rabbit is accommodated in a specially designed box, provides maximum comfort for the animals and combines with that essential, minimum opportunity for unnecessary and undesirable movement.

Smears of circulating blood were prepared from each rabbit immediately prior to, and about $3\frac{1}{2}$ hours after, injection of pyrogen, and stained with Giemsa's Stain. We have found this stain satisfactory if used in a dilution of 1 in 10 and applied after fixing the rapidly dried smear for 3 minutes in pure methanol. Polynuclear and differential counts were performed on each smear. In performing the polynuclear count we adopted the grouping suggested by Cooke and Ponder[5] in their practical modification of the count introduced by Arneth, and the average number of lobes per neutrophil over 100 neutrophils was calculated for each rabbit (described by these authors as the "weighted mean"). These suggestions of Cooke and Ponder were adopted because they lead to a rapid and sensitive assessment of the overall nuclear picture of the neutrophils.

Before commencing the investigation the rabbits were conditioned to the procedure to be adopted in the tests and normal values were worked out from smears taken during this conditioning period under the experimental conditions prevailing during the subsequent tests, i.e., with thermocouples inserted. We found that this approximately constant degree of stress to which they were subjected did not deflect the count or upset the rectal temperature of the animals beyond normal limits.

Normal values. Average number of lobes per neutophil:—Mean value for 30 rabbits, 2·30; Coefficient of variation = 10 per cent.

It was found that some animals gave consistently lower values than others, e.g., the group of 5 Beveren rabbits gave a mean normal value of 2·13, whereas the 5 Dutch gave a mean normal value of 2·59. The means for the other 4 groups lay between the values for the 2 groups cited. It

was also found that within the range of these normal values, percentage changes in the average number of lobes per neutrophil, observed for each breed after the intravenous injection of any one dose-level of pyrogen, did not differ significantly.

The error observed in counting one smear several times was shown to be less than that involved in counting smears prepared from the blood of one rabbit at different times (weekly intervals). Both of these errors in turn were less than the difference between counts performed on smears prepared before and after injection of the smallest dose-level of pyrogen used in the investigation.

Results

Total white cell counts were not performed at each test because of the time-consuming technique, especially when 15 animals were being used at once, and when the information provided by them was not required in the calculation of change occurring in the average number of lobes per neutrophil. Several were made, however, and the results obtained are recorded in Table I. The post-injection counts were made between 3 and 4 hours after injection of pyrogen.

TABLE I

INCREASES IN TOTAL NUMBERS OF LEUCOCYTES IN RABBITS, 3 TO 4 HOURS AFTER INJECTION OF VARIOUS DOSE-LEVELS OF PYROGEN. TEMPERATURE RESPONSES ARE ALSO GIVEN

Dose, ml./kg.	Increase in white blood cells/cu. mm.	Rise in rectal temperature, °C.
0·2	14,400	1·22
	14,800	1·57
	6,600	1·30
0·1125	7,200	1·17
	6,400	1·49
	13,200	1·10
0·05324	3,000	0·77
	8,000	1·18
	13,000	0·81
0·02	1,400	0·34
	—	0·95
	800	0·45

Preliminary investigation showed that maximal deflection of the average number of lobes per neutrophil could be expected to occur between 3 and 4 hours after injection of pyrogen. Then, after a period of instability, the count commenced an upward return to normal and attained a steady pre-injection level about 4 to 6 days later (see Fig. 1).

Our end-point was therefore the value given by a smear taken about 3½ hours after injection. We have measured the response as the percentage fall in the average number of lobes per neutrophil, i.e., the difference between pre- and post-injection readings expressed as a percentage of the pre-injection reading. The deflection of the count can be shown to be due to the addition of 1- and 2-lobed neutrophils—mostly 1-lobed—to the circulating blood giving as a result, a lower value for the average number of lobes per neutrophil.

It was observed that, when a double-peaked temperature response occurred, it was advisable to wait until after the second peak had passed before removing the sample of blood, otherwise the smear contained very few white blood cells (about 200 or less white blood cells in one smear—which made counting extremely tedious and difficult—as opposed to 60 to 80 per strip of smear in one taken after the second peak). When the biphasic temperature response occurred, the second peak appeared about 3 hours

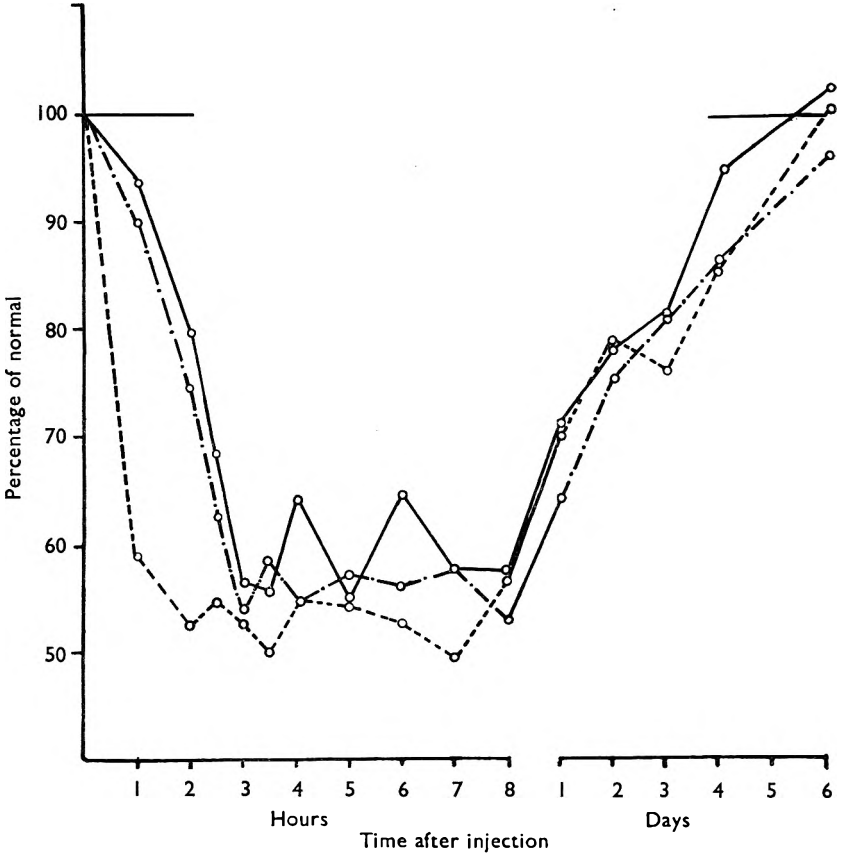

FIG. 1. Graph showing depression in average number of lobes per neutrophil after injection of pyrogen (0·2 ml./kg.) and the course of return to normality.

○——○ Rabbit number 1
○—·—○ ,, ,, 4
○ - - ○ ,, ,, 5

after injection. After the second peak had passed, an abundance of young neutrophils made their appearance.

COMPARISONS OF INDICES OF PYROGENIC ACTIVITY INVESTIGATED

(See Tables II, III and IV and Figures 2, 3 and 4)

The results for each dose level are means of 90 readings (i.e., 360 experiments) and in each case the means can be shown to differ significantly from each other. Significant correlation was found to exist between each of the 3 different indices and the logarithms of the doses and also between temperature response and both white blood-cell responses. Analyses of variances showed that, in the case of all 3 indices, between-rabbit variance exists significantly. The comparison of different pyrogenic preparations would therefore be more accurate if the same rabbits were used, provided

they are not used often enough or within close enough intervals to establish a significant degree of tolerance.

Inspection of the regression lines suggests that, to a first approximation, straight lines fit the 3 sets of data. Analyses of variances to check linearity give the following values for F ($n_1 = 2$, $n_2 = 356$):—small lymphocyte percentage fall:—2·53; percentage fall in average number of lobes per neutrophil:—3·46; average maximum rise in temperature:—6·2. It is seen that 2·53 is just below the 5 per cent. probability level, 3·46 is just above it and 6·2 between the 1 per cent. and 0·1 per cent. levels.

TABLE II

TEMPERATURE RESPONSE IN THE RABBIT TO VARIOUS DOSE-LEVELS OF PYROGEN

Dose, ml./kg.	Average maximal rise in rectal temperature	
	Mean °C.	Coefficient of variation per cent.
0·2	1·38	21
0·1125	1·25	21
0·06324	0·95	30
0·02	0·61	36

TABLE III

LEUCOCYTE RESPONSE IN THE RABBIT TO VARIOUS DOSE-LEVELS OF PYROGEN PERCENTAGE FALL IN AVERAGE NUMBER OF LOBES PER NEUTROPHIL

Dose, ml./kg.	Percentage fall in average number of lobes per neutrophil	
	Mean per cent.	Coefficient of variation, per cent.
0·2	35·6	22
0·1125	29·8	28
0·06324	25·2	33
0·02	16·8	48

TABLE IV

RESPONSE IN THE RABBIT TO VARIOUS DOSE-LEVELS OF PYROGEN SMALL LYMPHOCYTE PERCENTAGE FALL (6)

Dose, ml./kg.	Small lymphocyte percentage fall	
	Mean	Coefficient of variation, per cent.
0·2	56·5	27
0·1125	49·8	39
0·06324	44·8	41
0·02	27·0	62

The ratio of the standard deviation of the scatter about the regression line to the difference between the means for maximum and minimum doses was calculated for each index of activity. This confirmed the impression given by the coefficients of variation that the order of accuracy for the 3 indices is: rise in temperature, percentage fall in the average number of lobes per neutrophil, and small lymphocyte percentage fall.

TOLERANCE

It was observed after the investigation had terminated that 8 of the population of 30 rabbits were beginning to show diminished responses to the highest dose level of pyrogen. These responses differed significantly from those elicited by the same dose at the end of the test. Rabbits which had been rested for 3 months gave the expected response. Advantage was taken of this condition of incipient tolerance in the 8 rabbits and their sera were tested for antibodies using the white-ring precipitation test. The presence of antibodies to our pyrogen could not be demonstrated in any of the 8 sera. We conclude therefore that for this level of tolerance the presence of antibodies to the pyrogen is not essential.

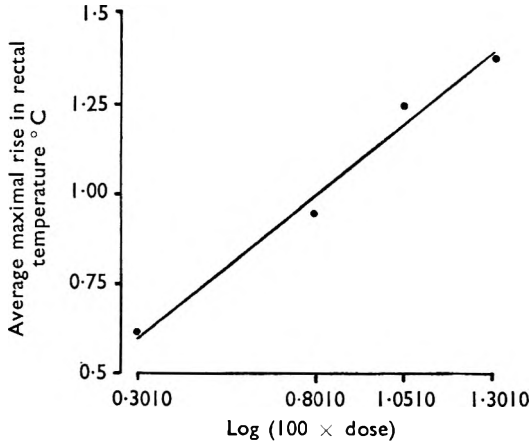

FIG. 2. Log dose response curve for pyrogen. Temperature response. Each experimentally determined point represents the mean of 90 observations.

$$Y = 0{\cdot}36 + 0{\cdot}79x \qquad \sigma_r = 0{\cdot}27$$

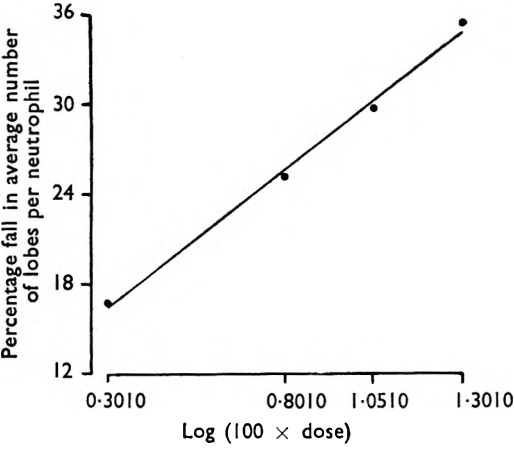

FIG. 3. Log dose response curve for pyrogen. Response measured as percentage fall in average number of lobes per neutrophil. Each experimentally determined point represents the means of 90 observations.

$$Y = 12{\cdot}83 + 18{\cdot}33x \qquad \sigma_r = 8{\cdot}24$$

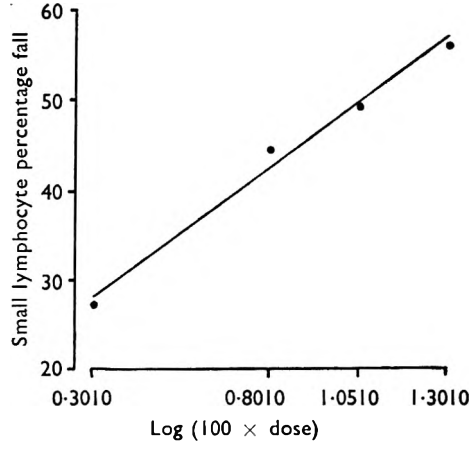

FIG. 4. Log dose response curve for pyrogen. Response measured as small lymphocyte percentage fall. Each experimentally determined point represents the mean of 90 observations.

$$Y = 19{\cdot}46 + 29{\cdot}03x \qquad \sigma_r = 17{\cdot}6$$

DISCUSSION

In the light of recent views[7,8] which have cast doubt upon the production of temperature-rise as a direct effect of pyrogen, it seems reasonable to attempt a measurement of response using an index based on an effect which, in view of these doubts, is not less likely to be attributable, directly or indirectly, to pyrogen. It appears from our results that in estimation of

pyrogenic effect, measurement of the average maximum temperature rise in rabbits affords accuracy not exceeded by any other method of estimation hitherto investigated, but, on the other hand, our results also suggest that an alternative method possessing an almost equivalent degree of accuracy exists in measuring the left-handed deflection of the polynuclear count, by computing the percentage fall in the average number of lobes per neutrophil which occurs in rabbits about 3½ hours after injection of pyrogen.

The active material is admittedly impure, as are most other preparations in use at the present time, and the extent of impurity is unknown, but we offer the fact that there is correlation of the 3 effects between each other over 4 dose-levels, as evidence that these effects derive from the injection of pyrogen. Furthermore, in preliminary investigations using a more highly purified pyrogen from *Proteus vulgaris* (and one from *E. coli*) we have observed that all 3 responses are equally evident after injection.

Summary

1. The effect of 4 dose-levels of pyrogen from *Proteus vulgaris* on the polynuclear count in rabbits has been investigated quantitatively and the effect measured as the percentage fall in the average number of lobes per neutrophil.
2. The simultaneous effect of pyrogen on temperature and on the percentage of small lymphocytes was also recorded.
3. Correlation was found between the 3 effects.
4. The merits of each of these 3 indices of pyrogenic activity have been assessed comparatively.
5. The presence of antibodies to pyrogen could not be demonstrated in rabbits which showed a small degree of tolerance.

It gives us pleasure to express our indebtedness to Miss Anne C. Inglis, B.Sc., M.P.S., for her patient assistance in performing many of the counts and to Mr. J. C. Eaton, M.A., for advice on the statistical analysis of the results.

One of us (W. A.) thanks the Council of the Pharmaceutical Society for the provision of an Educational Grant during the tenure of which this work is being carried out.

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DISCUSSION

The paper was presented by MR. W. ANDERSON.

DR. J. G. DARE (Leeds) said that this was the third paper by Professor Todd and his colleagues in which they had discussed the small lymphocyte percentage fall as a measure of pyrogenic activity. In the first paper they

had found no correlation between temperature and percentage lymphocyte fall; in the second paper they had found partial correlation, and in the present paper complete correlation. No reference was made in the paper to the different results obtained previously. Although the paper dealt mainly with a different index, in view of the discrepancy in the results with the other index the authors should offer a rational explanation as to why the present findings for this correlation should be accepted. In temperature response one often found double peaks; and in looking for the maximum response one must take the higher figure. The frequency of the occurrence of this double peak with *Proteus vulgaris* was related to the dose. Did the authors find a higher incidence of double peaks as the dose increased or was the incidence randomly scattered throughout the doses?

DR. G. E. FOSTER (Dartford) raised the question of the detection of pyrogens in antipyretic drugs, for which the method given in the paper might be useful. It would be interesting if some of Professor Todd's pyrogen could be mixed with such a drug to see whether the pyrogen could be detected by its effect on the white cells.

MR. T. D. WHITTET (London) said that at the Symposium held the previous December it was stated that calcium gluconate caused a temperature fall. This had been confirmed. He had found that chlorpromazine would completely abolish the pyrogenic response. Was the blood picture affected by chlorpromazine? Referring to Menkin's pyrexin, he said that, in America, strongly pyrogenic tissue exudate had been produced, free from bacterial pyrogen. Did the same effects occur with such substances as pyrexin and the leucocyte extract of Bennett?

MR. A. BRAGG (Liverpool) asked the age of the culture used for the preparation of the freeze-dried material. What were the time and temperature factors involved in the freeze-drying treatment?

MR. ANDERSON, in reply, said the object of the paper was to investigate the effect on neutrophils and had the authors wished to hide their previous conclusions they would not have referred to the small lymphocytes at all. A careful inspection of the various relationships between the three indices would show that what had changed was the temperature response. An improvement in the temperature response had resulted in correlation being established between temperature and small lymphocyte percentage fall. Replying about the double peaks, he said that in calculating the results he had used the maximum temperature attained, as shown in the tables. He agreed that the highest dose level of pyrogen elicited the greatest number of double peaks. The smallest dose gave an incidence of 8 per cent. of double peaks, the middle two doses gave 35 per cent. and the highest dose gave 28 per cent. This last result was surprising, and they concluded that the slightly smaller incidence at the highest dose level was probably due to the fact that the first peak was so high that there was then a flattening-out effect. They had visualised the admixture of their pyrogen with several types of drugs, notably those which themselves produced a white cell effect and those which produced a temperature effect. It had been reported that chlorpromazine produced an effect on white cells. In

regard to the age of the culture, he said that the organism was grown for three days at 37° C. in the medium stated.

PROFESSOR J. P. TODD, in reply, commenting on Dr. Dare's references to their previous work, said that Mr. Anderson had employed the term correlation with a different meaning from that used by Miss Dawson in one of the earlier papers.

THE PHOTOMETRIC DETERMINATION OF QUATERNARY AMMONIUM SALTS AND OF CERTAIN AMINES BY COMPOUND FORMATION WITH INDICATORS

PART I. QUATERNARY AMMONIUM SALTS

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INTRODUCTION

METHODS available for the determination of quaternary ammonium salts have been reviewed by Dubois[1,2] and by others[3,4]. The now well-known reineckate, ferricyanide and indicator extraction methods have been adopted by standardising bodies such as the Association of Official and Agricultural Chemists of U.S.A. and the various pharmacopœia authorities. In the British Pharmacopœia the ferricyanide method is specified for cetrimide and the reineckate method for decamethonium iodide. The indicator extraction method has the advantages of high sensitivity, rapidity and partial selectivity and appeared to be of potential value for application to pharmaceutical preparations. Considerable attention has been given to this method in the literature, but very varying conditions have been laid down by different workers (Table I).

TABLE I

EXTRACTION METHOD—DIFFERENT CONDITIONS USED BY DIFFERENT WORKERS

Reference	Indicator	*p*H	Solvent	Method of clarifying	Applicability
7	Bromophenol blue	11·5	Ethylene dichloride	Anhydrous sodium sulphate	$R_1R_2R_3R_4NX$ where R = $C_6H_5CH_2$– or C_4H_9 or derivative thereof
8	,,	,,	Benzene	Centrifuging	,, ,,
9	Bromocresol purple	7·0	Chloroform	,,	Tetraethylammonium bromide
10	Bromophenol blue	—	Ethylene dichloride	,,	Hexamethonium and tetraethylammonium bromides
11	Bromothymol blue	8·4	Chloroform	Filter paper	Quaternary ammonium and amine salts
12	,,	7·6	,,	Centrifuging	Hexamethonium bromide and decamethonium iodide

REVIEW OF THE LITERATURE

Methods for the determination of amine and quaternary ammonium salts involving the formation of salts or complexes with indicators are of 4 types. The first and most widely applicable type involves extraction and is based on Prudhomme's[5] discovery in 1938 that alkaloids form chloroform-soluble complexes with acid dyes such as eosin, the reaction being

quantitative.[6] Auerbach[7] in 1943 described a method using bromophenol blue which is applicable to many quarternary ammonium salts, but not to amines. In this method, as first described by Auerbach,[7] a buffered solution of the quaternary ammonium compound and bromophenol blue, is extracted with ethylene dichloride to give a coloured solution of the complex which is clarified by a drying agent or by centrifuging[8] before final measurement of its optical density. Other workers since have varied indicator, *p*H, concentrations of reagents, solvent and method of clarifying (Table I) but the reasons underlying the selection of working conditions are not always clear.

Extraction methods employing different conditions have been described for tertiary amines[11,13,14,15], alkaloids[16,17], long chain surface active[18,19] and other high molecular weight quaternary ammonium compounds[11,20] as well as for short chain salts such as tetraethylammonium bromide[9,10] and hexamethonium bromide[10,11,12]. No methods have been described for gallamine triethiodide and pentapyrrolidonium salts.

Two types of method have been based on Hartley's[21] observations of the effects of surface active quaternary ammonium salts such as cetylpyridinium bromide and cetrimide on various indicators, the colour of sulphonphthaleins for example being "not only displaced to the alkaline side, but also qualitatively changed." Hartley and Runnicles[22] in 1938 used this effect to determine the concentration of cetylpyridinium ion in a solution by titration with sodium cetylsulphate using bromophenol blue as indicator. Methods published since[23,24,25,26,27] use other indicators but with one exception both reactants are surface active agents. Carkhuff and Boyd[27], however, apply their method to a tertiary amine and claim that it is suitable for the determination of most quaternary ammonium salts and certain types of amine. A variation of the method involves titration of surface active agent with a suitable indicator[28]. The second of the two types of method based on Hartley's observations was provided by Colichman[29], who in 1947 applied the indicator displacement effect to the direct photometric determination of quaternary ammonium salts by using an excess of bromophenol blue and measuring the colour of the aqueous solution. He applied the method to two surface-active compounds and implied that it was applicable to quaternary ammonium salts in general. Other workers[4,30] using bromocresol purple applied similar methods to cetylpyridinium chloride and they also suggested a wider application.

The fourth type of method is applicable only to organic solvent solutions of bases and consists in adding a solution of indicator in either aqueous buffer[31,32,33,34], in which case shaking is necessary, or in organic solvent[35], to a solution of the base in a halogenated solvent, followed by photometric measurement of colour produced. This method, which was first described by Brodie and Udenfriend[31], is based on observations by LaMer and Downes[36] in 1933, supplemented later by Griffiths[37] and reaffirmed by others[38,39], that addition of bases to organic solvent solutions of indicators results in changes of colour proportional to the concentration of base.

The titration and direct photometric methods appeared to have such considerable advantages as regards simplicity and rapidity that it was

clearly desirable to determine their range of applicability. If this were found to be limited then it was decided to elucidate the principles underlying the indicator extraction method sufficiently to facilitate selection of optimum conditions for determination of particular compounds either alone or in the presence of other substances.

EXPERIMENTAL

1. *Titration Method*

Titration of a 0·1 per cent. solution of cetylpyridinium chloride with a 0·12 per cent. solution of sodium lauryl sulphate using methyl yellow, in the presence of chloroform, as indicator under the conditions laid down by Carkhuff and Boyd[27] gave a sharp end-point. With cetrimide the end-point was less sharp and with *p*-nitrophenoxymethylbenzyldimethylammonium chloride a diffuse and very premature end-point was obtained. With salts of hexamethonium, pentapyrrolidinium and gallamine the chloroform layer became coloured after the addition of as little as 3 per cent. of the expected volume of titrant.

2. *Direct Photometric Method*

Following Colichman's method[29], addition of 0·5 mg. of cetylpyridinium chloride to a 0·0016 per cent. solution of bromophenol blue buffered with sodium carbonate gave an increase in optical density of 0·28 at a wavelength of 645 mμ, which is the λ_{min} for the indicator alone. At the λ_{max} of 585 mμ, which was not used by Colichman, a decrease in optical density of 0·49 occurred, but the reagent blank was large. With cetrimide a small increase in optical density was obtained at 645 mμ and no increase with gallamine; at the λ_{max} small decreases were obtained with both. Using bromothymol blue the corresponding changes in optical density were even smaller.

3. *Extraction Method*

Simple preliminary tests having indicated that chloroform was a better solvent for the complex than ethylene dichloride, a systematic study of conditions was commenced.

(a) *Choice of Indicator*

Working with cetrimide, pentapyrrolidinium and pentamethonium, tests were made in nearly neutral, slightly acid and slightly alkaline solutions with more than 40 anionic indicators to see whether a chloroform-soluble complex was formed. Indicators tried included halogenated and unhalogenated sulphonphthaleins, phthaleins, fluoresceins and azo-dyes. Extractable complexes formed most readily with cetrimide and least readily with pentamethonium, cetrimide giving extractable complexes with 28 indicators but pentamethonium with only 4, bromothymol blue, phenyl-α-naphthylamineazobenzene-*p*-sulphonic acid, dibutylaminoazobenzene-*p*-sulphonic acid[40] ("butyl orange"), and tetrachlorotetraiodofluorescein, most colour being obtained with bromothymol blue. Furthermore, as expected from Gottlieb's[11] results, complexes formed more readily in neutral or slightly acid solution than in alkaline solution.

(b) *Choice of Solvent*

Working with dimethylaminotoluene methiodide and bromothymol blue at *p*H 9·2, extraction of complex with one portion of chloroform being incomplete, single extractions were made with 24 solvents including halogenated and non-halogenated aliphatic, alicyclic and benzenoid hydrocarbons, alcohols, esters and nitrated hydrocarbons such as nitrobenzene, the latter being chosen because of its high dielectric constant. Alcohols and esters gave a large reagent blank, whilst unhalogenated hydrocarbons extracted no complex. The best solvents were nitrobenzene, acetylene tetrachloride, chloroform and methylene dichloride, the extinction values obtained being 0·33, 0·206, 0·123 and 0·118 respectively. Nitrobenzene, however, was rejected because of toxic hazards and tendency to form persistent emulsions. Using acetylene tetrachloride a solvent phase was obtained which could not be clarified with glass wool and hence chloroform was chosen for further work. Solubilities of the complexes of 3 quaternaries in certain solvents are given in Table II.

TABLE II

SOLUBILITIES OF QUATERNARY AMMONIUM COMPLEXES OF BROMOTHYMOL BLUE

Quaternary ammonium cation	Complex in 100 ml. of solution				
	Chloroform, g.	Ethylene dichloride, g.	Acetylene tetra-chloride, g.	Carbon tetra-chloride, g.	Benzene, g.
Cetyltrimethylammonium ..	More than 60	More than 50·0	—	4·6	40·0
Gallamine..	43	20·0	38	Insoluble	0·01
Hexamethonium	34	0·14	14	,,	0·04

(c) *Phase Volume Ratio*

To avoid emulsions it was necessary to have a volume ratio of the aqueous phase to the chloroform of not greater than 1·5 to 1. To obviate the measurement of small volumes of reagents and of sample the aqueous phase was fixed at about 25 ml. and the solvent phase at 20 ml.

(d) *Effect of Time and Technique*

The complex appeared to form rapidly[4] in the aqueous phase and not slowly as believed by Marshal and Rogers[16]. Phase equilibrium was complete after 2 minutes shaking. Separation of the phases was complete after 2 minutes, but the upper portion of the chloroform phase was more intensely coloured than the lower. This resulted from the "foam" (chloroform dispersed in aqueous phase) hanging from the upper aqueous phase into the chloroform, complex being adsorbed at the large interfacial surface as in the case of indicators alone[34]. Reproducibility was obtained by swirling the chloroform to mix before running it off.

(e) *Method of Clarifying Chloroform Solution of Complex*

Different types of filter paper tried removed up to 20 per cent. of colour from the chloroform solution for one paper, additional colour being removed with each filtration through a fresh paper; Munktell No. 0 filter

papers used by Gottlieb[11] were not available at this stage. Sodium sulphate, used initially by Auerbach[7] and later by Wilson[41] removed complex at the rate of about 12 per cent. of colour for each 1 g., the amount increasing with time of contact. Several other substances such as silica[42], celite and calcium oxide were also found unsatisfactory. However 0·06 g. of glass wool clarified 25 ml. of moist chloroform solution of complex in 10 minutes, no loss of colour occurring on standing for a further 15 minutes or on adding another 0·04 g.; even with 0·2 g. only a 5 per cent. loss of colour resulted. A slight fall in optical density during actual clarification was attributed to removal of the traces of indicator present in the dispersed water droplets since the glass wool acquired a greenish-blue colour removable by alcohol or chloroform B.P., but not by alcohol-free chloroform or by ethylene dichloride.

(f) *Stability of Chloroform Solution of Complex*

Use of a simple chloroform solution of the complex for optical measurements suffered from certain disadvantages such as adsorption of the complex on to the glass walls of flasks and cells and instability of colour arising from removal of alkali from the glass. A solution stable for at least one hour and free from adsorption effects was obtained by the addition of an ethanolic solution of boric acid containing a small but necessary amount of water; the concentration of boric acid and water chosen gave maximum sensitivity and small variations had no effect. Stronger acids such as hydrochloric lowered sensitivity whilst sulphonphthaleins tend to fade in alkaline solution[43].

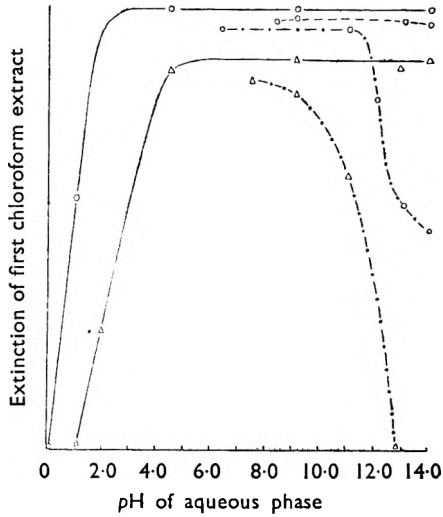

FIG. 1. Effect of *p*H of aqueous phase on the extraction of complex into chloroform using three different indicators.

———— methyl orange
– – – – bromophenol blue
– · – · – bromothymol blue
○ cetrimide
△ 2:4-dichlorophenoxymethyltriethylammonium chloride

N.B.—Extinction values were determined at different wavelengths and in solvents of different composition for each indicator. Arbitrary scales for the respective series of ordinates were chosen in order to obtain a clear diagram.

(g) *Purity of Reagents*

Some batches of both bromophenol blue and bromothymol blue failed to pass the purity test given by Wilson[44] and were purified as described by him. However, both original and purified indicators although differing appreciably in colour gave identical results in photometric work and the amount

of impurity was probably small. With the exception of trichlorethylene solvents were not specially purified; trichlorethylene was freed from amines present as stabilisers[45] by extraction with acid. Quaternary ammonium salts were recrystallised from ethanol, ethanol-ether or ethanol-benzene[46] mixtures.

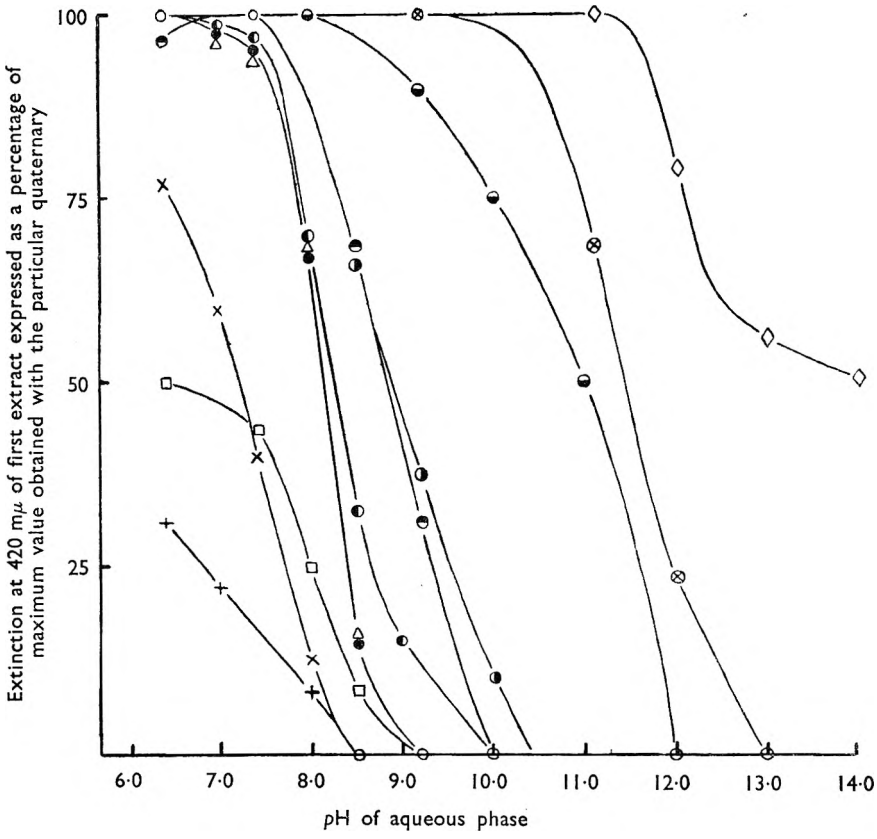

FIG. 2. Effect of *p*H of aqueous phase on the extraction of complex into chloroform using bromothymol blue and different quaternary ammonium salts.

+ pyridine methiodide
□ tetraethylammonium hydrogen tartrate
× hexamethonium bromide
△ decamethonium iodide
● pentapyrrolidinium bitartrate
◐ phenyltrimethylammonium chloride
◓ gallamine triethiodide
◑ diethylaminotoluene methiodide
◒ γ-phenoxypropyltriethylammonium iodide
⊗ *p*-nitrophenoxymethylbenzyldimethylammonium chloride
◊ cetrimide
○ is used where several lines pass through the same point and where the points lie on the base line.

(h) *Effect of* p*H*

The effect of variation of the *p*H of the aqueous phase on the extraction of the complex into chloroform was studied to a limited extent with different indicators (Fig. 1) and in greater detail with bromothymol blue (Fig. 2). McIlvaine standard buffers[47] containing varying ratios of citric acid and sodium phosphate were used for *p*H values between 6·4 and 8; hydrochloric and boric acids were used to obtain low *p*H values and solutions of sodium bircarbonate, borax, sodium carbonate and sodium hydroxide for the higher *p*H values. Extinction values (2 cm.) for the reagent blank (3 extractions) rose, with decrease of *p*H, from 0·008 at *p*H 7·6 to 0·32 at *p*H 5·8 using chloroform, and were rather less using ethylene dichloride.

(i) *Effect of Excess of Bromothymol Blue*

Using chloroform at *p*H 7·4 increasing the amount of indicator from 1 ml. to 5 ml. of 0·1 per cent. raised the reagent blank from 0·01 to 0·16. With quaternaries giving calibration curves of theoretical slope (Fig. 5) complete extraction of complex was obtained in the presence of an excess of indicator varying between 10 and 40 per cent. (Table III). Other compounds required a much greater and varying excess, the hexamethonium complex not being completely extracted even with 100 per cent. excess.

TABLE III

EXTENT OF LINEARITY OF CALIBRATION CURVES FOR QUATERNARY AMMONIUM COMPOUNDS

Compound	Extent of linearity, per cent.
Gallamine triethiodide	80
Pentapyrrolidinium bitartrate	60
2:4-Dichlorphenoxymethyltriethylammonium chloride	Greater than 70
Decamethonium iodide	70
3:5-Dimethyl-4-chlorphenoxymethyldimethylbenzylammonium chloride	85
Dimethylbenzyl-*p*-nitrophenoxymethylammonium chloride	90

(j) *General Method using Bromothymol Blue*

Apparatus. All apparatus, including separator stopcocks, must be completely free from grease.

Reagents

Bromothymol Blue. If this does not pass the purity test given by Wilson[44], purify as he describes but using a buffer of *p*H 7·5.

Bromothymol Blue Solution. Dissolve 0·15 g. of bromothymol blue and 0·15 g. of anhydrous sodium carbonate in water and dilute to 100 ml.

Buffer pH 7·5[47]. Dilute 7·5 ml. of freshly prepared citric acid solution 0·1 M (2·10 per cent.) to 100 ml. with sodium phosphate solution 0·2 M (7·16 per cent. $Na_2HPO_4.12H_2O$).

Boric Acid in Ethanol. Dissolve 5·0 g. of boric acid in dehydrated ethanol, add 20·0 ml. of water and dilute with dehydrated ethanol to 250 ml.

Procedure

To 20 ml. of chloroform B.P. in a separator add 5·0 ml. of solution containing the equivalent of up to 2 micromols of univalent quaternary ammonium salt, 1·0 ml. of bromothymol blue solution and 20·0 ml. of buffer. Shake vigorously for 2 minutes, allow to separate, slowly invert the separator to mix the chloroform phase and allow to stand for 2 minutes.

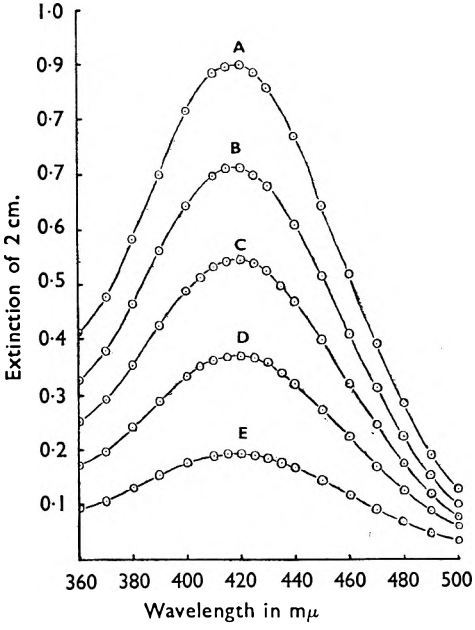

FIG. 3. Absorption spectra of solutions of bromothymol blue in chloroform containing 25 per cent. v/v of solution of boric acid in ethanol. A, 2·43 micromols; B, 1·94 micromols; C, 1·46 micromols; D, 0·97 micromols; E, 0·48 micromols.

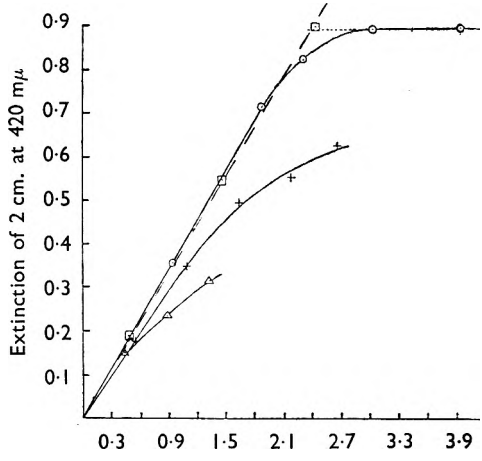

FIG. 4. Calibration curves for bromothymol blue, gallamine triethiodide and hexamethonium bromide.

—⊡— bromothymol blue in chloroform containing 25 per cent. v/v of solution of boric acid in ethanol

⊙ gallamine triethiodide by proposed general method

△ hexamethonium bromide by proposed general method

+ hexamethonium bromide by proposed general method but at *p*H 6·8.

Run off the chloroform into a stoppered flask containing 0·4 g. of glass wool and repeat the extractions and separations with 2 further portions of chloroform. Shake the bulked chloroform for 1 minute and allow to stand for 5 minutes. Decant the chloroform through a small plug of glass wool into a 100 ml. graduated flask containing 25·0 ml. of solution of boric acid in ethanol. Wash the flask and filter with successive small quantities of ethylene dichloride or ethanol-free chloroform, adding the washings to the main portion of filtrate and finally diluting to 100 ml. Measure the extinction of 2 cm. at 420 mμ against chloroform, and read off the corresponding amount of quaternary ammonium salt from a calibration curve.

(k) *Calibration Curves*

Solutions in chloroform (containing ethanol and boric acid) of the gallamine complex and of bromothymol blue, having the same concentration of indicator gave identical spectra. Absorption spectra for solutions covering a range of concentrations of indicator are shown in Figure 3. The extinction values at 420 mμ are shown plotted in Figure 4. The effect of variation in path length was small, extinction values for 1 cm., 2 cm., and 4 cm. being 0·179, 0·361 and 0·75. Calibration curves were linear from the origin for a varying range of extinction values, flattening off at a value which was a varying fraction of the extinction of 0·89 which corresponded to the total available bromothymol blue (Table III). The curves for gallamine triethiodide and hexamethonium bromide (Fig. 4) illustrate the types obtained with compounds of high and low molecular weight respectively. In the case of hexamethonium bromide the slope was affected by change of *p*H, being greatest at *p*H 6·8. These curves were plotted from values corrected for the reagent blank on the basis that this is greatest in the absence of quaternary and zero at the extinction (0·89) corresponding to complete extraction of bromothymol blue.

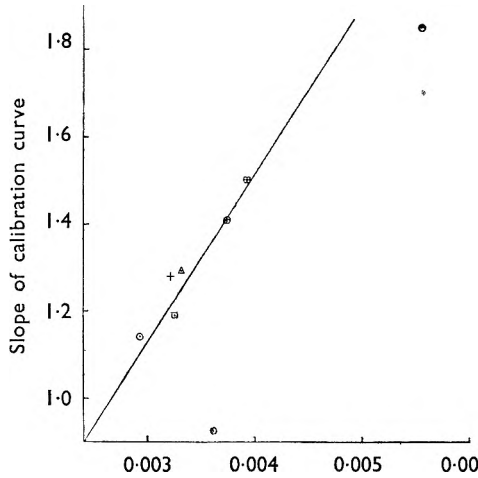

FIG. 5. Relationship between slope of calibration curve, expressed as extinction of 2 cm. at 420 mμ for 1 mg. in 5 ml. of sample solution, and reciprocal of molecular weight × number of quaternary nitrogen atoms per molecule.

△ gallamine triethiodide
⊕ pentapyrrolidinium bitartrate
⊡ decamethonium iodide
◓ hexamethonium bromide
◐ tetraethylammonium bromide
○ 3 : 5-dimethyl-4-chlorphenoxymethyldimethylbenzylammonium chloride
⊡ dimethylbenzyl-*p*-nitrophenoxymethylammonium chloride
\+ 2 : 4-dichlorphenoxymethyltriethylammonium chloride

For the high molecular weight compounds the slopes of the calibration curves, expressed as extinction/concentration (mg./100 ml.), were directly proportional to the number of quaternary nitrogen atoms per molecule and inversely proportional to the molecular weight (Fig. 5), the line in this figure showing the linear relationship which holds when complete extraction of complex is obtained.

Calibration curves set up by different workers at different times using different reagents agreed to within 1 per cent. Fuller information regarding the precision of the method when applied to pharmaceutical preparations will be given in a later paper.

(l) *Composition of Complex*

The composition of bromothymol blue complexes was determined in 3 different ways. (1) Assuming that for the linear portion of the calibration curve complete extraction of quaternary compound is taking place, then at each point the molar ratio of indicator to quaternary compound can be calculated. For gallamine, for example, the ratio is 3 to 1 and since there are 3 quaternary nitrogen atoms per molecule then one molecule of indicator is associated with each nitrogen atom. For methyl orange and 2:4-dichlorophenoxymethyltriethylammonium chloride a ratio of unity was also obtained. Similar results were obtained with the other relatively high molecular weight quaternary ammonium compounds. (2) In the case

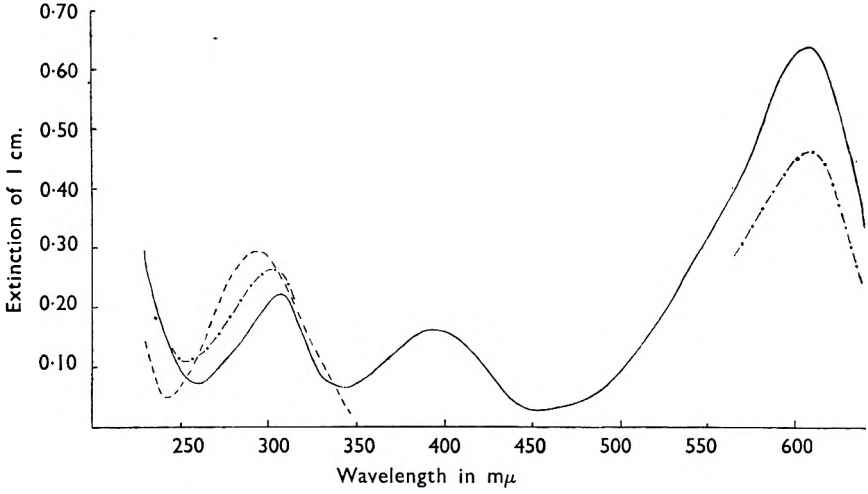

FIG. 6. Determination of composition of bromothymol complex of *p*-nitrophenoxymethylbenzyldimethylammonium chloride. Absorption spectra of about 0·001 per cent. solutions in 0·1 N sodium hydroxide of

——— bromothymol blue
– – – – *p*-nitrophenoxymethylbenzyldimethylammonium chloride
– · – · – complex.

of the *p*-nitrophenoxymethylbenzyldimethylammonium complex the composition of the chloroform extract was determined by extracting the chloroform with 0·1N sodium hydroxide and making extinction measurements at wavelengths of 295 mμ, 306 mμ and 610 mμ (Fig. 6). The amount of bromothymol blue was calculated from the extinction value at 610 mμ and the amount of quaternary from the remaining two values by the two-colour method. The ratio obtained was 1 to 1. (3) The hexamethonium complex was isolated, as described later, its indicator content calculated from the extinction at 420 mμ of its chloroform solution, and its quaternary content determined by the general method described in section (j). The ratio found was again unity.

That other ratios can exist was shown as follows. (1) The chloroform solution of the cetrimide-bromothymol blue complex obtained at *p*H 13 (Fig. 1) was equilibrated with a buffer solution at *p*H 8·4 containing

indicator. The extinction rose to that obtained normally at *p*H 8·4, the value being actually doubled, showing that the original ratio of indicator to quaternary was 1 : 2. (2) Using "butyl orange" a ratio of 4 : 1 was obtained with pentapyrrolidinium and with gallamine.

Complexes were obtained in the solid state in two ways. (1) Complexes of bromothymol blue with gallamine, hexamethonium and pentapyrrolidinium were obtained as orange-red to red-brown powders by evaporating chloroform solutions, obtained by extraction, to dryness. (2) Addition of the appropriate amount of bromothymol blue to a solution of quaternary hydroxide in methanol and evaporation to dryness gave the complexes of gallamine and hexamethonium as green powders. The ratio of indicator to quaternary compound was the same in both forms of complex.

(m) *Application of General Method in the Presence of Interfering Substances*

Possible interference by various substances in the concentrations likely to be encountered in pharmaceutical preparations was investigated. Sodium sulphite (5 mg.), chlorbutanol (0.06 mg.), dextrose (0·1 g.), lactose (0·1 g.), and polyvinylpyrrolidone (2·7 mg.) had no effect but sodium lauryl sulphate (0·2 mg.) lowered the extinction from 0·35 to 0·30. The effect of inorganic salts was to increase the amounts of both free indicator and complex extracted, the effect on the reagent blank being greatest at low *p*H values (Table IV). Series of tests using different inorganic salts

TABLE IV

EFFECT OF SODIUM CHLORIDE ON EXTRACTION OF INDICATOR ALONE AND OF INDICATOR-QUATERNARY COMPLEX

Compound extracted	Sodium chloride, g.	Extinction of first extract	
		*p*H 7·4	*p*H 8·5
Bromothymol blue alone	—	0·01	0
	0·2	0·035	0
	1·0	0·68	0
	2·0	1·07	0·035
	4·0	Greater than 1·07	1·1
Pentapyrrolidinium complex	0	—	0·096
	1·5	—	0·148

showed that those yielding doubly charged ions such as sulphate had a greater effect, the effect being actually related to the ionic strength of the solution.

DISCUSSION

Titration and Direct Photometric Methods

The failure of the titration method of Carkhuff and Boyd[27] when applied to hexamethonium, pentapyrrolidinium and gallamine may be attributed to the relatively greater stability of the complex formed with methyl yellow in chloroform solution compared with that formed with sodium lauryl sulphate in aqueous solution. Barr *et al.*[25] obtained divergent results with dodecyltriethylammonium bromide using methylene blue, whilst Epton[24] obtained quantitative results with cetylpyridinium bromide only when the

carbon chain length of the alkyl sulphate was greater than eight. Alkyl sulphates with even greater chain length may be necessary with the lower molecular weight quaternaries. Different workers have used different indicators and different *p*H conditions, but the theoretical principles underlying the method do not appear to have been worked out. According to Hartley and Runnicles[22] end-points may be obtained with bromophenol blue in the aqueous phase at concentrations well below the critical micelle concentration (C.M.C.) for the cetylpyridinium ion and hence the undoubtedly higher C.M.C. values for the lower molecular weight quaternaries are probably not the reason for failure of the method. Systematic studies of this potentially valuable method are in progress.

According to Colichman[46] the colour change of bromophenol blue in the presence of long chain cations results from electrostatic interaction between the quaternary cations and the indicator anions; conductivity results obtained were in accordance with the complete formation of non-conducting ion-pairs even in highly dilute solutions. Interfacial tension studies[48] also indicated ion-pair formation in aqueous solution followed by micellisation. According to Lewis *et al.*[49,50] ion-pair formation in itself does not alter the spectral absorption of associated ions and Colichman[51] in a later spectral study concluded that the ion-pairs were at least partially in the form of micelles even below the C.M.C. for the quaternary. Failure to obtain a colour change with the lower molecular weight quaternary compounds may be attributed to absence of micelles, bromothymol blue being less useful than bromophenol blue for the high molecular weight quaternaries, as was found also in interfacial tension titrations, because the C.M.C. for the metathesis compound is higher[48].

Extraction Method

Applicability. The general method described in section (j) is applicable to all except the low molecular weight quaternaries such as tetramethylammonium and choline salts. However, it cannot be regarded as entirely satisfactory for hexamethonium and tetraethylammonium salts since calibration curves of theoretical slope are not obtained owing to incomplete extraction of complex. Almost complete extraction of hexamethonium has been obtained using acetylene tetrachloride at *p*H 6·6 and it is hoped that a method incorporating these and other modifications will be more suitable for all low molecular weight quaternaries. Although calibration curves of theoretical slope are obtained with the remaining compounds a separate calibration curve for each is necessary on account of the varying extent of linearity.

The method is liable to interference from (*a*) anionic surface-active agents, proteins[52], peptones and other substances which may react with the quaternary, (*b*) substances such as albumin[53] and polyvinylpyrrolidone which may react with the indicator and (*c*) substances such as ointment bases and salts which may affect the partition of the indicator or of the complex between the two phases. Anionic detergents may possibly be removed by extraction with chloroform as the methylene blue complex[54]. Although polyvinylpyrrolidone combines with certain dyes[55] particularly

brilliant vital red, a sulphonated azo-dye used in a method for its determination[56], in the concentration tried it did not interfere with the extraction method. Modifications necessary in the presence of certain interfering substances will be given in a later paper.

Selectivity. The general method, like those of many other workers, is applicable to certain amines, but these may be readily separated from quaternary compounds by extraction from alkaline solution. It should be possible to differentiate quaternary compounds by taking advantage of such factors as (*a*) the greatly differing solubility of the complexes in different solvents, benzene for example being a poor solvent for complexes of gallamine and hexamethonium, but a good one for that of cetrimide, (*b*) differences in behaviour with different indicators, the lower molecular weight quaternary compounds not giving extractable complexes with bromophenol blue, and (*c*) the effect of *p*H, only the high molecular weight quaternaries being extracted at the higher *p*H values.

Theoretical Aspects. A considerable amount of work remains to be done before the theoretical basis of the method can be fully established. Probably the most important relationship to be further studied is that between the constitution of a quaternary compound or amine and the *p*H range over which it can be extracted. It seems probable that the reacting species in the case of amines is the cation $RR'R''NH^+$ formed in acid solution by the addition of a proton. Preliminary experiments have shown that amine-indicator complexes are extractable over different *p*H ranges extending into the alkaline side where, however, complexes may not form, the amine itself being extracted and then causing partition of indicator between the two phases. The limited *p*H ranges over which the complexes of different quaternaries can be extracted may be accounted for by the formation of undissociated quaternary hydroxide in sufficiently alkaline solutions.

Summary

1. Published methods for the determination of quaternary ammonium compounds by (*a*) titration with anionic surface-active agents, and (*b*) measuring the change in colour intensity produced in an indicator in aqueous solution, have been found inapplicable to compounds of molecular weight lower than that of cetrimide.

2. The indicator extraction method has been studied in some detail and the results of variations of conditions and the effects produced by interfering substances described and discussed.

3. A general indicator extraction method is proposed which is applicable to all quaternary compounds except those of very low molecular weight.

The authors wish to thank the Directors of Messrs. May and Baker, Ltd., for permission to publish this paper.

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DISCUSSION

The paper was presented by MR. J. ISAACS.

DR. D. C. GARRATT (Nottingham) said the authors had not sufficiently stressed the purity of the indicators used for the reaction. He had been unable to obtain English bromophenol blue which was sufficiently pure for spectrophotometric work, but American material gave good results. The chemical method was just as quick as the spectrophotometric method.

DR. G. E. FOSTER (Dartford) remarked that he had used this type of method for the determination of tubocurarine. There was some difficulty in the standardisation of cetrimide, as the substance supplied by different makers did not seem to be of uniform composition. Had the authors been able to detect any variation?

DR. W. MITCHELL (London) said that the authors had selected chloroform as a solvent in preference to nitrobenzene or acetylene tetrachloride, one reason being that nitrobenzene presented a toxic hazard. This was not mentioned as a reason for the rejection of acetylene tetrachloride, which was probably the most toxic of the chlorinated hydrocarbons.

MR. H. E. BROOKES (Nottingham) said that in certain formulations of cetrimide, due to inactivation of the quaternary compound, the microbiological determination did not agree with the chemical method, although the latter still gave the expected figure. Had the authors any information on this subject?

MR. ISAACS, in reply, referring to the purity of the indicators, said that they discontinued the use of bromophenol blue because bromothymol blue was found to be superior in many respects. They purified the bromothymol blue by Wilson's method, but towards the end of the work they checked 6 or 7 samples of the commercial indicator and found the unpurified material to be as satisfactory as the purified. It seemed that purification by Wilson's method might be unnecessary, but by this means one was certain of having satisfactory material. The general method could be applied to tubocurarine fairly well. It was true that cetrimide was not uniform, and for that reason in most of their quantitative work they had used quaternary compounds of known composition. Replying to Dr. Mitchell, he said that acetylene tetrachloride was possibly more toxic than nitrobenzene but, in view of the odour of the latter, workers preferred to face the greater dangers of the former. The general method did not apply too well to hexamethonium bromide, but using acetylene tetrachloride and an alteration in volume-phase ratios, quantitative extraction had been obtained and it seemed that this solvent should be used until something better was found.

SYNTHETIC ANALGESICS: STEREOCHEMICAL CONSIDERATIONS

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In a review article[1] in 1952 on "Analgesics," one of the present authors stated "it appears probable from a consideration of the diverse types of compounds which have an analgesic activity equal to, or greater than, that of pethidine, that the minimum requirement for activity may be a hydrophobic group (or collection of groups) containing a basic centre with an overall optimum spatial arrangement." Further "it is possible that the stereochemical configuration of the drug must be complementary to that of a certain tissue surface or enzyme system." Since the appearance of this article, much evidence has been forthcoming supporting the above statements. The purpose of this paper is to correlate the relevant information, to present stereochemical evidence obtained in our own laboratories, and to postulate the type of drug-receptor mechanism involved and the nature of the receptor surface.

The importance of the spatial configuration in analgesics is most clearly demonstrated in those compounds possessing one asymmetric carbon atom. In all cases where optical enantiomorphs have been prepared and tested, one is always more active than the other of each pair, and, in many cases, the whole of the activity of the racemic mixture resides in one of the isomers. In Table I the analgesic activities of a number of enantiomorphic pairs and the nature of their rotations are presented.

The significance of the difference in activities of optical isomers in drug-receptor mechanisms only becomes established when the configurations of the isomers are elucidated. We have determined the configurations of a number of these optical isomers, some of varying chemical types; the more analgesically active isomers of each enantiomorphic pair have identical spatial configurations which are related to that of D-(−)-alanine (see Fig. 1). (Figure 2 shows the outline of the reactions used in establishing the configurational relationships; full chemical details will be published elsewhere.)

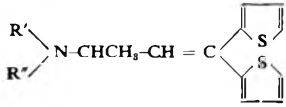
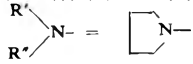
The difference in biological activity exhibited by isomers of enantiomorphic pairs can be attributed to one or more of the following factors:

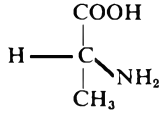
1. difference in their distribution;
2. difference in their rates of metabolism;
3. preferential adsorption of one of the isomers by an optically active constituent in the body before it can reach the site of action;
4. difference in their ease of adsorption upon a complementary receptor surface;
5. difference in the nature of the drug receptor combination.

TABLE I

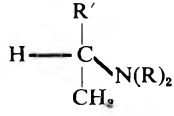
ANALGESIC ACTIVITIES OF ENANTIOMORPHIC PAIRS OF COMPOUNDS

The figures given for analgesic activity are approximate and relate to the activity measured on rats. They have been calculated from results published by various workers (see references 2 to 9).

Analgesic compound	Configuration	Rotation	Analgesic activity (Related to (±)-methadone = 100)
$R_2N{-}CHCH_3{-}CH_2{-}C(C_6H_5)_2{-}R'$			
Methadone: R = CH_3, R′ = $-COC_2H_5$	D	−	180
	L	+	10
Sulphone analogue of methadone: R = CH_3; R′ = $-SO_2C_2H_5$	D	−	180
	L	+	10
			
R′ = CH_3 R″ = CH_3	D	+	170
	L	−	30
R′ = C_2H_5 R″ = C_2H_5	D	+	120
	L	−	20–50
		+	90
		−	50
Isomethadone		−	80
		+	5
3-hydroxy-*N*-methyl morphinan (levorphan)		−	400
3-hydroxy-*N*-methyl morphinan (dextrorphan)		+	0
3-methoxy-*N*-methyl morphinan (levomethorphan)		−	40
3-methoxy-*N*-methyl morphinan (dextromethorphan)		+	0



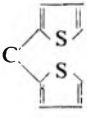
D-(−)-alanine



Optically active analgesics (D-configuration)

(*a*) ((−)-isomer) R = CH_3; R′ = $-CH_2(C_6H_5)_2COC_2H_5$.

(*b*) ((−)-isomer) R = CH_3; R′ = $-CH_2(C_6H_5)_2SO_2C_2H_5$.

(*c*) ((+)-isomer) R = CH_3; R′ = 

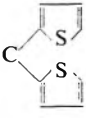
(*d*) ((+)-isomer) R = C_2H_5; R′ = 

FIG. 1.

The possibility of the first 3 factors being involved in the difference of activity amongst enantiomorphs with analgesic action appears remote, because it has been shown that the distribution of (+)-and (−)-methadone within the body is the same[10], and that the excretion of (+), (−) and (±) 3-hydroxy-*N*-methylmorphinan is similar[11]. Furthermore, rat

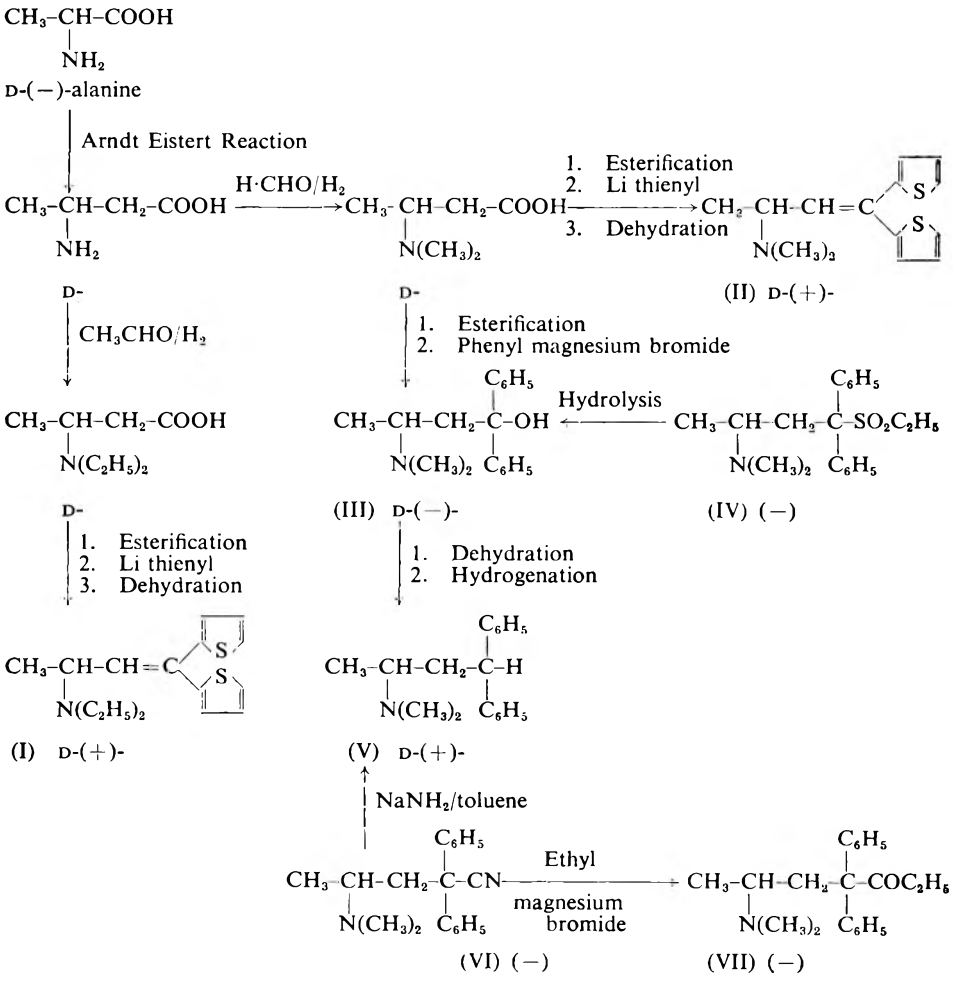


FIG. 2. The determination of the configuration of certain analgesically active isomers.

liver slices (the liver being the chief organ for the metabolism of methadone) have the same effect upon both methadone isomers[10].

Indications that a particular receptor surface is involved in the mediation of analgesic action by a drug can also be obtained by consideration of analgesic antagonists. *N*-allylnormorphine (nalorphine) has been shown to antagonise the analgesic action of compounds of various chemical structures, i.e., morphine, methadone, isomethadone, the

dithienylbutenylamines, acetylmethadols, phenadoxone, 3-hydroxy-*N*-methylmorphinan, pethidine[12,13,14,15,16]. Nalorphine possesses slight analgesic action itself and, when injected into mice after a dose of morphine, rapidly abolishes the analgesic effect of the latter and reduces the pain perception to the level given by nalorphine alone[17]. It also antagonises the respiratory depressant properties of morphine[18]. The replacement of the CH_3– of the N–CH_3 group of morphine by various alkyl groups yields compounds showing a gradation of analgesic and anti-analgesic action; some exhibited both effects[19]. *N*-propylnormorphine, and its diacetyl derivative, antagonised the analgesic action of methadone, pethidine and thiambutene in addition to that of morphine[19]. Substitution of allyl, methallyl, *n*-propyl and *iso*butyl groups for methyl in morphine, dihydro-, desoxy- and dihydrodesoxymorphines and various dihydromorphinones give compounds which antagonised the analgesic effect of morphine[20]. 3-Hydroxy-*N*-allylmorphinan (racemic mixture and (−)-isomer) antagonise the actions of morphine and synthetic analgesics[21,22].

The stereochemical factors involved in the "fit" upon the complementary receptor surface are further emphasised by the following facts concerning analgesic antagonists:

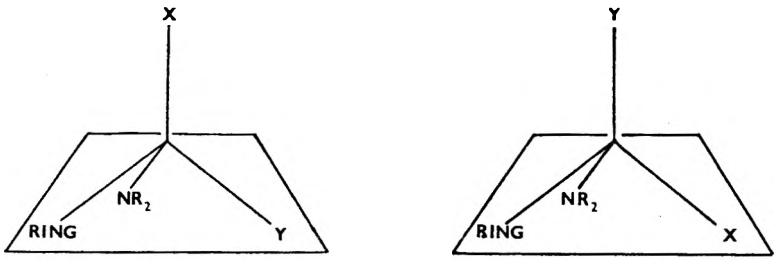
1. Morphine and its antagonist nalorphine have identical configurations.

2. (−)-3-Hydroxy-*N*-methylmorphinan (levorphan), a potent analgesic, is antagonised by (−)-3-hydroxy-*N*-allylmorphinan of identical configuration but not by the (+)- allyl compound possessing the same configuration as the almost inactive (+)-3-hydroxy-*N*-methylmorphinan (dextrorphan)[21].

3. *N*-Methyl*iso*morphinan, essentially without analgesic activity, yields a *N*-allyl compound which is inactive as an anti-analgesic[20].

It may be assumed, therefore, that a "fit" at a receptor surface is involved in analgesic action and that stereochemical factors are extremely important. However, it is not intended to imply that adsorption at a particular receptor site, of necessity, mediates an analgesic effect. It is postulated that analgesic compounds, and their antagonists, "fit" the receptor surface, the former initiating a reaction sequence (or interfering with a reaction sequence), the outcome of which effect is analgesic action, while the latter, because of slight difference in their structure, fail to evoke, or only partially evoke, the reaction sequence. An antagonist can thus compete with an analgesic for the receptor site and, in suitable concentration, completely prevent the analgesic being adsorbed upon the essential site with consequent blocking of its pharmacological effect. By considering the structural surfaces common to different chemical types of analgesics, by bearing in mind the established stereochemical requirement of the synthetic analgesics, and assuming that there is no deformation on adsorption on the receptor surface, certain tentative conclusions can be drawn as to the complementary fine structure of the latter.

Features which are common to all analgesics and their antagonists are (1) a tertiary basic group and (2) a flat aromatic ring structure, e.g., a 6-membered ring possessing 3 double bonds or a 5-membered ring with 2 double bonds. It is probable that these two essential groupings become associated with specific receptor sites in a cellular boundary representing the primary site of action. Substituent groups are not essential because *N*-methylmorphinan, an analgesic, is simply a three dimensional structure of aromatic and hydroaromatic rings possessing one basic centre. Because in certain enantiomorphic pairs, one isomer is analgesically active while the other is inactive, a third grouping consisting of a hydrocarbon moiety must be involved to form a third reaction point at the surface, with the result that only one of the stereoisomers presents the three groups in the correct relative positions. (Figure 3 illustrates that if the (−)-isomer is analgesically active and structure X is involved at the surface as well as the basic group and the aromatic ring, then the (+)-isomer can only present two of the three essential groups in the correct orientation to the receptor surface.)



(+)− isomer (−)− isomer

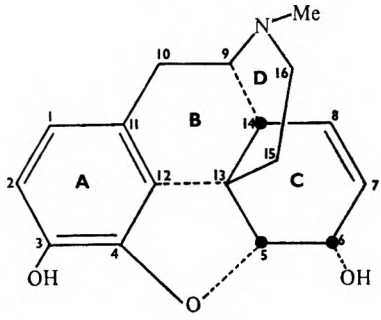
FIG. 3.

Both ease of attachment of the drug to the receptors and the nature of the drug-receptor combination appear to be important in analgesic activity. Inactive isomers, although incapable of coming into 3-point contact with the receptors, can reduce the activity of the active isomers, when added together, by a partial "blocking" effect. It has been shown, for instance, that dextrorphan tartrate and (+)-methadone (inactive isomers) can reduce the analgesic effects of their respective active enantiomorphs[23,24]. Furthermore, the analgesic effects of levorphan and (−)-methadone hydrochloride are inhibited by the inactive (+)-isomers of the other compound to the same degree as by their own inactive isomers, while the combination of half the effective dose of each (−)-isomer is additive in nature[24].

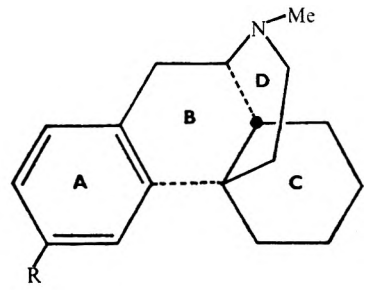
Morphine and the morphinans have rigid structures in which the relationships of the various asymmetric centres have been established[25,26] (see VIII and IX for conventional line representation). They were used as models for a consideration of the most probable surface for presentation to a receptor (a flat ring and the basic centre being regarded as two essential moieties).

The nature of the conformation of the reduced rings of these substances is therefore important. Bose[27] has presented evidence from which he has

concluded that, in morphine (VIII) the alicyclic rings B, C and the piperidine ring D are all in the chair form. Although agreeing with his general conclusions, the present authors consider that ring C in morphine is a boat form and not a chair, because of the steric requirements of the 4 — 5 ether bridge. This steric constraint is not present in the morphinans



(VIII)
morphine

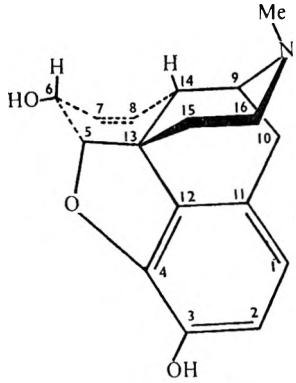


(IX)
N-methyl morphinan (R = H)
racemorphan (dromoran) (R = OH)

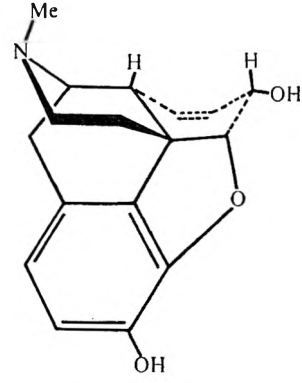
(IX) where it is concluded that rings B, C and D are of the chair conformation. Either conformation of ring C, however, satisfies the points raised in the following discussion. Figure 4 shows a diagramatic representation of the three dimensional arrangement of these structures. Because the relationship of the configuration of morphine and the morphinans to the amino-acids has not been established, morphine can be represented by structure (X) or (XI) and (—)-3-hydroxy-*N*-methyl morphinan by (XII) or (XIII). It is concluded that the structures, as shown (X and XI), represent the surface of the drug which can fit at a complementary receptor surface. The flat aromatic ring A and the basic group lie in almost the same plane, with the $-\overset{15}{C}H_2-\overset{16}{C}H_2-$ part of the piperidine ring D projecting slightly in front, and to the side, of the line between the centre of ring A and the basic group. If a cavity is present in the receptor to accommodate the projecting $-CH_2-CH_2-$ portion, then the electrostatic attraction for the basic group as a cation (at physiological *p*H morphine (pK_a 7·88)[28] will be approximately 80 per cent. ionised) for an anionic site in the receptor could be reinforced by the collective van de Waals' forces between benzene ring A and a flat portion of the receptor surface (e.g. see Albert[11]). If it is assumed that the configuration of morphine is (X) rather than (XI), its mirror image, then (XIV) would represent a diagramatic representation of the complementary surface of the receptor. (If morphine is configuration (XI) then the receptor surface will be the mirror image of the one shown.)

It is postulated, therefore, that the receptor surface by which analgesic action is mediated has the following three essential sites:

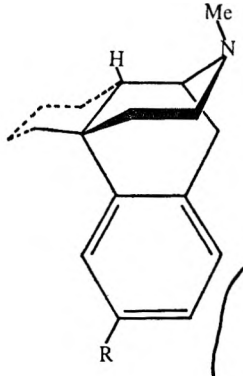
1. A flat portion allowing of van de Waals' forces binding the aromatic ring of the analgesic drug;
2. An anionic site;



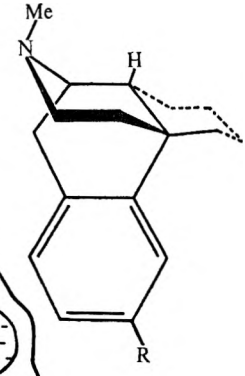
(X) Morphine



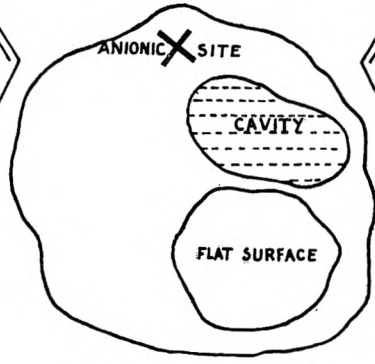
(XI)



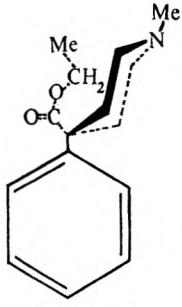
Levorphan (XII)



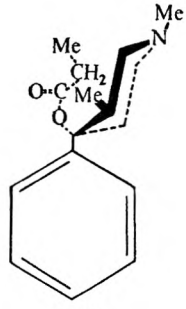
(XIII)



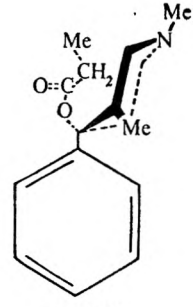
(XIV) Receptor surface



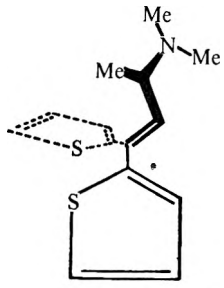
(XV) Pethidine



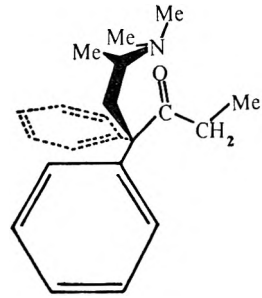
(XVI)



(XVII)



(XVIII)



(XIX) Methadone

FIG. 4. Diagrammatic representation of the three dimensional arrangement of analgesics and the "analgesic receptor surface." The diagrams represent the lower surface of the drug and the upper surface of the receptor, i.e., complementary surfaces. In front of, behind, and in the plane of the paper are represented by ———, ·····, and ——— respectively.

3. A cavity suitably orientated with sites 1 and 2 as diagramatically represented in (XIV).

The association of drug donor groupings with sites 1 and 2 then represents the primary site of analgesic action whereas correct alignment of a projecting hydrocarbon residue with the cavity in one enantiomorph can enhance the drug-receptor contact and consequently the analgesic activity, and, in the opposite enantiomorph, the projecting group will impair the drug receptor contact. The difference in activity between levorphan (XII; R = OH) and dextrorphan (XIII; R = OH) is explicable in terms of the above receptor surface hypothesis. The replacement of the *N*-methyl group of morphine or levorphan by the *N*-allyl group will not impair the fit at the receptor surface. Yet the alteration in structure is enough to make these substances sufficiently dissimilar from their parent compounds to cause them to fail to carry out the normal reaction of the latter when adsorbed. The reason for the simultaneous mild analgesic and anti-analgesic action exhibited by certain of the *N*-allyl*nor*morphine type compounds and the lack of anti-analgesic action in (+)-*N*-allyl-3-hydroxymorphinan is also explicable.

The implications of the presence or absence of phenolic or other groups and their effect upon partition coefficients and dissociation constants, etc., will be described in detail in a subsequent publication.

The synthetic analgesics of less rigid structures may be considered in terms of association with the essential receptor sites specified above.

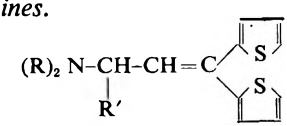
Pethidine and related structures.

The most stable conformation of pethidine will be a chair form for the piperidine ring with the large phenyl group probably in the equatorial and the ester group in the axial position. This structure fits the essential three receptor sites (see XV and XIV). The greater activity exhibited by the pethidine type compound (XVI), in which the methyl and propionoxy groups are *cis*, compared with compound (XVII) in which they

are *trans*[29] becomes explicable in terms of the *cis* methyl (equatorial) fitting the receptor cavity better than the *trans* methyl (axial).

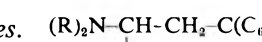
The configuration of the two isomers XVI and XVII was not established unequivocally. If the assignments are incorrect, compound XVII would be the more active isomer and its most stable conformation would probably be that in which the phenyl group was in the axial, and the propionoxy and methyl groups in the equatorial positions. The resulting three dimensional arrangement would be nearer to that of morphine than the one diagrammatically represented in XVII. This point will be discussed more fully in a later publication when further experimental evidence has been obtained.

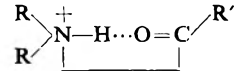
Dithienylbutenylamines.



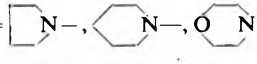
(XX)

The use of molecular models (Catalin) clearly reveals that, if R and R′ = CH_3, the D-(+)-isomer can have one thienyl ring and the basic group fitting at the flat surface and the anionic site of the receptor respectively, while the methyl group will fit into the cavity (see Fig. 4, XVIII and XIV). In the L-(−)-isomer the methyl group is not quite correctly orientated although there is not a great difference between the "fit" of the two isomers. These facts are compatible with the fact that the D-(+)-isomer is more active than the L-(−)-isomer although both exhibit analgesic activity. The same explanation and correlation applies to the observed structure and activities when R = C_2H_5 and R′ = CH_3. The use of molecular models also revealed that increase in size of group R′ would result in a less favourable alignment of the thienyl ring and basic centre with the receptor sites; this observation is compatible with the pharmacological results of decrease in activity with increasing size of group R′[6].

Methadone and related structures.  (XXI).

In methadone (XXI; R = CH_3; R′ = CH_3; R″ = COC_2H_5) the bulky groups attached to the quaternary carbon atom impose severe restraint to free rotation in the molecule. The planar phenyl groups will tend to positions of maximum clearance, that is to positions corresponding to the sides of a trihedral angle with the quaternary carbon at the apex (an effect termed "trihedralisation"[30]). The other bulky groups impart a certain rigidity to the molecule, especially since the carbonyl oxygen atom and the nitrogen atom can approach so close to each other that it seems probable that, when this basic group is in the cationic form, hydrogen bonding of the type  occurs. The molecule can be regarded

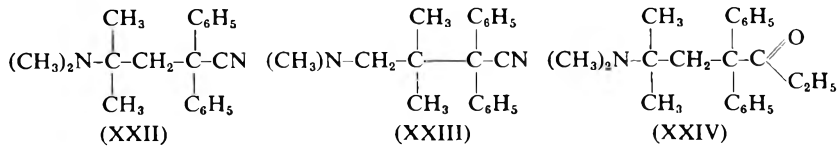
as semi-rigid and the D-(−)-isomer of methadone has then a correct alignment if one phenyl ring, the basic group and the CH_3– group fit the flat surface, the anionic site and the cavity of the receptor surface respectively (see Fig. 4, XIX and XIV). Models reveal that the L-(+)-isomer would be expected to fit at the same receptor surface, although not so well as the D-(−). It is significant that, although the former isomer is almost completely devoid of analgesic activity, it reduces the activity of the latter when both are given together, indicating that both ease of attachment and the nature of the drug–receptor combination are important in analgesic activity. The difference in activities between the D-(−)- and the L-(+)-isomers of the sulphone analogue of methadone (XXI; $R = CH_3$, $R' = CH_3$, $R'' = SO_2C_2H_5$) and of the (+)- and (−)-isomers of isomethadone can also be explained as above. Reduction of isomethadone yields the isomethadols which would be expected to fit the same receptor surface as the active analgesic. This conclusion is supported by the fact that analgesically inactive α-isomethadol, in large doses, partially blocks the analgesic activity of isomethadone; if methadone and the slightly active α-methadol are given together a sum of the effects of the two compounds given separately is obtained.[31] In methadone compounds, the significance of the dependence of analgesic effect upon configuration of the isomer is not so clear as in the cases which have been discussed previously, e.g., α-(−)-methadol, α-(−) and β-(+)-acetylmethadol, all derived from the relatively inactive L-(+)-methadone, possess a fair degree of activity[32]; the L-(+)-ester (XXI; $R = CH_3$, $R' = CH_3$, $R'' = COOC_2H_5$), although possessing only weak analgesic activity, is more active than its D-(−)-isomer.[5] However, these compounds can "fit" the analgesic receptor surface (Fig. 4. XIV) and consequently the nature of the drug–receptor combination is the most important factor involved in their activity. There are indications that there are limitations to the size of basic groups permissible if the drug is to combine with the receptor because, although 5- and 6-membered basic ring structures (XXI);

$(R)_2N =$  in amidone analogues are active, 7- and

8-membered rings yield inactive products[33].

The fact that D-(−)-methadone possesses high analgesic activity while its L-(+)-isomer is almost inactive made the preparation of "6-methylmethadone" (6-dimethylamino-6-methyl-4 : 4-diphenylheptan-3-one) (XXIV) of interest. If one phenyl group, the basic group and one methyl group (on C 6) in this compound are correctly orientated towards the receptor surface (as in D-(−)-methadone) then the other C 6 methyl group will be positioned as in L-(+)-methadone with phenyl and basic group fitting at the appropriate receptor sites. This substance has been prepared and has been shown to be devoid of analgesic activity indicating that, despite the correct configurational arrangement of the 3 essential analgesic groupings, an incorrectly positioned methyl group can prevent the correct drug–receptor combination.

"6-Methylmethadone" (XXIV) was prepared by treating 3-dimethylamino-3-methyl-1 : 1-diphenyl-butyl cyanide (XXII) with ethyl magnesium bromide. This cyanide was formed together with the isomeric 3-dimethylamino-2 :2-dimethyl-1 : 1-diphenyl-propyl cyanide (XXIII) by the condensation of 1-chloro-2-dimethylamino-2-methyl propane with diphenylmethylcyanide.



(XXII) (XXIII) (XXIV)

It has been observed that condensations between 2-amino-1-chloropropanes, or 1-amino-2-chloropropanes (e.g., $(CH_3)_2N\,CH_2{\cdot}CH(Cl){\cdot}CH_3$, and diphenylmethyl cyanide yield pairs of isomeric cyanides[33,34,35,36], the cyanide group of one member being hindered as shown by the isolation of a stable ketimine from the cyanide-Grignard complex and the difficulty of hydrolysis of the cyanide group. The two cyanides obtained in the present work (XXII and XXIII) varied greatly in their reactions. Ethylmagnesium bromide yielded a ketone with one cyanide (A) but failed to react with cyanide (B). The latter was also recovered unchanged after treatment with lithium ethyl and attempted hydrolysis with 20 per cent. hydrobromic acid. These facts demonstrate the hindered nature of the cyanide group in (B) and consequently structure (XXII) has been allocated to the less hindered cyanide (A) and structure (XXIV) "6-methylmethadone" to the derived ketone.

Conclusion

It is postulated that, if an organic compound is to exhibit high analgesic activity, the following essential features are necessary:

1. A basic centre which is ionised as a cation at physiological *p*H, in order that it may be able to associate with an anionic site in the receptor surface.
2. A flat aromatic structure in the molecule to allow of a strong collective van der Waals' force bonding to a flat portion of the receptor reinforcing the ionic bond mentioned in (1) which otherwise would not be sufficiently permanent because of ion exchange under biological conditions.
3. The basic group and the flat structure to be almost in the same plane; this to be accomplished by a completely rigid molecule or a slightly less rigid one held in the correct configuration by steric or other constraints.
4. A suitably positioned projecting hydrocarbon moiety to form, with the basic centre and flat aromatic structure, a three dimensional geometric pattern indicated in Fig. 4.

Experimental

All m.pts. are uncorrected.

Equivalent weights of the bases were determined by titration with 0·02N perchloric acid in glacial acetic acid using crystal violet as indicator. Titration of the hydrohalide salts was carried out in non-aqueous media in the presence of mercuric acetate by the method described by Pifer and Wollish[37].

Configuration of certain analgesics. (−)-Methadone, (−)-ethyl 3-dimethylamino-1 : 1-diphenylbutyl sulphone, (+)-3-dimethylamino-1 : 1-di-(2′-thienyl)-but-1-ene and (+)-3-diethylamino-1 : 1-di-(2′-thienyl)-but-1-ene have been shown to possess identical configurations related to that of D-(−)-alanine (full details will be published elsewhere).

2-*Dimethylamino*-2-*methylpropan*-1-*ol.* This was prepared in 83 per cent. yield by the method described by Rosnati[38]; Equiv. wt. 117 (theory 120), methiodide m.pt. 237° to 238° C. (decomp.).

1-*Chloro*-2-*dimethylamino*-2-*methylpropane hydrochloride.* Thionyl chloride (7·5 ml.) was added slowly, with stirring, to a solution of 2-dimethylamino-2-methylpropan-1-ol (5·85 g.) in benzene (50 ml.) cooled in ice, and the mixture then refluxed for 1 hour. The crystals (7 g.) which separated upon cooling were washed with benzene and recrystallised from acetone-methanol to yield 1-*chloro*-2-*dimethylamino*-2-*methylpropane hydrochloride* as colourless platelets m.pt. 166° to 167° C. Found: C, 41·9; H, 8·5; N, 8·5; Cl, 41·2 per cent. Equiv. wt., 168. $C_6H_{15}NCl_2$ requires C, 41·8; H, 8·7; N, 8·1; Cl, 41·3 per cent. Equiv. wt., 172.

Condensation of 1-*chloro*-2-*dimethylamino*-2-*methylpropane with diphenylmethyl cyanide.*

Sodamide (4 g.) was added to a solution of diphenylmethyl cyanide (19·3 g.) in dry benzene (150 ml.) and the mixture stirred until no further darkening occurred. A solution in benzene (150 ml.) of 1-chloro-2-dimethylamino-2-methylpropane, freshly liberated from the hydrochloride (17·2 g.), was added drop by drop and the mixture stirred overnight. The bases were extracted with dilute hydrochloric acid, the acidic extracts made alkaline with solution of ammonia, and the liberated bases extracted with ether. The ethereal extract was dried (sodium sulphate), and the solvent removed to yield a yellow oil (23 g.) which, after dissolving in boiling light petroleum (b.pt. 40° to 60° C.) (10 ml.) and then cooling, gave pale yellow crystals of *cyanide A*, m.pt. 73° C. Found: C, 82·4; H, 8·25; N, 9·4 per cent. Equiv. wt., 290. $C_{20}H_{24}N_2$ requires C, 82·2; H, 8·2; N, 9·6 per cent. Equiv. wt., 292. The *hydrobromide* crystallised from acetone/ether as colourless needles mp.t 183° C. Found: C, 63·7; H, 6·7; N, 7·2; Br 21·0 per cent. Equiv. wt., 367. $C_{20}H_{25}N_2Br$ requires C, 64·3; H, 6·7; N, 7·5; Br, 21·5 per cent. Equiv. wt., 373.

The mother liquors, after removal of the solvent, were treated with excess of ethanolic hydrobromic acid. A white solid precipitated and was recrystallised from acetone to yield *cyanide B hydrobromide* (10·5 g.), m.pt. 239° C. (d). Found: C, 64·0; H, 6·7; N, 7·7; Br, 21·4 per cent.

Equiv. wt., 376. $C_{20}H_{25}N_2Br$ requires C, 64·3; H, 6·7; N, 7·5; Br, 21·5 per cent. Equiv. wt., 373.

6-Dimethylamino-6-methyl-4 : 4-diphenylheptan-3-one (XXIV).

Cyanide A (2 g.) in dry toluene (15 ml.) was added to ethylmagnesium bromide in ether (10 ml.) prepared from magnesium (0·75 g.) and ethyl bromide (3·4 g.). The ether was distilled off, the mixture refluxed for 3 hours and then added to crushed ice and concentrated hydrochloric acid (8 ml.). The solid which separated was washed, the base liberated with dilute solution of ammonia and extracted with ether. After drying (sodium sulphate) the solvent was removed to yield a pale yellow oil (1·6 g.) which crystallised on standing, and was recrystallised from ethanol to give colourless crystals of *6-dimethylamino-6-methyl-4 : 4-diphenylheptan-3-one*, m.pt. 81 to 82° C. Found: C, 82·1; H, 8·95; N, 4·3 per cent. Equiv. wt., 320. $C_{22}H_{29}NO$ requires C, 81·7; H, 9·0; N, 4·3 per cent. Equiv. wt., 323.

Reactions of cyanide B. The cyanide was recovered unchanged when subjected to the above reaction, and when treated with a fourfold equivalent of lithium ethyl. It was not hydrolysed upon boiling with 20 per cent. aqueous hydrobromic acid for 3 hours.

Pharmacological results.

The method of testing used was a tail reflex method[39] which gives results exactly corresponding to the tail pain method described by Grewal[40].

(+)-3-Diethylamino-1 : 1-di-(2′-thienyl)-but-1-ene is as active, and the (±)-compound slightly more than 50 per cent. as active, as (±)-methadone. 6-Dimethylamino-6-methyl-4 : 4-diphenylheptan-3-one is devoid of analgesic activity.

Summary

1. The evidence is presented for the probability of the activity exhibited by analgesics and their antagonists being due to their association with a specific receptor surface.

2. The configurations of those isomers exhibiting nearly all the activity of certain racemic mixtures are identical, and related to that of D-(−)-alanine; the stereochemical requirements of analgesics are considered.

3. Active analgesics are shown to have structures which enable them to present similar surfaces to allow of their association with a proposed "analgesic receptor surface," the essential features of which are described.

4. A number of essential structures are outlined as prerequisites for compounds designed as analgesics.

The authors wish to express their thanks to Dr. M. F. Lockett for carrying out the pharmacological testing.

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DISCUSSION

The paper was presented by DR. A. H. BECKETT.

DR. F. HARTLEY (London) said that many denied that any substantial correlation had been found between chemical structure and physiological action, but it was desirable that, as far as possible, attempts should be made to correlate some aspects of chemical structure, however simple, with activity, in order to formulate a working hypothesis. The authors had selected an excellent test case in 6-methylmethadone. The extra methyl group should interfere with the analgesic behaviour, and, as had been shown, the compound was inactive. This might be regarded as a critical test, but it was unfortunate that in preparing 6-methylmethadone the reaction of the diphenylacetonitrile with the chlorodimethylamino-alkyl-derivative was ambiguous, as the reaction could proceed in two ways. Although Dr. Beckett had advanced *prima facie* evidence as to why the structure he had assigned to the compound was correct, it was important that the critical example should be beyond reproach. In order to establish the structure with certainty Dr. Beckett should degrade his compound to establish the position of the methyl groups beyond doubt.

DR. G. E. FOSTER (Dartford) said he was not clear how the authors explained why nalorphine was an antagonist to morphine and other synthetic analgesics when they all fitted the proposed receptor surface.

DR. J. B. STENLAKE (Glasgow) expressed the opinion that the conformations suggested for pethidine and the two esters XVI and XVII were open to question on the grounds that the propionoxy and phenyl groups were of comparable size. A pethidine molecule with a conformation having an axial 4-phenyl group bore a far more striking resemblance to the 3-dimensional structure of morphine than did the alternative conformation in which the 4-phenyl group was equatorial. He considered that the conformation suggested for the ester XVII with an axial 3-methyl group was improbable, and that in fact the most likely stable conformation would be the alternative one in which both methyl and propionoxy groups were equatorial and the phenyl group axial. He agreed that in XVI the phenyl group was most probably equatorial. He went on to point out that the configurations of XVI and XVII had not been rigidly assigned, and suggested that the known greater ease of hydrolysis of the least active isomer was open to the alternative interpretation that in this isomer (actually XVI) the propionoxy group was axial. In consequence the phenyl group would be equatorial and the molecule would be in an unmorphine-like conformation. Its more stable and more active isomer would then be that in which the phenyl group was axial with the molecule in a morphine-like conformation (XVII). He expressed the hope that the authors would seek further experimental evidence of the configuration of these two isomers.

DR. G. BROWNLEE (London) said that although nalorphine antagonised some of the effects of morphine, it did not antagonise all of them, which seemed to indicate that there was more than one receptor site. This did not detract from the value of the hypothesis that there was not a single simple receptor site for morphine. It was possible that 6-methylmethadone should also have some functions which might be measured. Did it reverse the action of morphine? What action did it have on smooth muscle?

DR. BECKETT, in reply to Dr. Hartley, said that work was in progress to establish the constitution of the product obtained by the condensation of diphenylacetonitrile with 1-chloro-2-dimethylamino-2-methylpropane in an unequivocal manner. The lack of analgesic activity of the final ketone, however, supported the hypothesis irrespective of whether it was 6-methyl methadone or 6-methyl-*iso*methadone. Replying to Dr. Foster, he said that nalorphine and certain other substances in which the *N*-methyl group of morphine and related compounds was replaced by *N*-allyl and other groups could 'fit' the proposed receptor site. A gradation of analgesic and anti-analgesic action was produced. Replying to Dr. Stenlake, he said that he agreed that there was some doubt concerning the correctness of the configuration assigned by the American workers to the isomers XVI and XVII. Work was in progress to establish their configurations. If the configurational assignments were in fact reversed, it would then

be reasonable to postulate the most stable conformation for the more active isomer with an axial phenyl and the methyl and propionoxy groups in the equatorial position. It could then be postulated that the phenyl group of pethidine was axial when the molecule was adsorbed on the receptor surface because "fit" at the surface under biological conditions could favour the adoption of the authors' conformation in preference to an alternative one of approximately equal stability as the contact points were coming together. However, the general hypothesis was unaffected by the problems still requiring solution in connection with these pethidine type compounds. In reply to Dr. Brownlee, he said that he would be disappointed if 6-methylmethadone reversed the activity of morphine because the evidence suggested that it did not "fit" at the proposed analgesic receptor surface.

THE ASSAY OF BENZATHINE PENICILLIN BY TITRATION IN A NON-AQUEOUS SOLVENT

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Since the description of *NN'*-dibenzylethylenediamine dibenzylpenicillin (benzathine penicillin) by Szabo, Edwards and Bruce[1], this material has become widely used as a repository-type penicillin. Since it is only very slightly soluble in aqueous media, although this property is made use of in therapeutics, its chemical assay is somewhat difficult. As has been pointed out by Parker and Donegan[2] it is impossible to obtain an aqueous solution of sufficient strength for a trustworthy assay by the normal iodimetric method. Since benzathine penicillin contains four secondary nitrogen atoms, it was thought likely that these would be readily titratable, in a suitable non-aqueous system. This was found to be the case, a solution of the amine in glacial acetic acid titrating readily with 0·1N perchloric acid in glacial acetic acid.[3]

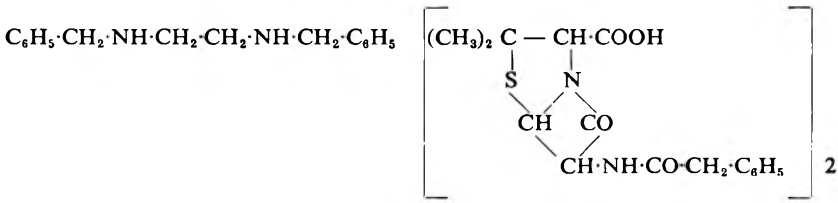
Method

Standardisation of the perchloric acid solution

Dissolve about 0·4 g. of A.R. potassium hydrogen phthalate, accurately weighed, in 70 ml. of glacial acetic acid containing 2 per cent. of acetic anhydride, by gently refluxing. Cool the solution, add 0·2 ml. of 0·1 per cent. aqueous solution of crystal violet and titrate with 0·1N perchloric acid to the first disappearance of the violet tinge.

Determination of the purity of the sample

Transfer 500 mg. to a conical flask and dissolve in 70 ml. of glacial acetic acid containing 2 per cent. of acetic anhydride. Titrate with 0·1N perchloric acid in glacial acetic acid, using crystal violet as indicator and titrating to the same colour as obtained in the standardisation of the perchloric acid. The formula for benzathine penicillin is



and its molecular weight 909·1 so that 1 ml. of 0·1N perchloric acid is equivalent to $\frac{909{\cdot}1}{40{,}000}$ g. of anhydrous benzathine penicillin. To calculate the purity of the sample as anhydrous compound:—

$$\text{Percentage} = \frac{\text{ml. of 0·1N perchloric acid} \times 909{\cdot}1 \times 100}{\text{g. of sample} \times 40{,}000}$$

The potency in I.U./mg. is given by:—percentage purity × 1307 (theoretical potency 1307 I.U./mg.).

A series of comparative determinations was run using this method, the two methods of Parker and Donegan[2], a method based on the determination of the optical density in absolute methanolic solution at 264 mμ[4] and a biological assay using *Staphylococcus aureus*. The results given in

TABLE I

COMPARATIVE ASSAYS OF BENZATHINE PENICILLIN

Batch	Water (Karl Fischer)	100-Water	Microbiological		Iodimetric		Optical density		Base extraction		Non-aqueous titration	
			Potency, I.U./mg.	Purity, per cent.	Potency, I.U./mg.	Purity, per cent.	Potency, I.U./mg.	Purity, per cent.	Potency, I.U./mg.	Purity, per cent.	Potency, I.U./mg.	Purity, per cent.
414	7·5	92·5	1284	98·2	1203	92·0	1177, 1139	90·0, 87·1	1188, 1191	90·9, 91·1	1207	92·3
415	7·0	93·0	1283	98·2	1204	92·1	1177, 1233	90·0, 94·3	1190, 1195	91·0, 91·4	1188	90·9
416	7·2	92·8	1240	94·9	1194	91·4	1215, 1233	93·0, 94·3	1200, 1203	91·8, 92·0	1196	91·5
59	7·3	92·7	1208	92·4	1183	90·5	1195	91·4	1185, 1177	90·7, 90·0	1164	89·2
60	7·2	92·8	1268	97·0	1203	92·0	1195	91·4	1191, 1186	91·1, 90·7	1169	89·4
61	7·3	92·7	1234	94·4	1186	90·7	1203	92·0	1199, 1187	91·7, 90·8	1172	89·7
62	7·5	92·5	1243	95·1	1191	91·1	1139	86·5	1195	91·4	1189	91·0
63	7·4	92·6	1220	93·3	1207	92·3	1225, 1207	93·7, 92·3	1202	92·0	1193	91·3
65	7·4	92·6	1220	93·3	1203	92·0	1225, 1233	93·7, 94·3	1204	92·1	1195	91·4
66	7·5	92·5	1216	93·0	1204	92·1	1233, 1215	94·3, 93·0	1198	91·7	1198	91·7
67	6·8	93·2	1218	93·2			1232, 1194	94·3, 91·4			1194, 1187	91·4, 90·9
68	7·0	93·0	1156	88·4			1262	96·6			1191, 1203	91·2, 92·1
69	6·6	93·5	1220	93·3			1266	96·9			1195	91·5
70	6·0	94·0	1220	93·3			1240	94·9			1179	90·3
71	7·1	92·9	1201	91·9			1240	94·9			1211	92·7
72	7·1	92·9	1220	93·3			1157, 1164	88·6, 89·1			1198	91·7
73	6·3	93·7	1213	92·8			1194, 1202	91·4, 92·0			1198	91·7
74	6·9	93·1	1220	93·3			1194, 1176	91·4, 90·0			1204	92·3
411	7·3	92·7	1255	96·0			1194	91·4			1183	90·6
412	7·3	92·7	1207	92·4			1325	101·4			1195	91·5
413	7·1	92·9	1264	96·7			1150, 1145	88·0, 87·7			1211	92·7
410	7·3	92·7	1235	94·5			1204	92·2			1203, 1207	92·1, 92·4
409	6·8	93·2	1196	91·5			1194	91·4			1191, 1194	91·2, 91·4
58	7·0	93·0	1286	98·4			1187	90·9			1178, 1173	90·2, 89·8

Table I show that the proposed method gives results which are in reasonable agreement with those given by other methods, and is rapid.

SUMMARY

1. A method for the assay of benzathine penicillin using titration with perchloric acid in glacial acetic acid is described.

2. Results are set out and show favourable comparison with four other methods.

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DISCUSSION

The paper was presented by MR. W. H. STEPHENSON.

Mr. F. A. J. TALMAN (Liverpool) asked whether the authors had any information on the use of this assay for preparations such as suspensions and tablets.

DR. F. HARTLEY (London) said that penillic acid had about the same strength as penicillin acid, and asked what would happen if they were dealing with a partly degraded benzathine penicillin. Iodimetric determination of the degradation product would then appear to be advantageous over the recommended titration method.

DR. A. H. BECKETT (London) said that 2 per cent. of acetic anhydride had been added to glacial acetic acid. Knowing that excess acetic anhydride immediately acylated secondary or primary amines, it was possibly a little dangerous to have this amount present. Usually it was customary, when titrating amines, secondary or primary, to make sure that a trace of water was present. Possibly the lower results in Table I by titration, as compared with the optical density method, could be explained in terms of a trace of acylation. The end-point colour for the standardisation using potassium hydrogen phthalate and the colour in the actual determination were stated to be the same. Had this been checked potentiometrically? When potassium ions were present, there was precipitation of potassium perchlorate, which altered the colour at the end-point.

DR. G. E. FOSTER (Dartford) said that some analysts had difficulty in determining the end-point with crystal violet. He had used quinaldine red. Had the authors used any other indicators?

MR. STEPHENSON, in reply, said their method was used with benzathine penicillin itself. In suspensions it was necessary to carry out a blank on the suspending gel. They were in the experimental stage of their work on tablets. He had no analytical evidence with penicillic acid. He agreed that the 2 per cent. excess of acetic anhydride might be responsible for the somewhat lower results. A potentiometric titration had been carried out to check the colorimetric end-point. This might be preferable, since crystal violet was not an ideal indicator. They had not used quinaldine red.

THE CHEMISTRY OF *ARISTOLOCHIA* Spp.

PART I. THE PETROL-SOLUBLE FRACTION FROM *Aristolochia reticulata*

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SPECIES of *Aristolochia* have been widely used in medicine since Greek and Roman times, though they have now largely fallen into disuse. Numerous observations have been recorded on the action and chemical constituents of *Aristolochia* of many different species (for references *see* Rosenmund and Reichstein[1]), but for the most part these studies are incomplete. At least one bitter principle, aristolochic acid, from which the *Aristolochia* are thought to derive their action, appears to be common to most species. The constitution of this acid is unknown, though Rosenmund and Reichstein[1] and others[2,3,4,5,6,7,8] have examined some of its reactions. The presence of an ethereal oil in *A. serpentaria* was mentioned first by Bucholz[9] and again, later by Spica[10], who recorded the presence of borneol. Peacock[11] examined the ethereal oil from *A. reticulata* and found it to consist mainly of an oily substance, $C_{15}H_{25}O_2$ which gave borneol and an acid (probably $C_5H_9O_2$) on saponification. A monoterpene, b.pt. 157° C., a fraction $C_{18}H_{29}O$, b.pt. 240° C., and a blue fluorescent oil were also obtained. More recently, 3 sesquiterpene fractions, designated ishwarene, ishwarol and ishwarone respectively have been found in the oil from the roots of *A. indica*[8]; the chemical structures of these substances have not yet been elucidated.

The present studies are concerned with the petrol-soluble fraction isolated from a batch of *A. reticulata* (serpentary root). The oil (3·9 per cent.), obtained by cold percolation of dried powdered serpentary root with light petroleum, was dark brown in colour, and of pleasant odour, similar to that of the root itself. Acidic substances were first removed by extraction of the oil in light petroleum, with saturated aqueous sodium bicarbonate, as a glassy resinous solid (0·25 per cent. of the oil), of high equivalent weight (*ca.* 1400), which exhibited a violet fluorescence under ultra-violet light.

Steam distillation of a small portion of the residual oil yielded, in the later runnings, a small quantity of a colourless crystalline solid. A much larger quantity of this same material was slowly deposited from the main bulk of the oil when it was cooled and seeded with crystals of the solid. This substance, a hitherto unknown lactone $C_{15}H_{20}O_2$, designated *aristolactone*, was crystallised in colourless platelets, m.p.t. 110·5° to 111° C. and will be described in greater detail in later communications.

Ketonic material present in the oil did not form a sodium bisulphite addition complex, but was readily extracted with Girard's reagent-T as a brownish oil (3 per cent.), which was strongly dextrorotatory, ($[\alpha]_D^{17°\,C.} + 170°$). A small portion of this ketonic oil was further resolved into two components, a brownish-yellow viscous semi-solid ($[\alpha]_D^{13°\,C.} + 62°$), insoluble in light petroleum, and a pale yellow oil $[\alpha]_D^{13°\,C.} + 179°$, soluble

in light petroleum. The insolubility of the former fraction in light petroleum is of interest and suggests that it may be an artefact, though the possibility that it was solubilised in the original extract by the presence of various terpene fractions cannot be discounted entirely. None of the usual crystalline derivatives could be isolated from either of these two ketone fractions, which therefore appear to require further fractionation.

Steam distillation of the oily residue yielded a number of volatile fractions (Table I).

TABLE I

Fraction	Weight, g.	Characteristics
I	200·0	Colourless mobile liquid (mainly light petroleum and ether) but containing traces of oil.
II	19·0	Colourless mobile liquid (mainly light petroleum).
III	22·4	Almost colourless fragrant oil.
IV	30·9	Pale yellow oil, $d_{15^\circ C.}^{15^\circ C.}$ 0·960, $[\alpha]_D^{16^\circ C.}$ − 6·94°.
V	7·0	Yellow oil, $d_{15^\circ C.}^{17^\circ C.}$ 0·962, $[\alpha]_D^{17^\circ C.}$ + 33·0°.

The oil present in fraction I could not be separated from solvent by fractional distillation and was isolated as a dibromide after treatment with bromine. The product, a colourless oil, yielded a small quantity of a colourless crystalline solid, m.pt. 87° to 88° C. when chromatographed from light petroleum on alumina. This substance was undoubtedly the same as the dibromide subsequently isolated from fractions A and B as described in the sequel. Repeated refractionation of fractions II and III gave further fractions as shown in Table II.

Unfortunately, fractionation on this small scale was not complete and most of the fractions were found to be mixtures. We have, however, succeeded in identifying a number of the components. Analysis of the physical data recorded for the various fractions suggested the presence of at least 3 components, a monoterpene (A and B), an ester (C and D) and a sesquiterpene (E and F).

Few monoterpenes have densities as high as those recorded for fractions A and B ($d_{15^\circ C.}^{15^\circ C.}$ 0·856 and 0·882 respectively) and contamination with denser ester fractions present in C and D was suspected. However, saponification revealed the presence of only traces of esters and chromatography of the neutral material gave an oil, $d_{15^\circ C.}^{15^\circ C.}$ 0·859, $n_D^{15\cdot5^\circ C.}$ 1·4742, $[\alpha]_D^{17^\circ C.}$ −59°, $[R_L]_D$44·59. These constants are substantially in agreement with those for (+)-Δ^4-carene (I), which are given[12] as $d_{30^\circ C.}^{30^\circ C.}$ 0·8552, $n_D^{30^\circ C.}$ 1·4740, $[\alpha]_D^{20^\circ C.}$ + 62·2°, $[R_L]_D$ 44·60, the optical exaltation of the latter (0·75), over the theoretical value for a bicyclic monoethenoid terpene $C_{10}H_{16}$, $[R_L]_D$ 43·85 being due to conjugation of the ethylenic bond and *cyclo*propane ring. The presence of a single double bond in the molecule of our terpene was confirmed by an iodine value and by the low intensity ultraviolet absorption maximum at 210mμ (ϵ 4200), characteristic of a trisubstituted ethylenic bond[13,14]. Unfortunately Δ^4-carene does not yield any well authenticated crystalline derivatives. Reaction with bromine is said to give

(I)

a viscid oil, which shows no sign of crystallising. Bromination of our purified monoterpene, similarly gave a colourless viscous oil, but which, after chromatography yielded a colourless crystalline dibromide, $C_{10}H_{16}Br_2$, m.pt. 89° C., $[\alpha]_D^{17° C.}$ + 98° identical (m.pt. and mixed m.pt.) with that obtained from fraction I. Further work on this terpene fraction is at present restricted from lack of material, but it is interesting to note, however, in connection with its tentative recognition as (−)-Δ^4-carene, that, to date, only the dextrorotatory form of this terpene has been isolated from natural sources.

Constants for fraction C were in reasonable agreement with literature figures for (−)-bornyl formate ($d_{4° C.}^{20° C.}$ 1·006, $n_D^{15° C.}$ 1·4708, $[\alpha]_D$−49°), but saponification with ethanolic potassium hydroxide gave the equivalent weight as 276, equivalent to only about 70 per cent. of bornyl formate. Extraction of the neutralised saponification liquors yielded neutral material, which was separated chromatographically into (−)-borneol, and a small quantity of a colourless oil, $C_{15}H_{24}$. (−)-Borneol, was confirmed by conversion to its *p*-nitrobenzoate and by oxidation to camphor. Physical constants of the oil, $C_{15}H_{24}$ ($d_{16° C.}^{16° C.}$ 0·913, $n_D^{16° C.}$ 1·4955, $[\alpha]_D^{15° C.}$ + 1·6°, $[R_L]_D$ 65·46) were characteristic of a bicyclic sesquiterpene ($[R_L]_D$ 66·1) with two ethylenic bonds (*see* Simonsen and Barton[15]), and the presence of these two ethylenic bonds was confirmed by an iodine value. We have designated this sesquiterpene, *reticulene*.

The aqueous liquors remaining after the extraction of borneol and reticulene exhibited reducing properties typical of formic acid. However, the immediate formation of a buff precipitate on addition of ferric chloride solution was atypical, and acidification of the solution caused the deposition of oily droplets of a second (water-insoluble) acid. The latter was extracted with light petroleum, whilst the light petroleum-insoluble formic acid was isolated from the residual liquors by steam distillation. Neutralisation and concentration of the steam distillate gave crystalline sodium formate, identified by conversion to the corresponding *p*-bromophenacyl ester, by a determination of sulphated ash and by its conversion to sodium oxalate when heated rapidly to 360° C.

The water-insoluble acid was obtained as a colourless oil, which gave a pale buff precipitate with ferric chloride and a white gelatinous precipitate with silver nitrate. The equivalent weight was found by titration to be 165. A more reliable figure of 169, obtained by gravimetric determination of barium (as sulphate) in the crystalline barium salt was in agreement with its formulation as $C_{10}H_{16}O_2$. The acid was unsaturated, containing a single ethylenic bond as indicated by the uptake of hydrogen on microhydrogenation. This conclusion was confirmed by the iodine value, equivalent to one double bond. The acid formed a crystalline benzyl-*iso*thiouronium salt and with diazomethane it yielded a pleasant smelling (camphoraceous) liquid ester unfortunately, insufficient in quantity for proper characterisation.

Constants for the acid ($n_D^{20° C.}$ 1·5019; $d_{15° C.}^{15° C.}$ 1·050; $[R_L]_D$ 47·29) and for its reduction product ($d_{15° C.}^{15° C.}$ 1·030) suggested that the acid should be formulated with a monocyclic ($[R_L]_D$ 47·25) rather than an open chain structure

($[R_L]_D$ 49·45). This conclusion was supported by the molecular formula $C_{10}H_{16}O_2$. The low intensity ultra-violet absorption maximum at 215mμ (ϵ 2970), which was absent from the spectrum of the corresponding saturated acid, suggests that this oily acid may well be a mixture of related α,β- and β,γ-unsaturated acids. This view is supported by the fact that on long standing the oil slowly crystallised, to yield an acid, which in the purest form obtained, melted at 72° to 73° C. This substance exhibited a high intensity ultra-violet absorption maximum at 206mμ (ϵ 9980), characteristic of an α,β-unsaturated acid. The crystalline acid is similar to and is possibly identical with the acid isolated by Peacock,[11] which melted about 65° C. and gave a flesh-coloured precipitate with ferric chloride. Its formulation by Peacock as $C_5H_9O_2$ can be explained by the fact that it was based not upon a direct analysis of the acid, but merely on an analysis of the parent bornyl ester fraction, which had also been shown to contain acetic acid.

Saponification of fractions D and E, and treatment as above yielded further small quantities of the water-insoluble acid. The neutral fractions, isolated after saponification contained considerable proportions of the sesquiterpene reticulene. Fraction F was almost pure reticulene.

Experimental

Melting points are uncorrected. Rotations were determined in absolute ethanol (unless otherwise stated) in a 1 dcm. tube. Ultra-violet absorption spectra were determined in absolute ethanol in 1 cm. cells using a Hilger Uvispek photoelectric spectrophotometer.

Extraction of Serpentary Root. The dried root (28 lb.) in No. 60 powder, was extracted with light petroleum (b. pt. 40° to 60° C.) by cold percolation until the percolate was just very pale yellow. Concentration of the percolate gave a dark greenish-brown oil (700 g.), still containing some solvent and having a pleasant odour similar to that of the root.

Removal of free acids. The oil from the above extraction was diluted with light petroleum and extracted with saturated aqueous sodium bicarbonate. The acids, recovered by acidification of the aqueous solution and extraction with light petroleum were obtained as a pale brown viscous oil (0·51 g.).

Aristolactone (a) The oil from a premilinary small scale extraction of *A. reticulata* (6 lb.) was steam distilled and the distillate collected in 27 fractions, each of 350 to 500 ml. The oily material present in each fraction of distillate was extracted with light petroleum. Fractions 6, 7, 9 and 10 on evaporation yielded pale yellow oils, from which aristolactone separated in varying amount, as a colourless solid, m.pt. 104° to 108° C. raised by recrystallisation from acetone-water to 110·5° to 111° C., $[\alpha]_D^{14° C.}$ + 156·4° (C, 1). Found: C, 77·5; H, 8·8 per cent. $C_{15}H_{20}O_2$ requires C, 77·6 H, 8·7 per cent.

(b) the bulk sample of oil (700 g.) when concentrated to remove the last traces of light petroleum and seeded with a single crystal of aristolactone and cooled (refrigerator, 2 days) gave crude crystalline aristolactone (16 g.). Seeding is essential, cooling alone being insufficient.

Isolation of Carbonyl Compounds

(a) *Sodium Bisulphite Addition Compounds.* A light petroleum solution of the oil remaining after the separation of aristolactone was shaken with saturated aqueous sodium bisulphite continuously for 4 days. Small quantities of amorphous material separated at the interphase; further treatment with sodium carbonate failed to yield carbonyl compounds.

(b) *Extraction with Girard's Reagent-T.* The extract (100 g.) was refluxed for 1 hour with a solution of Girard's Reagent-T (10 g.) in a mixture of glacial acetic acid (10 ml.) and absolute ethanol (90 ml.).

After cooling and diluting with iced-water (to reduce the ethanol content of the solution to 10 per cent. w/v) neutralisation of 90 per cent. of the acetic acid (N sodium hydroxide; 158 ml.) and extraction of non-ketonic material with light petroleum, the aqueous solution of ketonic material was treated with sulphuric acid to give an approximately normal solution. This solution was allowed to stand for 1 hour and extracted with solvent ether. The ethereal solution was dried (sodium sulphate), treated with magnesium oxide (to remove acetic acid) and evaporated to give a pale brownish-yellow oil (3·14 g.), $[\alpha]_D^{17^\circ C.} + 170^\circ$ (C, 2·38).

(*c*) *Fractionation of the Ketonic Oil.* The oil (0·2 g.) was shaken with light petroleum (50 ml.). Insoluble material remained as a brownish-yellow viscous semi-solid (0·042 g.), $[\alpha]_D^{13^\circ C.} + 62^\circ$ (C, 0·428). Evaporation of the light petroleum gave a pale yellow oil (0·146 g.), $[\alpha]_D^{13^\circ C.} + 179^\circ$ (c, 2·07).

Steam Distillation of the Residual Oil. The oil, maintained at 120° to 130° C. was steam distilled and collected first in fractions of 1 l. (I-III) and later of 2 l. (IV and V). The oils, which readily separated were dried over sodium sulphate. Descriptions of these fractions are recorded in Table I.

Fraction I. The liquid (*ca.* 200 g.) consisting mainly of light petroleum, but containing traces of lævorotatory oil, was treated with bromine until the reagent was no longer decolourised. Evaporation of the solvent gave a trace of colourless oil (0·1 g.) which when, chromatographed on alumina from light petroleum, gave 3 fractions. The first and last fractions yielded colourless oils, which were not identified; the second fraction was obtained as a colourless, dextrorotatory crystalline solid, m.pt. 87° to 88° C. (from light petroleum).

Fractional Distillation of II and III. Fractions II and III from the steam distillation were combined and submitted to repeated fractional distillation under reduced pressure. Separation into fractions was followed by measurement of refractive index, density and optical rotation of each fraction obtained throughout the process. Constants for the 6 main fractions are given in Table II. Minor fractions, not specified in detail in Table II were also obtained at various stages of the fractionation procedure (Table III).

Fraction A. The oil (0·355 g.) was dissolved in light petroleum and treated with bromine until the reagent was no longer rapidly decolourised. Evaporation of the solvent gave a colourless oil, which when chromatographed on alumina from light petroleum gave 3 fractions, of which the second was obtained as a colourless crystalline dibromide (0·123 g.),

TABLE II

Fraction	B.pt., ° C./mm.	$n_D^{20°\,C.}$	$d_{15°\,C.}^{15°\,C.}$	$[\alpha]_D^{13°\,C.}$	Weight, g.
A	106° to 140° C. atm.	1·4770	0·856	−30° (at 16°)	1·0
B	105° to 110° C. (bath)/75	1·4755	0·882	−50°	1·43
C	114° C. (bath)/17	1·4765	1·000	−44·4°	4·8
D	120° to 126° C. (bath)/18	1·4841	0·961	−23·3°	3·8
E	130° to 132° C. (bath)/18	1·4910	0·937	−8·3°	3·1
F	132° to 136° C. (bath)/18	1·4971	0·942	0°	2·4
Minor and intermediate fractions					8·0
Residues					3·5

TABLE III

B.pt. ° C./mm.	$n_D^{20°\,C.}$	$d_{15°\,C.}^{15°\,C.}$	Weight
			g.
109° to 110° C. (bath)/17	1·4755	0·992	4·0
111° to 114° C. (bath)/17	1·4764	0·998	4·0
114° to 120° C. (bath)/17	1·4802	0·979	1·0

m.pt. 89° C., $[\alpha]_D^{17°\,C.}$ + 98° (C, 0·46). Found, C, 40·65; H, 5·5; Br, 54·5 per cent. $C_{10}H_{16}Br_2$ requires C, 40·6; H, 5·5; Br, 53·9 per cent. The remaining two fractions were obtained as colourless oils, the first (0·152 g.) being odourless, and the third (0·118 g.) having a distinct camphoraceous odour.

Fraction B. (a) The oil (0·93 g.) was refluxed for 30 minutes with ethanolic potassium hydroxide (0·66N; 10 ml.) and neutralised with 0·5N hydrochloric acid. Extraction of the solution with light petroleum yielded a colourless oil, which when chromatographed on alumina from light petroleum yielded two fractions, of which the first was a colourless oil (B′;0·4 g.); $d_{15°\,C.}^{15°\,C.}$ 0·859, $n_D^{17°\,C.}$ 1·4742, $[\alpha]_D^{15°\,C.}$ − 59° (C, 6·2) λ max. 210 mμ, $E_{1\,cm.}^{1\ per\ cent.}$ 313 (ε 4260 based on $C_{10}H_{18}$). The second fraction was identified as (−)-borneol. (Note: Poor recoveries were due to deliberate loss by evaporation to ensure complete removal of light petroleum).

(b) *Iodine Value* (B′). B′(13 mg.) was treated with pyridine bromide reagent (5 ml.) for 10 minutes by the official method, and, after the addition of potassium iodide, titrated with 0·02N sodium thiosulphate. Halogen uptake was equivalent to 1·05 double bonds.

(c) *Bromination (B′).* B′(0·2 g.) was dissolved in light petroleum and treated with bromine until the reagent was no longer rapidly decolourised. Evaporation of the solvent gave a colourless oil, which when chromatographed on alumina from light petroleum gave 3 fractions, of which the second was obtained as a colourless crystalline dibromide, m.pt. 87·5° to 88° C. undepressed on admixture with that obtained from A, above.

Fraction C. (a) *Saponification.* The oil (4 g.) was refluxed for 30 minutes with ethanolic potassium hydroxide (0.66N; 50 ml), and neutralised by titration with 0·5N hydrochloric acid. Equiv. wt. 276 (equivalent to *ca.* 70 per cent. of bornyl formate). Extraction of the solution with light petroleum yielded an oily semi-crystalline solid, which when chromatographed from light petroleum on alumina yielded two fractions, of which the first, *reticulene*, was obtained as a colourless oil

(0·18 g.), $n_D^{16° C.}$ 1·4955; $d_{16° C.}^{16° C.}$ 0·913; $[\alpha]_D^{15° C.}$ + 1·6° (C, 4·47). Found C, 88·35; H, 11·6 per cent. $C_{15}H_{24}$ requires C, 88·2; H, 11·8 per cent. The second fraction, a colourless crystalline solid, was identified as (—)-borneol, m.p. 206° C. (from light petroleum); *p*-nitrobenzoate, m.p. 134° to 135° C., $[\alpha]_D^{19° C.}$ — 34·7° (C, 1·3 in chloroform). (Hückel and Kaluba[16] give m.p. 136° C, $[\alpha]_D^{20° C.}$ — 34·2° {c, 4 in chloroform}). Found: C, 67·9, 67·9; H, 7·3, 6·8; N, 4·8 per cent. Calc. for $C_{17}H_{21}O_4N$, C, 67·3; H, 7.0; N, 4·64 per cent. Oxidation with chromic acid gave camphor, m.p. 176° C., 2:4-dinitrophenylhydrazone, m.p. 174° to 175° C.

(b) *The Water-insoluble Acid.* (i) *Isolation.* Acidification of the aqueous liquors from the saponification, extraction with light petroleum, drying (sodium sulphate) and evaporation of the solvent yielded a colourless, oily, optically inactive acid (0·29 g.), $n_D^{20° C.}$ 1·5019, $d_{15° C.}^{15° C.}$ 1·050, λ max. 215 mμ $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 177. The oil on standing for several weeks slowly crystallised. Chromatography of the crystalline material from light petroleum on a column consisting of a mixture of activated charcoal (1 part) and Whatman filter paper pulp (3 parts) yielded a colourless crystalline acid, m.p. 72° to 73° C., λ_{max} 206 mμ $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 594 (ϵ 9980 based on $C_{10}H_{16}O_2$). Equiv. wt. (titration) 165; benzyl*iso*thiouronium salt, m.p. 124° to 125° C.

Ferric Salt, formed as a buff precipitate on addition of ferric chloride solution to an aqueous solution of the sodium salt.

(iii) *Silver Salt*, formed as a white gelatinous precipitate on the addition of silver nitrate solution to an aqueous solution of the sodium salt.

(iv) *Barium Salt*, formed as a microcrystalline solid (from aqueous ethanol) by neutralisation of an ethanolic solution of the acid with 0·05N barium hydroxide. Found (sulphated ash): Ba, 49·26 per cent. $C_{10}H_{15}O_2Ba_{\frac{1}{2}}$ requires Ba, 49·43 per cent.

(v) *Hydrogenation* of the acid (113 mg.) at a palladium charcoal catalyst gave a saturated acid, obtained as a colourless oil, $d_{15° C.}^{15° C.}$ 1·030. The absorption of hydrogen was equivalent to 1·2 mols.

(vi) *Iodine Value.* The acid (15·1 mg.) was treated with pyridine bromide reagent (3 ml.) for 10 minutes by the official method and, after the addition of potassium iodide, titrated with 0·02N sodium thiosulphate. Halogen uptake was equivalent to 0·89 double bonds.

(c) *Identification of Formic Acid in the Residual Saponification Liquors.* Distillation of the residual liquors gave an aqueous solution, which exhibited the typical properties of formic acid. The acid was isolated as its sodium salt and characterised by (*a*) reduction of neutral silver nitrate solution, (*b*) production of a red colour with ferric chloride solution (buff precipitate on heating with excess reagent), (*c*) conversion to sodium oxalate by rapid heating to 360° C., (*d*) *p*-bromophenacyl ester, m.p. 137° to 139° C. undepressed on admixture with the *p*-bromophenacyl ester of an authentic sample of sodium formate (m.p. 135° to 138° C.). Found (sulphated ash): Na, 32·8 per cent. Calc. for H.COONa; Na, 33·8 per cent.

Fraction D. Saponification of the oil (3·714 g.) and separation of neutral and acidic fractions, as described above for fraction C, gave a further

0·353 g. of water-insoluble acid. The neutral fraction, after chromatography from light petroleum on alumina as described above, gave borneol (0·51 g.) and 3 oily fractions (2·5 g. in all; the first of these oils (1·0 g.) after repeated chromatography from light petroleum on alumina gave reticulene (0·409 g.), $n_D^{15°C.}$ 1·4944, $d_{15°C.}^{15°C.}$ 0·912 $[\alpha]_D^{15°C.}$ + 1·5° (C, 3·92).

Fraction E. Saponification of the oil (3·075 g.) and separation of neutral and acidic fractions, as described above for fraction C, gave a further 0·272 g. of water-insoluble acid. The neutral fraction, after chromatography from light petroleum on alumina as described above, gave reticulene (1·794 g.), $n_D^{16°C.}$ 1·4955, $d_{16°C.}^{16°C.}$ 0·913, $[\alpha]_D^{15°C.}$ + 1·6° (C, 4·47).

Fraction F. Saponification of the oil (1·897 g.) and separation of neutral and acidic fractions, as described above for Fraction C, gave only traces of water-insoluble acid. The neutral fraction, after chromatography on alumina from light petroleum, as described above, gave reticulene (1·189 g.), $n_D^{14°C.}$ 1·4972, $d_{15°C.}^{15°C.}$ 0·914, $[\alpha]_D^{15°C.}$ + 1·1° (C, 4·2).

Iodine Value of Reticulene. Reticulene (58·8 mg.) was treated with pyridine bromide reagent (30 ml.) and, after the addition of potassium iodide, titrated with 0·1N sodium thiosulphate. Halogen uptake was equivalent to 2·2 mols.

Summary

The light petroleum-soluble fraction from a batch of *Aristolochia reticulata* has been examined and found to contain the following:

1. A ketonic oil, consisting of at least 2 unidentified components.
2. A crystalline lactone, $C_{15}H_{20}O_2$, m.pt. 110·5° to 111° C., designated aristolactone.
3. A bicyclic monoterpene, which has been tentatively identified as (−)-Δ^4-carene.
4. A mixture of esters, which on saponification yields (−)-borneol formic acid and an unidentified cyclic unsaturated acid, $C_{10}H_{16}O_2$.
5. A bicyclic sesquiterpene, $C_{15}H_{24}$, designated reticulene.

We wish to thank The British Drug Houses, Ltd. for a gift of serpentary root, Mr. J. Chilton for assistance in preparation of the root for extraction, and Mr. W. McCorkindale and Dr. A. C. Syme for the microanalyses.

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DISCUSSION

The paper was presented by DR. J. B. STENLAKE.

DR. W. M. MITCHELL (London) said the authors took the view that the light petroleum-insoluble part of the ketonic fraction was an artefact. He thought it more likely that it was an original constituent retained in petroleum solution by the presence of other constituents of the oil, a not unusual happening when fractionating natural products.

DR. J. W. ROWSON (London) asked whether it had been confirmed that the commercial sample used was an authentic specimen of *Aristolochia reticulata?*

DR. G. E. FOSTER (Dartford) asked whether there was any evidence that the bitter principle was an alkaloid.

DR. STENLAKE, in reply, said that they were undecided whether the suggestion made by Dr. Mitchell was correct. On re-examination recently, they noted that the oily fraction had undergone considerable decomposition and deposition of solid matter, and it would be investigated further. Two batches of material were used in the work and samples from each examined and the authenticity of the material confirmed. In their petroleum extracts they had found traces of basic material. In other experiments, where they had further extracted the defatted drug, a basic material of fairly high molecular weight was present in quite small amounts, being about 0·1 or 0·05 per cent. of the total solid matter.

DEMINERALISED WATER FOR PHARMACEUTICAL PURPOSES

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The process of purifying water by means of ion exchange resins has been known for 20 years. In the last few years, the technique has been so improved that it can be used for the large-scale preparation of water. It is a matter of some importance to decide whether this "demineralised" water, as water purified by ion exchange resins is generally called, is of sufficient purity to replace distilled water of the British Pharmacopœia, for some pharmaceutical purposes. Demineralised water is considerably cheaper than distilled water and it has the great advantage that large volumes can be rapidly prepared from tap-water. Its value to the manufacturing pharmacist should be considerable.

The two resins of principal interest in the purification of water are the strong cation exchanger, containing sulphonic acid functional groups ($RSO_3^-H^+$, where R stands for the resin structure) and the strong anion exchanger containing quaternary ammonium groups ($R.NZ_3^+OH^-$, where Z stands for an aliphatic group). These ion exchange resins consist of hard organic polymer particles, each containing ionised, functional groups of a single type distributed throughout the mass of the resin. The ionised groups fixed in the resin structure, have oppositely charged ions held loosely to them and it is these loosely-held ions which are able to exchange with similarly charged ions in a solution in contact with the resin. The resins are insoluble in water, but the dry materials swell up when wetted.

If a dilute solution of sodium chloride is passed through a column of the above cation exchange resin in the hydrogen form, the sodium ions from the solution are exchanged with hydrogen ions from the resin giving an effluent consisting of hydrochloric acid.

$$Na^+Cl^- + RSO_3^-H^+ \longrightarrow H^+Cl^- + RSO_3^-Na^+.$$

The exchange is in fact, reversible, but by the use of a column and an excess of resin, it can be driven completely in the direction shown. If the effluent from this column is passed through a column of the second type of exchanger, the chloride ions are removed giving a pure water effluent.

$$H^+Cl^- + RNZ_3^+OH^- \longrightarrow H_2O + RNZ_3^+Cl^-.$$

If the original solution consists of ordinary tap-water, the two columns remove the dissolved salts, giving a water of sufficient purity for many industrial purposes. The efficiency of the salt removal is most conveniently assessed by measuring the specific electrical resistance of the effluent. This decreases when the water is allowed to stand, owing to carbon dioxide absorption from the atmosphere.

If the two resins are mixed together in a single column, a considerable improvement in demineralising efficiency is obtained. Water of specific resistance of 10 megohm. cm. is readily prepared, comparable only with

conductivity water prepared by repeated distillation in a quartz still. Until recently, the mixed resin column method has been unsuitable for large-scale work owing to the difficulty of regenerating the resins, when spent. However, with the development of the quaternary ammonium anion exchangers, regeneration has become possible because this exchanger is considerably less dense than the cation exchanger. If a rapid backflush of water is passed up through the mixed resin column, the two resins separate and a sharp line of demarcation appears between them. They can then be regenerated separately with sodium hydroxide for the upper layer of anion exchanger and hydrochloric acid for the lower layer of cation exchanger. After washing, the resins can be remixed by blowing air up through the column.

The chief disadvantage of demineralised water is that although it is almost completely free from salts, it may contain non-ionic and colloidal impurities, incompletely removed from the feed water by the resins.

The suitability of demineralised water for pharmaceutical purposes was examined by Harrison, Myers and Herr[1]. In 1943, they reported that their product, obtained from New Brunswick tap-water by treatment with cation and anion exchangers contained in separate columns, was within the specification of distilled water of the United States Pharmacopoeia. They also showed that the resin treatment did not enhance the pyrogenic effect of the feed-water although they pointed out that it should not be presumed that strongly pyrogenic water would be purified by treatment with the exchangers.

Reents and Kahler[2] showed that water from a mixed resin ion exchange column, collected only when its specific resistance exceeded 1 megohm cm. contained 0·02 parts per million (p.p.m.) or less of calcium, magnesium, sodium, chloride and silica; sulphate, nitrate and iron were undetectable.

Bütikofer and Ammann[3] in 1952 concluded that demineralised water was not suitable for use in place of aqua destillata of the Pharmacopoea Helvetica V. They used the resins in both separate and mixed columns and found that it was not always possible to obtain a product which passed the permanganate test (a less exacting test than that imposed by the British Pharmacopœia, the Swiss requiring only 3 minutes of boiling). They also stated that the treated water had a disagreeable taste, that it often failed the chloride test and that the residue after evaporation exceeded the limit of 1 mg. per 100 ml. A detailed examination of this paper reveals that the authors do not appear to have had any criterion of electrical resistance for acceptance or rejection of the demineralised water, they only mention that commercial equipment gives a warning when the resistance (specific resistance?) of the water drops below 50,000 to 100,000 ohms. This rejection figure is too low for demineralised water, the limit should be at least 1 megohm. cm. Lack of resistance control of the effluent would account for their positive chloride and residue results. The presence of detectable amounts of chloride in mixed resin, demineralised water is a good indication that the process is not being carried out correctly. Their permanganate reaction is due partly to the same cause and partly to the

fact that they carried out only 6 regenerations of the resins. They noted that the quality of the water improved as the number of regenerations increased. There is no doubt that fresh resins do contain organic impurities which are only slowly eliminated with use. This problem is overcome by some manufacturers by cycling the resins between active and inactive forms several times, before marketing them. The work of Bütikofer and Amman is a good example of the way in which a poor quality product results from the use of a faulty demineralising technique. The results they obtained with the mixed resin column were better than those with the separated resin columns.

Eisman, Kull and Mayer[4] have described some bacteriological experiments with demineralised water. They used two demineralising plants, each having separate columns for anion and cation exchangers. The first plant was kept in continuous operation and was regenerated at least every 4 days. The output from this plant was substantially sterile, a bacterial count gave an average figure of 1 organism/ml., the feed water having an average count of 50 organisms/ml. The second plant was used infrequently and was only regenerated every 20 days. Immediately after regeneration the demineralised water had a low count, but after standing without use for a few days, the count rose to 1500 or more organisms/ml., much higher than the feed water. The organisms present in the contaminated water were of the type normally present in fresh water, mainly *Pseudomonas*. From this work it appears that a resin bed which is allowed to stand without renenera-tion can form a culture medium, from which bacteria are flushed out when the unit is set in operation. A demineralised water of very low bacterial count can be obtained if the ion exchangers are used daily and are frequently regenerated. Assuming that *Pseudomonas* is the only impurity present in the water, that each organism occupies a mean volume of $1{\cdot}3\mu^3$ and has a loss of 90 per cent. on drying with a 10 per cent. nitrogen and 50 per cent. carbon content (dry weight basis), then the following numbers of organisms/ml. will cause the water to fail the chemical tests.

100 million/ml. for the residue test (BP. 1953).
30 million/ml. for the oxidisable matter test (B.P. 1953).
5 million/ml. for the albuminoid nitrogen test (p. 1019).

Other workers[5] have reported that freshly regenerated resins can have a sterilising effect on slightly contaminated waters.

Cruickshank and Braithwaite[6] have examined bacterial growth on ion exchange resin columns and have studied methods suitable for sterilising them. They concluded that there is no evidence that bacteria use the resins as a nutrient and they consider that the growth is due to a filtering effect, both bacteria and suspended organic matter being filtered off from the feed water, the latter providing the food for the former to grow on the column. They considered that the most suitable sterilising agent for a contaminated cation exchanger column was formaldehyde. 3 to 5 hours contact of a 0·25 per cent. solution with the resin gave complete sterilisation without damage to the resin. Subsequent washing gave a complete removal of the formaldehyde.

Experimental

Demineralised water was prepared by treatment with mixed exchange resins contained in a Pyrex tube, 120 cm. long and 7·5 cm. in diameter (see Fig. 1). The resins rested on a stainless-steel gauze A; below this there was a reducing adaptor B and the bottom outlet tube C was closed with a seasoned rubber tube and a screw clip. The upper end of the Pyrex tube was closed by means of a bung through which tubes for the feed water and regenerating liquids passed, these tubes were closed at their lower ends by distributors consisting of sintered glass discs, 3 cm. in diameter.

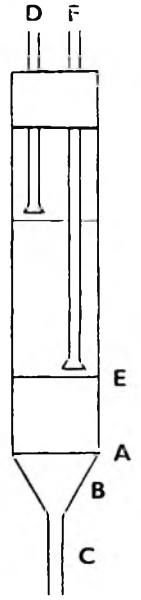

FIG. 1. Demineralising column ready for regeneration.

The large Pyrex tube was two-thirds filled with a mixture of one part of Zeo-Karb 225 (cation exchanger) and two parts of De-Acidite FF (anion exchanger) these being equivalent proportions of the two materials. The resin bed volume was approximately 2 l.

To activate the resins and to regenerate them when spent, a fairly rapid stream of water was passed up through the outlet tube C and run to waste through D, after a few minutes a sharp line of separation E appeared between the two resins. The water flow was then stopped, the top of the large Pyrex tube being left full of water. The upper, anion exchange layer was then treated with 2 l. of 5 per cent. sodium hydroxide solution which was introduced through tube D and withdrawn through tube F whose distributor was placed about 2 cm. above the line of separation of the resins, E. The upper layer was then washed with demineralised water. The lower, cation exchange layer was similarly regenerated with 9 l. of 1·5 per cent. sulphuric acid introduced through F and withdrawn from C. After washing this lower layer, most of the water above the resin bed was allowed to flow away and the resins were remixed by blowing a current of air up through C for about 5 minutes. The remaining liquid above the resins was drained rapidly away and the column was allowed to stand for several hours before use. The regeneration could be completed in about $1\frac{1}{2}$ hours.

To prepare demineralised water, London Metropolitan Water Board tap-water was run in at the top of the resin column and the purified water was removed through the tube C. This effluent passed through a conductivity cell fitted with platinum electrodes whose resistance measured directly the specific electrical resistance of the treated water.

The first runnings from the column after regeneration or after standing had a low specific resistance. The effluent was rejected until the value of

this property rose to 1 megohm cm., usually about 20 l. of water were rejected in this way. After collection was started, the specific resistance often rose to about 15 megohm cm. before declining again. When it had fallen to 1 megohm cm., collection of demineralised water was stopped and the resins were regenerated. The rate of flow of water through the column was adjusted to 20 to 30 l./hr., but much higher rates could be used.

With a moderately hard tap-water feed, the sulphuric acid regenerant for the cation exchanger was found to be unsatisfactory, fine precipitates of calcium sulphate formed in the column being carried through by the feed water. 2 l. of 5 per cent. hydrochloric acid was therefore used for most of the cation exchanger regenerations, After about 15 regenerations, the volume of acceptable demineralised water obtained between regenerations decreased quite considerably. To overcome this, the resin bed was washed through completely with 18 l. of 5 per cent. hydrochloric acid solution. A vigorous evolution of carbon dioxide from the anion exchanger occurred, suggesting that the loss of de-ionising efficiency was mainly due to the high affinity of this resin for carbonate ions, these ions not being completely removed by regeneration with sodium hydroxide.

Over a 5-month period, an average volume of 90 l. of acceptable, demineralised water was obtained between regenerations. Initially the column was run to exhaustion and regenerated 6 times before samples were collected for examination by the British Pharmacopoeia, 1953 tests for distilled water.

The results of these tests are summarised below.

Description

The demineralised water was invariably clear and colourless. The accepted water was also tasteless, but the early runnings from the column which had been standing for some time in contact with the resins, had a slight unpleasant taste. This water was, however, rejected by the specific resistance test.

Copper, Iron and Lead. Not detected in any samples.

Chloride. Not detected in any of the samples examined.

Sulphate. Not detected.

Ammonia. No colouration with Nessler's reagent.

Oxidisable Matter. All samples of specific resistance above one megohm cm. passed this test. Some early runnings from the column, of unacceptable specific resistance, were at the limits of the B.P. test (note that this requires 10 minutes boiling with permanganate, whereas the Pharmacopœa Helvetica test used by Bütikofer and Ammann, requires only 3 minutes boiling).

Non-Volatile Matter. This is probably the most critical of the B.P. tests in relation to demineralised water. Accepted samples of demineralised water passed the test, but water of specific resistance of 0·5 megohm cm. gave a residue of 2 mg./100 ml., well above the prescribed limit of 0·001 per cent.

After the column had had about 30 regenerations, a sample of water was taken at a specific resistance of just over 1 megohm cm. This was analysed

according to the "Approved Methods for the Physical and Chemical Examination of Water," published by the Institution of Water Engineers, 1949. The following results were obtained.

Heavy metals. At the limits of detection; 0·1 parts per million (p.p.m.) of zinc; 0·05 p.p.m. of copper, iron and lead.

Free ammonia. 0·01 p.p.m. as nitrogen. This is similar to the value obtained for "ammonia-free" water and is very much lower than the usual value for ordinary distilled water which is 0·2 p.p.m.

Albuminoid nitrogen. This is an important analytical result for demineralised water since it measures the amount of colloidal, nitrogenous material which passes through the resin column. With this sample of demineralised water the low value of 0·02 p.p.m. of albuminoid nitrogen, was obtained. The feed water had an average value of 0·07 p.p.m. so it seems that the resins have removed some of the albuminous colloids from the tap water. Albuminoid nitrogen is not usually detectable in distilled water.

Oxygen absorption. In this test a sample of water is heated for 4 hours at 27° C. with dilute potassium permanganate solution. The amount of permanganate used up by the reducing materials in the water is determined and the result is expressed as p.p.m. of oxygen. The demineralised water gave a figure of 0·04 p.p.m. of oxygen compared with an average value of 0·8 p.p.m. for the feed water. This test therefore also indicates that the resins do remove some organic matter from tap water. The average oxygen absorption for distilled water is much the same as that for the demineralised water.

A Suggested Amendment to the Monograph on Distilled Water in the the British Pharmacopoeia

The use of demineralised water is rapidly growing and it can be prepared in a state of purity equal to that of distilled water. It therefore seems desirable that demineralisation should be officially permitted as a method for purifying water for pharmaceutical purposes. To effect this, the author suggests that the present monograph in the British Pharmacopoeia dealing with distilled water should be modified as follows:—

(1) The title of the monograph should be changed to "Purified Water."

(2) The method of preparation should be altered to "Purified Water is prepared from a potable water either by distillation or by treatment with ion exchange resins." (With a specification for suitable resins in an Appendix.)

(3) All the existing tests should be retained with the following addition.

Albuminoid Nitrogen

To a 500 ml. sample of water contained in a 1 l. distillation flask, add 0·2 g. of magnesium carbonate and distil 200 ml. of the water. Add 25 ml. of alkaline permanganate solution (8 g. of potassium permanganate and 200 g. of sodium hydroxide are dissolved in 1 l. of ammonia free water; before use the solution is mixed with an equal volume of water and evaporated down to its original volume). Distil 100 ml. of water and to

this add 4 ml. of alkaline solution of potassium mercuri-iodide. The colour is not more intense than that given by 100 ml. of ammonia-free water containing 4 ml. of dilute solution of ammonium chloride (Nessler's). (This corresponds roughly to 0·06 p.p.m. of albuminoid nitrogen.)

This new test would guard against the inclusion of undesirable amounts of albuminous colloidal matter in the demineralised water.

The ion exchange method of water purification is unlikely to be suitable for the preparation of water for injection. Pyrogens are almost certainly colloidal in nature and although freshly regenerated resins may remove them almost completely from the feed water, as the resins become spent, they will pass increasing quantities of colloids.

Summary

1. Demineralised water, prepared from tap-water by treatment with mixed anion and cation exchange resins, has been examined by means of the tests of the British Pharmacopœia for distilled water. It has also been subjected to a more detailed analysis.

2. The results show that providing a correct demineralising technique is used and that only water of specific resistance greater than 1 megohm cm., is collected, the purity of the product is at least equal to that of distilled water, B.P.

3. It is suggested that the monograph on distilled water in the British Pharmacopœia should be amended so that demineralised water of suitable purity can be included within it.

The author thanks Mr. J. P. Vokes for carrying out much of the experimental work, the Permutit Co., Ltd., for the loan of the demineralising equipment and Professors H. Berry and W. H. Linnell for their interest and support.

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DISCUSSION

The paper was presented by THE AUTHOR.

MR. J. H. OAKLEY (London) said that he agreed in general with Dr. Saunders's findings. He would reserve judgment on the proposed limits for albuminoid nitrogen until the test had been worked over a longer period. The Swiss findings only emphasised how important it was to let the resins settle down and have many regenerations before using the water. He had met a difficulty with a new resin at an early stage when a Nessler ammonia test had indicated heavy contamination with ammonia. This was later found to be aldehyde derived from the resin. The author stated that the carbon dioxide content would increase on storage, but he

thought it would decrease. Toxicity tests had shown that there were no toxic materials added to the water. He agreed that the plant must be used frequently, preferably daily, and should not be allowed to lie stagnant for long periods. In this way the bacterial content of the water tended to be reduced. One difficulty arose through channelling, since this reduced the efficiency of demineralisation. The resins tended to act as a filter, and material was trapped in the upper parts of the resin, but this could be removed by backwashing, which also disturbed the bed and prevented channelling. If the plant were in the same building and near to a distilled water plant, sulphuric acid rather than hydrochloric acid should be used for regeneration.

MR. W. TRILLWOOD (Oxford) asked how the resins behaved if the water used were not of a good potable standard?

MR. D. N. GORE (Dorking) remarked that demineralised water was used in the laboratories with which he was associated, except for microbiological work.

MR. T. D. WHITTET (London) said that the American workers, Reid and Jones, had claimed that ion exchange resins would quantitatively remove pyrogens from tap water. He had tested demineralised water for pyrogens, and even when the feed water was strongly pyrogenic the treated water was apyrogenic. On the other hand, using the resins as columns, as suggested by the Americans, he had had no success in removing pyrogens. With bead resins he had been unable to remove pyrogens using columns, but with granular resins the pyrogenicity of the tap water was reduced. He had used a column 10 to 12 cm. long, and the amount of water passed through the column was very small compared with that used by Reid and Jones. There was need for many more experiments in connection with the removal of pyrogens. Demineralised water would be useful in a hospital pharmacy, for example, for washing ampoules.

MR. G. SYKES (Nottingham) asked whether the water obtained was bacteriologically satisfactory if the columns were used continuously. Was the bacterial content lower than that of the initial water? Was the bacterial content high if the column had not been used for a time?

DR. G. E. FOSTER (Dartford) observed that demineralised water might be cheaper than distilled water if prepared in a laboratory, but in a factory condensed waste steam produced water which was quite satisfactory for many purposes.

DR. R. E. STUCKEY (London) said that it was necessary to watch for organic materials, which were not removed by the normal deionisation mechanism of the columns but were removed by simple adsorption processes. Had the author any experience of putting water through with a high organic content? It was essential that the process should operate continuously in order to get a bacteria- and mould spore-free water. Had Dr. Saunders any suggestions to make regarding a specification for the resins?

DR. SAUNDERS, in reply, said the carbon dioxide content would not increase if the material were sealed, but if it were stored in contact with air it was possible to watch the conductivity rise as carbon dioxide was absorbed. It was essential to keep the plant in frequent use, so that the method was unsuitable for small-scale work. If all the water needed was run off after regeneration and the column left standing it could be sterilised, by running dilute formaldehyde solution through it and washing thoroughly before the next regeneration. He had done no work with water heavily contaminated with colloidal material, but the B.P. tests would give an adequate safeguard if the water were very bad. He had carried out no bacteriological tests, but other workers had confirmed that as long as the columns were kept in continuous operation the bacterial count of the water coming out was very low indeed. He had not drawn up specifications for the resins, but it would not be difficult to do so.

THE ENTRAINMENT OF LIQUID DURING DISTILLATION

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The process of distillation involves vaporisation of a liquid followed by removal and subsequent condensation of the vapour formed. In practice this is not always achieved, and it has been found that the distillate may be contaminated with the original liquid. This contamination has been traced to a variety of causes such as foaming and in particular to entrainment.

The term entrainment was used by Cessna and Badger[1] to signify the phenomenon whereby a small proportion of the liquor undergoing evaporation was carried over by the vapour as droplets into the condenser resulting in a loss of the material contained in the evaporator and contamination of the condensate. For example, in concentrating sugar juice, entrainment results in the loss of sugar from the evaporators[2] and in the production of distilled water the prevention of entrainment has been shown to be essential for precise *p*H work[3], conductivity experiments[4,5,6] and for water used for surface chemistry. In pharmacy it is important that water intended for the preparation of injection solutions should be free from pyrogens. The most probable source of pyrogen contamination of freshly distilled water is in the carry-over by entrainment of the water being evaporated. To reduce this to a minimum, the British Pharmacopæia directs that in preparing water for injection the still used should be fitted with an efficient device for preventing entrainment.

The mechanism whereby droplets of liquid and bubbles are thrown into the vapour above the boiling liquid has been studied and appears to be mainly due to the bursting of vapour bubbles and as the cavity left in the liquid surface collapses inwards droplets may be projected from the rising centre. If the boiling liquid foams small bubbles may be released into the vapour and also fragments from the breaking foam films.

The liquid droplets and bubbles formed are then carried away by the rising vapour and may thus reach the condenser. The factors governing the conveyance of the entrained droplets by the vapour have been the subject of much published work and variously ascribed to the rate of evaporation[2,7] the velocity of the vapour[1,8,9,10,11,12] and, particularly in the case of fractionating columns, to the vertical linear path of the vapour, i.e., plate spacing[8,9,10,11,12].

Much of the published work has been carried out on commercial and pilot plants which do not seem very suitable for a close control of the experimental conditions; we have, therefore, re-examined this problem using a relatively simple system, the entrainment being followed through straight vertical tubes.

Experimental

A very sensitive method of detecting entrainment was necessary since the contamination in the distillate represents only a very small fraction of the original liquid. Various methods have been reported, such as using electrolyte and detecting by conductivity methods[1,7] or by volumetric analysis[9,11]. These methods were not considered satisfactory in the circumstances, and it was decided to use fluorescein sodium as the indicator and to estimate this fluorimetrically.

A Spekker fluorimeter (model H.760)[13] was used for the estimations with a Wood's glass filter[14] on either side of the mercury-vapour lamp and

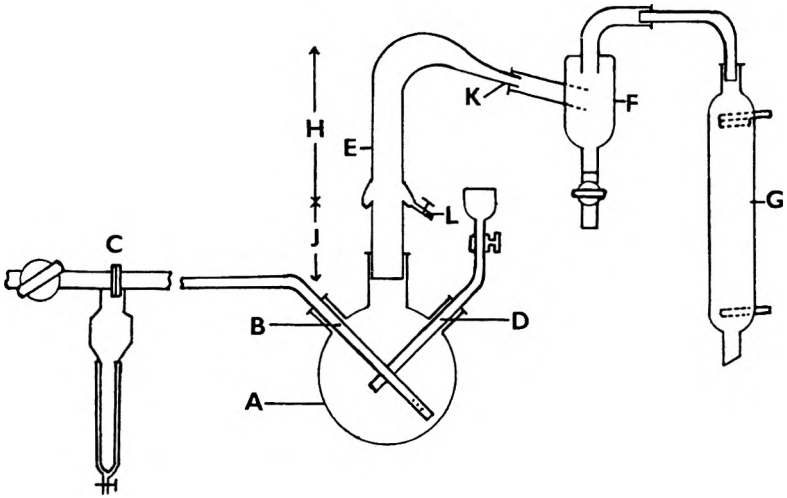

Fig. 1. Distillation apparatus.

A	5-l. flask.	D	Water inlet.
B	Steam inlet tube.	E	Stillhead.
C	Orifice meter.	F	Separator.
		G	Condenser.

Chances glass filters OG1 between the cuvette and the photocells. We found that fluorescein solutions exhibited the maximum fluorescence when buffered at *p*H 6. The fluorescein sodium used for the solutions was found to contain a small amount of fluorescent material volatile in steam and this was first removed by drying the solid at 105° C. to constant weight. Solutions containing 0·1, 0·01 and 0·001 per cent. of fluorescein sodium were unaffected by boiling under reflux for 80 hours, 8 hours and 8 hours respectively. Also exposure to diffuse daylight for 14 days did not affect the fluorescence of these solutions. For solutions containing 1·5 μg. to 0·02 μg. per ml. of fluorescein sodium the error of the estimation was found to be less than 6 per cent., but below 0·02 μg. per ml. the error was much greater. Fluorescence could be detected with concentrations as low as 0·001 μg. per ml.

The apparatus is represented diagrammatically in Figure 1. The 5-l. flask (A) was charged with 3000 ml. of a 0·1 per cent. w/v solution of fluorescein sodium in distilled water which was maintained at its boiling

point with a heating mantle having two heating circuits (500 W. and 300 W.). Steam was injected into the fluorescein solution through a $\frac{5}{8}$-in. glass tube (B), the rate of flow of the steam being measured by a simple orifice meter (C). 4 stainless-steel orifice plates were used with holes $\frac{1}{8}$ in., $\frac{1}{4}$ in., 5/16 in. and $\frac{3}{8}$ in. diameter for measuring distillation rates of 0 to 1·5, 1·5 to 4, 4 to 8 and 8 to 17 l. per hour. The tube (B) which dipped below the surface of the fluorescein solution was sealed at the end having 6 holes ($\frac{1}{4}$ in. diameter) in the side of the tube. It was considered that by these means, reproducible conditions in the still would be achieved and for a given rate of distillation the amount of entrainment produced in the still would be constant. The vapour rising from the solution passed through stillhead (E) into a centrifugal separator (F), where entrained droplets were collected, and then to the condenser (G). The still was constructed of Pyrex pipeline equipment, reduction joints were used to prevent the solution creeping along the walls and to avoid possible constrictions in the pipes by the gaskets used in butt-ended joints. Stillheads of 4 diameters, $\frac{5}{8}$ in., 1 in., $1\frac{1}{2}$ in. and 2 in. were used. The length of the stillheads (H) being 5 in., 10 in., 15in. and 30 in. for each diameter. Part J at the bottom was standard in all cases—2 in. diameter and 5 in. long; part K was $\frac{5}{8}$ in. diameter for all stillheads. The apparatus was assembled so that the stillhead was vertical and channel and tapping (L) was included so that the liquid trapped on the walls of the vertical section could be collected as it drained down. The still, stillhead and the separator were lagged to prevent undue condensation in the system.

The fluorescein sodium used in preparing the 0·1 per cent. w/v solution in distilled water was dried to a constant weight at 105° C. in order to remove moisture, which may be as much as 10 per cent., and to remove impurities which have been shown to be fluorescent and steam volatile. The still was heated by the mantle (800 W.) until the solution was at the boiling point, the loading on the mantle was then reduced to 300 W. and the level of the solution at 100° C. noted. A loading of 300 W. was sufficient to maintain the solution at the boiling point.

Steam was allowed to pass for 20 minutes to ensure equilibrium conditions in the apparatus as indicated by maintenance of water level in the boiler and a constant steam rate through the orifice meter. During an experiment the steam was fed into the still at atmospheric pressure at a controlled rate and the distillation timed for 20 minutes by a stop-clock. After the run the level of the liquid in the still was checked and if a variation occurred, corrections for the change in concentration of the solution were applied to the figures obtained for entrained fluorescein. Liquid was collected separately from the base of the stillhead, from the separator and from the condenser. The volumes were measured, and the concentration in each was estimated fluorimetrically by comparison with standard solutions of fluorescein, 2 standard solutions being used for the liquid from the separator. All solutions were buffered to *p*H 6 for the estimation.

The rate of distillation was expressed as ml. per hour, being the sum of the volumes of the liquids collected from the separator and condenser. It was found that the concentration of fluorescein in the liquid collected from

the condenser was constant for all rates of distillation irrespective of length and diameter of stillhead. Since this concentration was equal to the figure obtained when the volatility of dried fluorescein in steam was determined, it was assumed that the centrifugal separator was acting efficiently and separating the droplets from the vapour stream. Fluorescein equivalent to that collected during the run was returned to the still before another experiment was commenced.

Results

Figures 2, 3, 4 and 5 summarise the experimental results obtained and these curves represent a total of 1067 distillations. The points plotted on these curves are the averaged results for increments of 500 ml./hour in the distillation rate. By this means the scatter of the points around each curve was reduced, which simplified the drawing of the curves where they lie close together, but this did not alter their shape. The figures obtained for the concentration and quantity of fluorescein collected from the base of the stillhead varied very widely and showed little correlation with the rate of distillation. The quantity of fluorescein collected in the separator was expressed as μg./l. of distillate, which was a measure of the entrainment taking place during the distillation, this representing the degree of contamination of the distillate, each μg. of fluorescein being equivalent to 0·001 ml. of the original solution.

Discussion

The results have been treated in a number of ways in an attempt to demonstrate the effect of various factors influencing entrainment.

Rate of Distillation. This treatment is the simple experimental plot given in Figures 2, 3, 4 and 5. These curves may be divided into three distinct stages.

(*a*) Initially, entrainment increases linearly with distillation rate.

(*b*) The middle portion tends to a constant rate of entrainment which may be preceded by a region where entrainment decreases with increasing distillation rate.

(*c*) The third stage is characterised by a sudden and spectacular increase in the amount of fluorescein carried over. It was observed that this increase was due to a film of solution being swept along the walls of the stillhead by the vapour at high distillation rates. This phenomenon is referred to subsequently as "gross carry-over."

The slope of the line in the first part of the curve is related to the diameter and entrainment increases with increasing diameter. This is probably due to the greater radial path offering less chance for the ascending droplet to be trapped on the wall. In the second portion of the curve the entrainment tends to a value which is nearly constant for all diameters as the rate of distillation increases, except for the $\frac{5}{8}$-in. diameter stillhead, and this entails a reduction in entrainment for the $1\frac{1}{2}$-in. and 2-in. diameter stillheads. This "constant" value for entrainment was of the order of 8 μg. of fluorescein, although the stillheads 5 in. long and those $\frac{5}{8}$ in. diameter deviated from the figure. Rhodes and Slachman[15] obtained results which

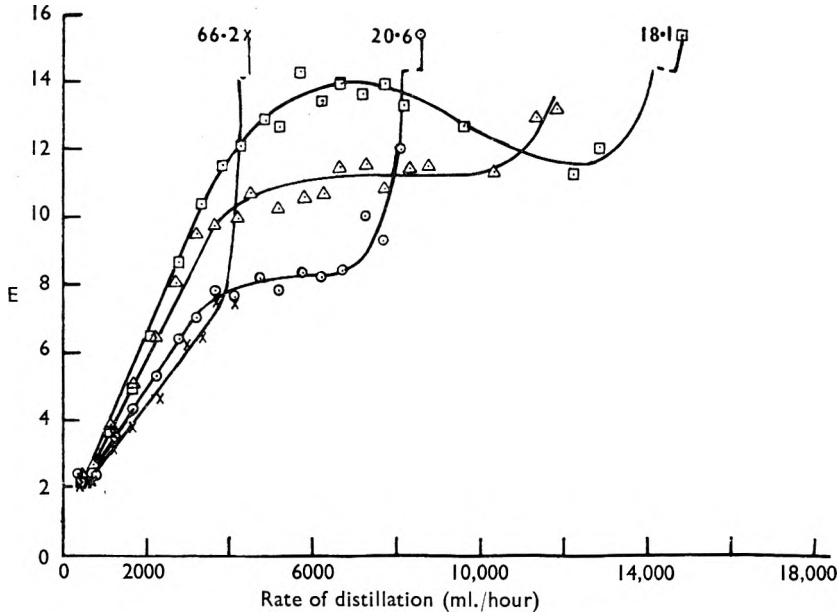

FIG. 2. The effect of the rate of distillation on entrainment in stillheads 5 in. long.
× $\frac{5}{8}$ in. diameter. △ $1\frac{1}{2}$ in. diameter.
⊙ 1 in. diameter. ⊡ 2 in. diameter.
E = entrainment as μg. of fluorescein sodium per litre.

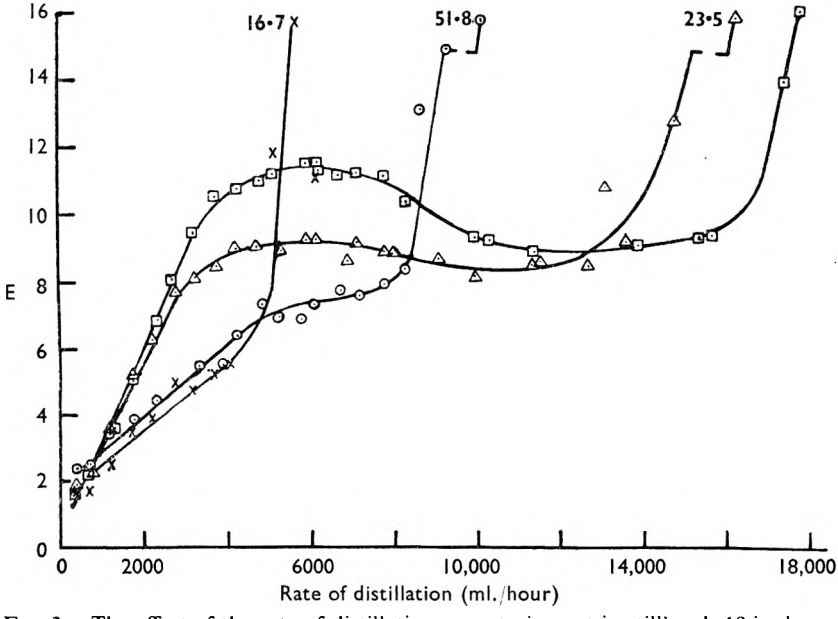

FIG. 3. The effect of the rate of distillation on entrainment in stillheads 10 in. long.
× $\frac{5}{8}$ in. diameter. △ $1\frac{1}{2}$ in. diameter.
⊙ 1 in. diameter. ⊡ 2 in. diameter.
E = entrainment as μg. of fluorescein sodium per litre.

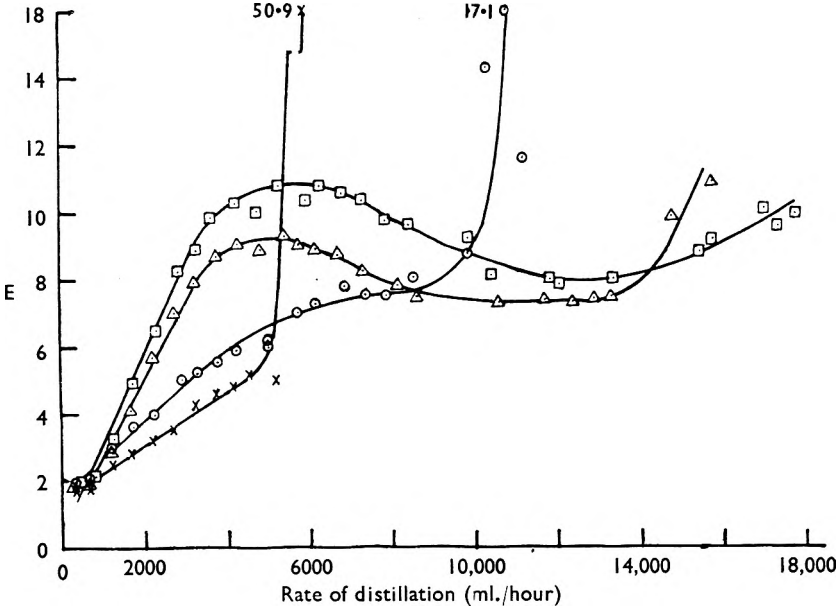

FIG. 4. The effect of the rate of distillation on entrainment in stillheads 15 in. long.
× $\frac{5}{8}$ in. diameter. △ $1\frac{1}{2}$ in. diameter.
○ 1 in. diameter. □ 2 in. diameter.
E = entrainment as μg. of fluorescein sodium per litre.

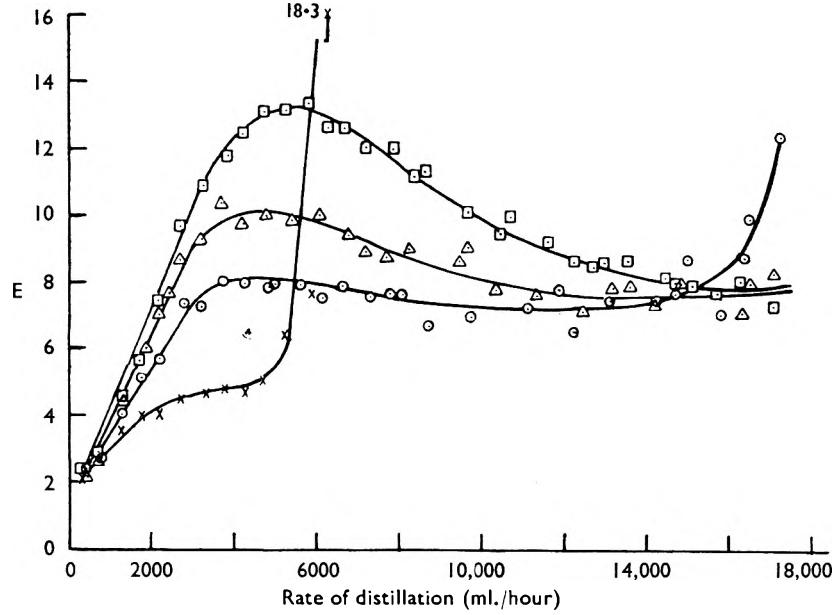

FIG. 5. The effect of the rate of distillation on entrainment in stillheads 30 in. long.
× $\frac{5}{8}$ in. diameter. △ $1\frac{1}{2}$ in. diameter.
○ 1 in. diameter. □ 2 in. diameter.
E = entrainment as μg. of fluorescein sodium per litre.

were very similar for the effect of the rate of distillation on entrainment, using benzene-toluene and also ethanol-water mixtures.

Effect of Vapour Velocity. The mean velocity of the vapour through each stillhead was calculated from the rate of distillation and Figure 6 illustrates the effect of vapour velocity on entrainment in stillheads 15 in. long. These curves are of the same general form as Figures 2, 3, 4 and 5, but emphasise more strongly the effect of the diameter of the stillhead on entrainment.

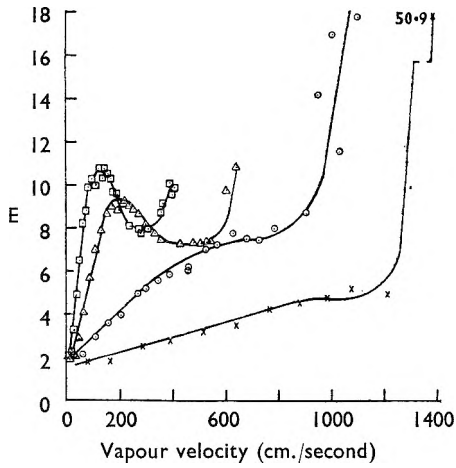

FIG. 6. The effect of vapour velocity on entrainment in stillheads 15 in. long.
× $\frac{5}{8}$ in. diameter. △ $1\frac{1}{2}$ in. diameter.
⊙ 1 in. diameter. ⊡ 2 in. diameter.
E = entrainment as μg. of fluorescein sodium per litre.

Sherwood and Jenny[11], using columns of 17·8 cm., 20·3 cm. and 25·4 cm. diameter, found that for a given gas velocity the entainment increased with increasing diameter, which is in agreement with our results.

Correlation with Reynolds Number. Reynolds[16] found that the flow of a fluid through a pipe is related to the density, viscosity and velocity of the fluid and also the diameter of the pipe in the following manner:—

$$\text{Reynolds Number: (Re)} = \frac{\rho d u}{\eta}$$

ρ = density of fluid.
d = diameter of pipe.
u = velocity of fluid.
η = viscosity of fluid.

For smooth-bore pipes such as the glass stillheads used the flow is streamline or laminar for a Reynolds number below 2000 to 4000, whereas above this range the flow becomes turbulent and full turbulence will develop when the Reynolds number reaches a value of 10,000 to 20,000.

The variation of entrainment with Reynolds number is given in Figures 7 and 8 for the 5 in. and 15 in. long stillheads respectively and here a relationship with the diameter of the stillhead is shown also since in these figures the entrainment divided by the diameter of the stillhead is plotted (i.e., entrainment per unit radial path). The curves for the 10 in. and 30 in. long stillheads are similar. From these curves it would seem that when the steam exhibits laminar flow, as indicated by Reynolds number, the entrainment increases linearly with Reynolds number and is greatest with the greatest diameter since if the wall of the tube is the main trapping

agency then the wider the tube the greater is the radial path a droplet must traverse to reach the wall. The sudden reduction in diameter of the stillheads of $\frac{5}{8}$ in. and 1 in. diameter may have induced some turbulence at the lower values of Reynolds number which would account for the lower values obtained for entrainment.

When Reynolds number is increased and the vapour in the stillhead becomes more and more turbulent the effect of the diameter is decreased

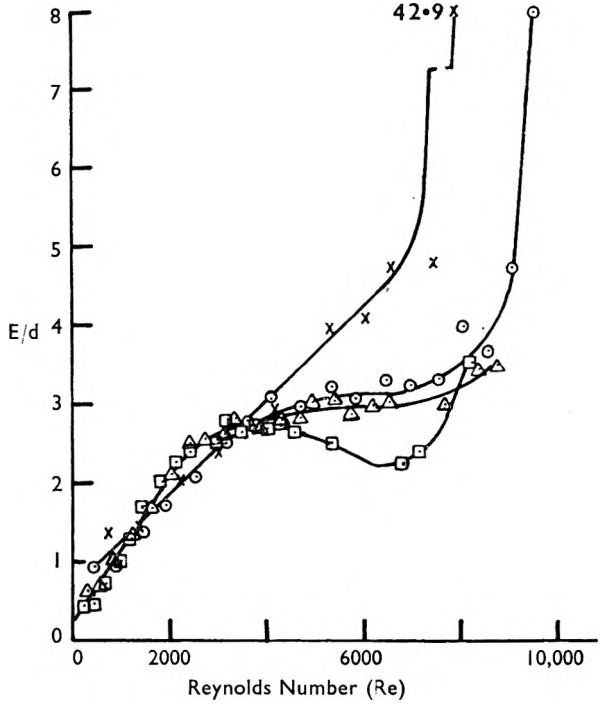

FIG. 7. The effect of Reynolds number on entrainment per unit diameter in stillheads 5 in. long.

× $\frac{5}{8}$ in. diameter. △ $1\frac{1}{2}$ in. diameter.
○ 1 in. diameter. ⊡ 2 in. diameter.

$$\frac{E}{d} = \frac{\text{entrainment as } \mu\text{g. of fluorescein sodium per litre.}}{\text{diameter of stillhead}}$$

so that as full turbulence is achieved the amount of entrainment is nearly the same for all diameters. This is shown for stillheads 15 in. long in Figure 9 and may be deduced for the 5 in., 10 in. and 30 in. long stillheads from Figures 2, 3 and 5.

The results published by O'Connell and Pettyjohn[7] have been converted into terms of Reynolds number and are shown in Figure 10 and 11. In drawing their curves these authors used only those points that are shown with continuous lines drawn through them and they ignored those points we have connected with a broken line. It is significant that the peaks in these curves occur at values for Reynolds number comparable with our

results. The apparatus was a semi-commercial horizontal tube evaporator using solutions of salts.

Effect of the Length of the Stillhead. A consideration of the factors governing the height to which a drop may be carried by vapour has been given by Hausbrand[17]. Figure 12 shows the effect of the rate of distillation on entrainment for stillheads of different lengths of 1 in. diameter. Curves obtained for $\frac{5}{8}$ in., $1\frac{1}{2}$ in. and 2 in. diameter stillheads are similar. At the

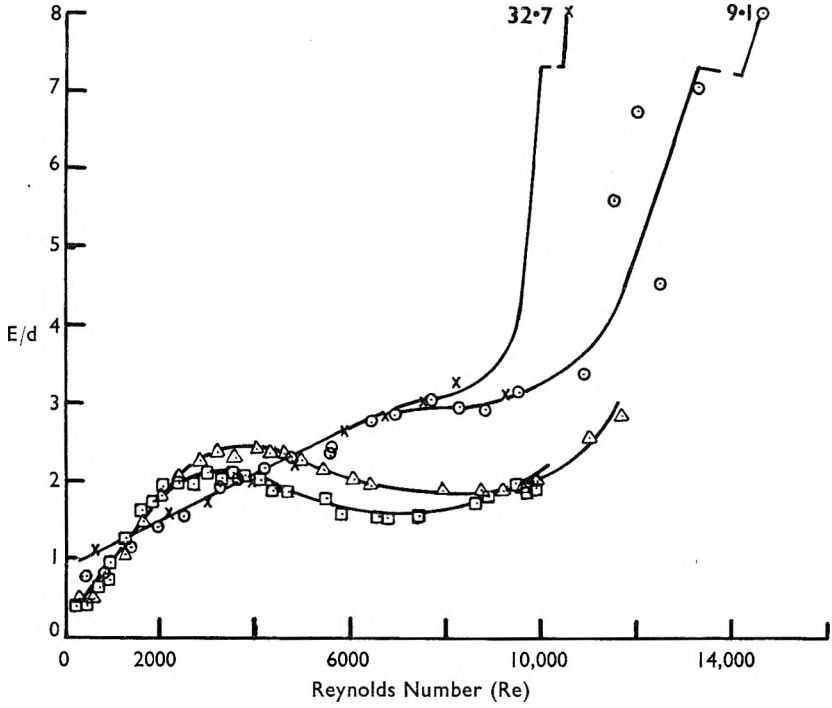

FIG. 8. The effect of Reynolds number on entrainment per unit diameter in stillheads 15 in. long.

× $\frac{5}{8}$ in. diameter. △ $1\frac{1}{2}$ in. diameter.
⊙ 1 in. diameter. ⊡ 2 in. diameter.

$$\frac{E}{d} = \frac{\text{entrainment as } \mu\text{g. of fluorescein sodium per litre}}{\text{diameter of stillhead}}$$

lower rates of distillation the amount of fluorescein entrained decreases as the length of stillhead increases from 5 in. to 15 in. as may be expected, but is greatest for the 30 in. stillhead. Then as the rate of distillation increases entrainment through the 30 in. stillhead falls to values below that of the others.

This apparent anomaly with the 30 in. stillhead at the lower rate of distillation is surprising, but was found to occur with the stillheads of $\frac{5}{8}$ in., $1\frac{1}{2}$ in. and 2 in. diameter also. Further work may give rise to a reasonable explanation.

Much of the published work on the effect of the vertical path has been carried out in fractionating columns of the bubble-cap type by varying the

distance between the plates[8,9,10,11,12]. In general an increase in the plate spacing produces a decrease in entrainment, but the conditions in a bubble-cap column will be very different from the conditions in our apparatus.

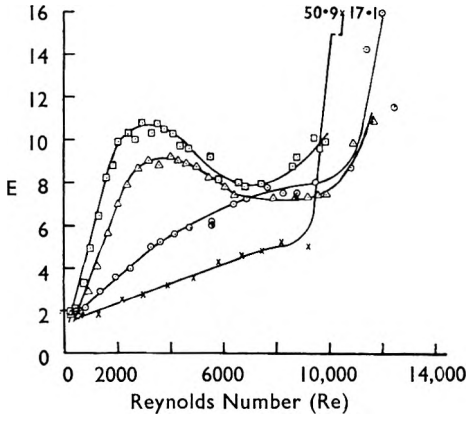

FIG. 9. The effect of Reynolds number on entrainment in stillhead 15 in. long.
× $\frac{5}{8}$ in. diameter. △ $1\frac{1}{2}$ in. diameter.
○ 1 in. diameter. ⊡ 2 in. diameter.
E = entrainment as μg. of fluorescein sodium per litre.

Gross Carry-Over. At the higher distillation rates it was observed that fluorescein solution was swept upwards along the wall of the stillhead resulting in a large increase in the quantity of fluorescein collected in the separator. The onset of this phenomenon was sudden and Table I gives in terms of Reynolds number the lowest experimental results at which an ascending film was obtained in each stillhead.

The maximum Reynolds numbers obtained for the $1\frac{1}{2}$ in. and 2 in. stillheads (30 in. long) were 12,735 and 9545 respectively and no gross carry-over occurred, these figures corresponding to the maximum distillation rate of the apparatus, approximately 17·1 l./hour.

For the 15 in. long stillhead of 2 in. diameter an increase in entrainment was found at Reynolds number 9870, but the rising film was not seen in the stillhead.

From Table I there is good agreement for the Reynolds number at which gross carry-over occurred for each length of stillhead except for those 30 in. long. For the two in which gross carry-over was obtained, however, the agreement was better if vapour velocity was compared. For the $\frac{5}{8}$ in. and 1 in. diameter stillheads the Reynolds numbers given correspond to vapour velocities of 1480 cm./sec. and 1507 cm./sec. respectively.

TABLE I

REYNOLDS NUMBERS AT WHICH GROSS CARRY-OVER OCCURRED CALCULATED FROM EXPERIMENTAL DISTILLATION RATES

Length of stillhead in.	Diameter of stillhead			
	$\frac{5}{8}$ in.	1 in.	$1\frac{1}{2}$ in.	2 in.
5	7,875	8,090	8,425	8,230
10	9,190	9,250	9,732	9,650
15	10,500	10,830	10,970	9,870
30	11,235	18,280	No gross carry-over	

SUMMARY

1. The entrainment of liquid droplets through straight vertical stillheads during distillation has been investigated using fluorescein sodium as an indicating substance.

2. When the Reynolds number for the vapour flowing through the stillhead is below 2000, indicating streamline or laminar flow, the entrainment has been found to be directly proportional to the rate of distillation

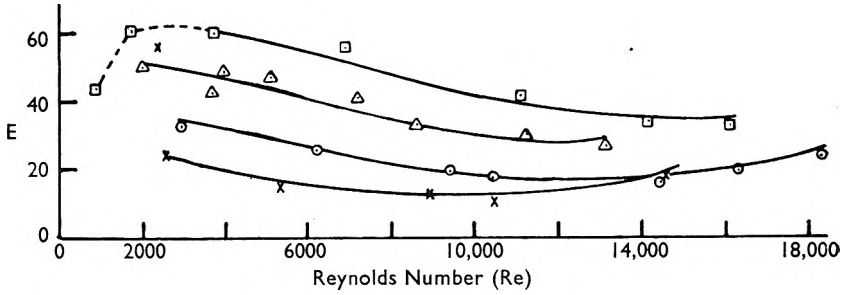

FIG. 10. The effect of Reynolds number on entrainment (O'Connell and Pettyjohn[7]). Distillation of 20 per cent. sodium chloride.

× = solution boiling at 140° F.
⊙ = solution boiling at 167° F.
△ = solution boiling at 190° F.
⊡ = solution boiling at 219° F.

E = entrainment as pounds of liquid per 10^6 pounds of vapour.

and to the diameter of the stillhead. As the wall of the stillhead constitutes the main trapping agency it is postulated that the entrainment will increase as the diameter increases since under these flow conditions the vapour has little radial movement. The entrainment per unit diameter is directly proportional to the Reynolds number in the 30 in. stillheads, but deviations occur in the shorter stillheads of $\frac{5}{8}$ in. and 1 in. diameter, which is probably due to the constriction from 2 in. inducing some turbulence.

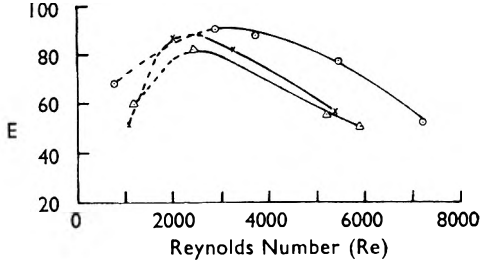

FIG. 11. The effect of Reynolds number on entrainment (O'Connell and Pettyjohn[7]). Distillation of solutions of sodium sulphate).

× = 20 per cent. sodium sulphate boiling at 140° F.
⊙ = 24 per cent. sodium sulphate boiling at 166° F.
△ = 24 per cent. sodium sulphate boiling at 140° F.
E = entrainment as pounds of liquid per 10^6 pounds of vapour.

3. As the Reynolds number is increased to 6000 to 8000 the entrainment approaches a constant value, irrespective of the diameter, for a given length of stillhead. Under these conditions the flow of the vapour is probably fully turbulent and has a high radial velocity which carries the droplets to the wall.

4. Entrainment has been found to decrease as the length of the stillhead increases from 5 in. to 15 in. for each diameter, but in the 30 in. stillhead entrainment was highest.

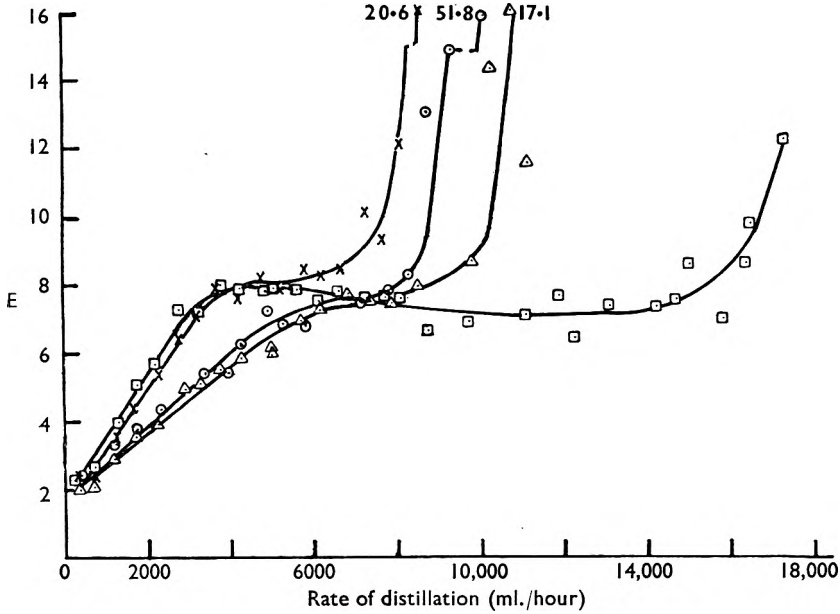

FIG. 12. The effect of the length of stillhead on entrainment in stillheads of 1 in. diameter.
× Stillheads 5 in. long.
⊙ Stillheads 10 in. long.
△ Stillheads 15 in. long.
⊡ Stillheads 30 in. long.
E = entrainment as μg. of fluorescein sodium per litre.

5. Gross carry-over (climbing film effect) occurred in the 5 in., 10 in. and 15 in. stillheads at an approximately constant value for Reynolds number for each length and was unaffected by the diameter. For the 30 in. stillheads gross carry-over was not achieved for diameters of $1\frac{1}{2}$ in. and 2 in. and in the case of the $\frac{5}{8}$ in. and 1 in. diameter tubes gross carry-over was more nearly related to vapour velocity.

We would like to thank Professor H. Berry for suggesting the use of fluorescein sodium, Dr. F. Wokes for advice on the fluorimetric estimations and Mr. D. Train for help and advice throughout this work.

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DISCUSSION

The paper was presented by MR. E. SHOTTON.

DR. W. MITCHELL (London) asked whether the results obtained would apply to apparatus of different design, in particular to large-scale apparatus? It would be of interest to know if the ordinary gross carry-over would be different if a material other than glass were used. This could be tested by replacing the glass stillheads—part E in Figure 1—by similar metal stillheads.

DR. F. HARTLEY (London) said that for the particular stillhead there might be a critical length between 30 in. and 15 in. at which entrainment began to increase. This might be due to the thermal losses occurring in the longer stillheads. Were the authors satisfied with the lagging of the stillheads? If lagging were inefficient and thermal losses high, a cloud would tend to build up and to adsorb the entrainment. The obvious pharmaceutical application of the work was in the design of stills for the preparation of pyrogen-free water. In industrial practice two stills were often used for this purpose, the distillate from the first being redistilled in the second. It seemed possible that they might find a critical length of stillhead for their equipment beyond which, relatively, no further reduction in entrainment occurred.

MR. W. C. PECK (London) described the paper as one of the most fundamental studies which had been made in the design of entrainment separators. It would affect not only the distillation of water but also the design of fractionating columns, and in fact all the processes where a vapour and a liquid were concerned. The relationship of the entrainment to the Reynolds Number was of first-rate importance. He suggested that dimensional analysis might have revealed that apart from the Reynolds Number there was another factor, the height of the column, which would have explained anomalous results. Some of the most economic processes for the production of distilled water, such as that of vapour recompression, had not been investigated in this country.

MR. J. H. OAKLEY (London) said that by widening the still orifice one would expect entrainment to be reduced because the rate of flow of the vapour is lowered, but at the same time condenser effect is also lowered, which would tend to increase entrainment. At the other extreme, if the orifice were too narrow one would reach a climbing film evaporator. Was the latitude between these two extremes very critical?

DR. A. F. S. A. HABEEB, in reply, said that the curves in Figures 10 and 11 showed results by the American workers, O'Connell and Pettyjohn, using a semi-commercial horizontal evaporator. After a critical Reynolds Number of about 2000 the amount of entrainment was reduced, which

was in agreement with their results, but in fractionating columns other workers got different results. The distances between the plates and the splashing of the liquid on the plates might account for these differences. Work on different materials for stillheads was in hand. Speaking of the abnormality of the curves for 30-in. stillheads, he said that if they had been able to see inside they might have seen whether there was clouding.

Note by the Editor.—Subsequent to the meeting the following note was received from MR. SHOTTON:—

"Although some condensation in the stillhead would take place, this would not be a governing factor since the entrainment falls with an increase in the distillation rate and vapour velocity, which is the reverse of what may be expected if the condensation due to heat loss was a governing factor. Regarding the point raised by Mr. Oakley, there was sufficient latitude between these two extremes, this latitude increasing with increasing diameter of pipe."

THE STABILITY OF AQUEOUS SOLUTIONS OF FERROUS GLUCONATE

By C. A. Johnson and J. A. Thomas

From the Laboratory of the Scientific Publications Department, Pharmaceutical Society of Great Britain

A demand for a liquid preparation of ferrous gluconate to be included in the British Pharmaceutical Codex and the National Formulary prompted this investigation into the conditions affecting the rate and extent of oxidation which might occur. It is generally held that ferrous iron is much more readily adsorbed than ferric and it is desirable therefore that any oxidation which may occur should be restricted to a minimum. That oxidation might occur fairly readily is evident from the high proportion of ferric iron permitted in the solid material by the specification of the United States National Formulary which allows over 17 per cent. of the total iron to be in the trivalent state. British manufacturers have been able to produce material which shows a considerable improvement on the United States specification, many commercial samples examined showing about 6 per cent. of the iron in the ferric state, whilst one manufacturer's product consistently contains as little as 2 per cent. or less. A further indication that considerable oxidation in solution is to be anticipated is afforded by the known fact that oxidation of ferrous iron depends upon the degree of ionisation of the dissolved salt, ferrous sulphate for example being about 10 times more stable than ferrous acetate.[1]

Experimental

In the first instance a number of elixirs were prepared according to different formulæ and were stored in well-closed amber glass containers varying from 20 to 40 fl. oz. in size, the ferrous iron content being determined from time to time. These preparations at first showed a steady deterioration in ferrous iron content, but in some of the smaller containers it was observed that oxidation became more rapid as the stock of solution diminished. It thus became obvious that the volume of air in the container was of prime importance in determining the extent to which oxidation would occur, and that a strict control of container size and of the volume of air above the liquid would be necessary in a comparison of the effect of different antioxidants and conditions of storage on the rate and extent of oxidation of ferrous gluconate in solution.

Details of tests. For each sample to be examined 15 × 2 fl. oz. containers fitted with metal screw caps containing a rubber liner were employed. One of these bottles was completely filled with the sample (a total volume of nearly 60 ml.) whilst 4 other bottles contained 48, 36, 24 and 12 ml. respectively. The 10 remaining bottles each contained 12 ml. of the sample, the volumes filled in being measured as accurately as possible. At the time of filling and closing the containers, portions of the sample were assayed for total iron and ferrous iron content by the methods described below. The bottles containing 12 ml. of sample were opened successively

at intervals of 24 or 48 hours and when the rapidly falling ferrous iron content had become relatively stationary the 5 bottles containing varying quantities of solution were opened and the ferrous iron content of each determined. The reproducibility of the test was examined by charging 5 sets of containers with the same preparation; Table I gives the figures

TABLE I

(*a*) SHOWING THE RATE OF OXIDATION OF FERROUS GLUCONATE WHEN 12 ML. OF AN AQUEOUS SOLUTION IS STORED IN A CLOSED 2 FL. OZ. CONTAINER

Total iron content of solution at time of preparation .. .. 0·798 per cent.
Ferrous iron content of solution at time of preparation .. .. 0·759 per cent.

Time (days)	Ferrous iron content (per cent.)				
1	0·674	0·665	0·670	0·682	0·668
2	0·616	0·609	0·600	0·612	0·614
3	0·585	0·590	0·578	0·580	0·592
4	0·576	0·575	0·568	0·580	0·570
6	0·567	0·570	0·571	0·569	0·565
7	0·564	0·564	0·560	0·568	0·564
9	0·570	0·558	0·564	0·564	0·560

(*b*) SHOWING THE EXTENT OF OXIDATION IN CLOSED 2 FL. OZ. CONTAINERS AFTER 10 DAYS

Volume of sample in container, ml.	Ferrous iron content (per cent.)				
Full (about 58 ml.)	0·755	0·760	0·756	0·758	0·758
48	0·732	0·740	0·728	0·736	0·734
36	0·702	0·706	0·696	0·700	0·706
24	0·664	0·648	0·656	0·662	0·650
12	0·560	0·564	0·572	0·566	0·551

obtained, which indicated that the method could be used with confidence to compare the stability of preparations. The conditions obtaining in this test were thought to be more comparable with those to be met with in practice than if the solution had been exposed to free access of air.

Methods of assay. The methods of assay used throughout the series of determinations were as follows:—

(i) *For total iron content.* A suitable portion of the solution was diluted with water to 75 ml. and 15 ml. of dilute sulphuric acid and 0·25 g. of zinc dust were added, the flask being immediately closed by means of a stopper fitted with a bunsen valve. After being set aside for 30 minutes the reaction liquid was rapidly filtered through an asbestos pad, flask and filter being washed with a dilution of 1 volume of dilute sulphuric acid with 9 volumes of water. The combined filtrate and washings, after addition of 3 drops of solution of *o*-phenanthroline-ferrous complex as indicator was titrated immediately with 0·1N ceric ammonium sulphate.

(ii) *For ferrous iron content.* A suitable portion of the solution was diluted with water to 75 ml. 15 ml. of dilute sulphuric acid and 3 drops of solution of *o*-phenanthroline-ferrous complex were added and the solution was titrated immediately with 0·1N ceric ammonium sulphate.

The application of this method of determination of iron to ferrous gluconate gives an end-point which fades back fairly rapidly from the greenish-blue indicative of complete oxidation to orange-red, but if the first sharp change is recorded as the end-point, results of a high degree of

reproducibility are obtainable. These methods of assay were chosen because of the ease and rapidity with which they could be carried out and because blank determinations showed that they were not invalidated by any of the ingredients employed in the formulation tests carried out. The applicability of the methods to the commercial samples examined was verified by a comparison of results for total iron content, obtained by the method described above, with results obtained by digesting the sample with nitric acid, precipitating and redissolving the iron and titrating with potassium dichromate.

This method of test was applied to liquid preparations of ferrous gluconate in order to determine the effects of formulation and of storage conditions upon stability.

Formulation. A number of solutions of ferrous gluconate containing added ingredients as antioxidants or buffers were prepared and examined together with 4 proprietary preparations containing ferrous gluconate in solution. Results are given in Table II. The effect of varying the concentration of ferrous gluconate in solution was also examined, results being recorded in Table III.

TABLE II

SHOWING THE RATE OF DETERIORATION OF LABORATORY FORMULATED AND COMMERCIAL SAMPLES WHEN 12 ML. OF THE SAMPLE IS STORED IN A CLOSED 2 FL. OZ. CONTAINER

The figures recorded represent percentage loss of ferrous iron

	Preparations				
Time (days)	1	2	3	4	5
1	16·6	9·6	11·2	17·4	15·6
2	22·1	14·2	15·4	22·8	20·4
3	25·8	18·3	20·2	25·6	23·8
4	24·6	21·4	22·6	25·1	24·8
5	25·0	23·5	24·8	25·4	25·8
7	25·6	24·6	25·7	26·1	25·3
8		24·7	25·0		25·6
9		24·9	26·1		
10		24·5	25·2		

Preparation 1. A 7·5 per cent. w/v solution of ferrous gluconate in water.
2. As (1) with the addition of 7·5 per cent. w/v of dextrose monohydrate.
3. As (1) with the addition of 20 per cent. w/v of dextrose monohydrate.
4. As (1) with the addition of 2 per cent. v/v of dilute hypophosphorous acid.
5. As (1) with citric acid and sodium citrate added to adjust to *p*H 4·5.

	Commercial samples			
	Manufacturer			
Time (days)	A	B	C	D
1	17·8	2·9	14·6	12·6
2	23·4	4·2	18·2	17·1
3	26·8	7·6	21·4	20·8
4	29·1	9·6	22·8	23·0
5	28·8	11·0	23·8	23·4
7	29·4	12·3	24·7	23·2
8	29·0	13·2	25·4	23·6
9		14·1	25·0	
10		14·9		

TABLE III

SHOWING THE EFFECT OF CONCENTRATION UPON PROPORTION OF FERROUS GLUCONATE OXIDISED

Original ferrous iron concentration	Final ferrous iron concentration	Percentage oxidation
1·03 per cent.	0·90 per cent.	12·6
0·52 ,, ,,	0·42 ,, ,,	19·2
0·21 ,, ,,	0·15 ,, ,,	28·6
0·10 ,, ,,	0·068 ,, ,,	32·0

Storage Conditions. The effect of storage in completely and partially-filled containers upon the ultimate extent of oxidation is already apparent from the figures given in Tables I and II, and

Figure 1, based upon the mean results given in Table I, shows this relationship more clearly. To determine whether the ratio of the volume of air to that of the solution plays any part in determining the rate at which oxidation occurs, a number of bottles were filled at each of the volume levels with a 7·5 per cent. solution of ferrous gluconate in distilled water, the liquid in one set of 4 bottles containing 48, 36, 24 and 12 ml. respectively being titrated daily. Only in the case of the bottle containing 12 ml. was any deterioration noted on the second day, so a further series was set up, titrations being carried out at 2-hourly intervals. The results of this series are shown in Table IV.

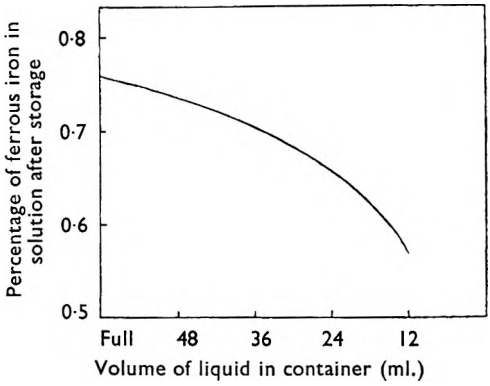

FIG. 1. Storage of ferrous gluconate solution in partially filled containers.

Finally, the effect of storage in daylight was examined. 15 containers were filled as previously described and placed on a window ledge facing south, the results obtained being recorded in Tables V and VI. These results show that after an initial oxidation the ferric iron became reduced, the ferrous iron content increasing beyond what was originally present until it approximated to the total iron content. The reduction was accompanied in the smaller volumes by the deposition of a brownish-yellow crystalline precipitate.

TABLE IV

SHOWING THE RATE OF DETERIORATION IN CONTAINERS CHARGED WITH DIFFERENT VOLUMES OF SOLUTION FERROUS IRON CONTENT OF THE SOLUTION AT TIME OF PREPARATION 0·764 PER CENT

Ferrous iron content of the solution at time of preparation 0.764 per cent

Time (hours)	Volume of solution in the container			
	48 ml.	36 ml.	24 ml.	12 ml.
2	0·753	0·754	0·750	0·758
4	0·744	0·740	0·741	0·737
6	0·746	0·730	0·733	0·732
8	0·740	0·718	0·722	0·721
10	—	0·709	0·705	0·714
12	—	0·703	0·691	0·698
26	—	0·706	0·648	0·626
28	—	0·704	0·643	0·611
30	—	—	0·649	0·597
32	—	—	0·646	0·583

The precipitate formed on exposure to light was prepared in greater yield by exposing a larger volume of considerably oxidised solution to the light, and isolated by filtration followed by washing with water, ethanol and ether. Pale brownish-yellow in colour, the separated residue was insoluble in water and organic solvents, but soluble in dilute hydrochloric acid, yielding a solution which gave reactions characteristic of ferrous iron. After removal of the iron the resulting neutralised solution yielded an immediate precipitate with calcium chloride, insoluble in acetic acid, but

redissolving in dilute hydrochloric acid. A portion of the calcium precipitate when heated with diphenylamine and syrupy phosphoric acid yielded a pale blue colour which was intensified by the addition of ethanol to the cooled reaction liquid. These reactions, characteristic of oxalates, gave reasonable grounds for believing that the precipitated material might be ferrous oxalate and a portion was assayed on this assumption. The iron was oxidised, precipitated as ferric hydroxide, ignited and weighed, whilst the filtrate and washings from the iron determination were just acidified with acetic acid and precipitated with calcium chloride, the residue, after washing, being titrated with potassium permanganate. The results obtained were as follows:— Iron, 30·3 per cent.; FeC_2O_4, $2H_2O$ (calculated from the permanganate titration), 97·8 per cent.

TABLE V

SHOWING THE EFFECT OF STORAGE IN DAYLIGHT

Time (days)	Ferrous iron in solution calculated as a percentage of the total iron content.
When prepared	94·2
1	88·0
2	84·8
3	82·6
4	83·8
5	86·6
7	93·0
8	94·8
9	97·5
10	99·6
11	99·3

TABLE VI

FERROUS IRON IN SOLUTION CALCULATED AS A PERCENTAGE OF THE TOTAL IRON CONTENT IN CONTAINERS CHARGED WITH DIFFERENT VOLUMES OF SOLUTION AFTER 5 WEEKS STORAGE IN DAYLIGHT

Volume of solution	Ferrous Iron	
Full	99·4	
48 ml.	99·7	
36 ml.	88·2	Pale brown crystalline deposit
24 ml.	79·8	
12 ml.	72·1	

The formation of this insoluble residue was reported as long ago as 1936 by Neiger and Neuschul[2], who carried out an elementary analysis, but did not identify the compound. The analytical figures quoted by these authors are compatible with the finding that the residue is ferrous oxalate dihydrate.

The possibility that oxalate may have been present in the ferrous gluconate used to prepare the solutions was considered, but of two batches of material from different manufacturers, both of which yielded the precipitate from aqueous solution in the light, neither gave a positive reaction when the test for oxalic acid of the United States National Formulary specification was applied.

Among other observations, Neiger and Neuschul stated that solution of ferrous gluconate in water containing appreciable proportions of invert sugar considerably retarded the deposition of the ferrous compound, although not its formation in solution. Accordingly, tests were carried out in which the ferrous gluconate was dissolved in a 20 per cent. solution of dextrose monohydrate and exposed to light in partially-filled bottles. No precipitate was formed in 17 days, while in the absence of dextrose a precipitate had formed in 9 days. No precipitate was formed in completely filled bottles, even in the absence of dextrose, during a period of 6 weeks.

Discussion of Results

1. *Factors affecting the rate of oxidation.* It is clear from the figures quoted in Table II that the traditional antioxidants associated with ferrous iron formulation exert little or no effect in preventing the oxidation of ferrous gluconate. A slight improvement might be claimed when a moderate percentage of dextrose is added, but no substantial effect is to be observed. Dilute hypophosphorous acid, instead of retarding the oxidation appears to encourage it—a result which parallels the experience of Huyck when examining solutions of ferrous sulphate.[3]

A solution buffered to *p*H 4·5 with citric acid and sodium citrate showed just as rapid a deterioration as an unbuffered solution, contrary to the finding of Stone[4]. This author also stated that the stability of ferrous gluconate solution is considerably improved as the *p*H is lowered, but he does not indicate the acid used to bring about the adjustment. If a strong mineral acid were introduced the ferrous iron would naturally be stabilised to a certain extent because of the increased ionisation to be expected. The inclusion of a small proportion of citric acid in a solution containing syrup of orange did, however, improve the flavour of the product.

Of the commercial samples examined one was found to deteriorate at a markedly slower rate than any other solution examined, although the loss was still considerable; the remaining commercial samples deteriorated at least as rapidly as the solutions prepared in the laboratory. The ratio of the volume of air to that of solution apparently played no part in determining the rate of oxidation, as is shown in Table IV.

2. *Factors affecting the extent of oxidation.* The ratio of the volume of air to the volume of solution has the most pronounced effect upon the extent of oxidation which will occur in a closed container as is shown in Figure 1. It is clear that the oxidation reaction proceeds until an equilibrium is set up, probably depending both on the oxygen tension and the ratio of ferrous to ferric iron. The concentration of iron in the solution also affects the extent to which oxidation will proceed. As is to be expected, the proportion of total iron which is converted to the ferric state increases as the concentration of total iron is decreased.

It is evident from the many samples examined that, providing the solution is kept in a well-filled and sealed container, no significant deterioration of ferrous iron content may be expected within 3 months. In practice this period may well be considerably longer and indeed Talman, in a recent review[5], mentions a period of 12 months. As soon as the container is opened and doses are removed the ferrous iron content begins to fall to an increasing extent as the volume of air trapped in the bottle becomes greater in proportion to the volume of liquid remaining.

3. *The effect of light.* The effect of light in arresting and ultimately reversing the oxidation of ferrous to ferric iron in solutions of ferrous gluconate which has been reported by Neiger and Neuschul[2] has been confirmed. The relatively insoluble ferrous compound which is deposited from solutions stored under such conditions has been identified as ferrous oxalate. It is a well-known fact that solutions of ferric oxalate, when stored in the light, are reduced to ferrous oxalate and this reaction has

formed the basis of a proposed test to assess the light-protecting properties of glass for medicine bottles[6]. It therefore seems possible that ferric oxalate may be formed in solution by the oxidative breakdown of ferrous gluconate. This theory has not been adequately tested, however, and it may well be that the oxalate is formed, as the result of a photochemical reaction, from some other product of the oxidation of ferrous gluconate. The possibility that the oxalate may have been present in the original material due to the method of manufacture has been considered, but has been disregarded since no reaction for oxalate was obtainable in the samples used. Moreover the formation of the precipitate only in those cases where considerable oxidation of the ferrous gluconate might be expected, i.e., in the bottles containing smaller volumes of solution, argues that the presence of the oxalate is connected with the oxidation reaction.

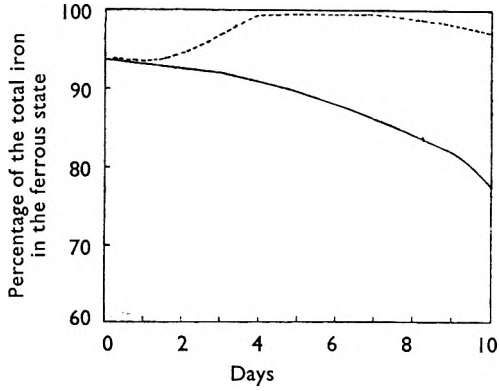

FIG. 2. Storage of ferrous gluconate elixir in 4 fl. oz. containers, withdrawing sample at the rate of 3 teaspoonfuls daily.

——— storage in dark
– – – – storage exposed to daylight.

The formation of this precipitate has been shown to be retarded by the presence of 20 per cent. of dextrose and a solution of ferrous gluconate in the presence of this sugar may be expected to remain stable in respect of ferrous iron concentration and freedom from deposit if stored in well-closed, completely filled containers exposed to direct daylight. Once the bottle is opened and doses are removed a suitably formulated preparation will retain its high ferrous iron content and remain free from deposit throughout the time that is required to consume 4 fl. oz. at the rate of 3 teaspoonfuls daily, provided that the container is kept well closed between withdrawals of doses and the bottle is stored in direct daylight. A comparison of the ferrous iron content of such a preparation stored in the daylight and protected from light throughout its period of use is shown in Figure 2. If it is argued that the presence of oxalate is undesirable then the question of the desirability of prescribing ferrous gluconate in solution under any conditions of storage, whether in the light or the dark, should be reviewed anew.

CONCLUSIONS

It is concluded from the foregoing experiments that:—

(i) solutions of ferrous gluconate should be dispensed only in completely filled containers of small size; the inadvisability of dispensing from a

Winchester stock is obvious since, when the Winchester is only partially full, portions withdrawn may already be some 30 to 50 per cent. oxidised.

(ii) the maximum concentration of ferrous gluconate compatible with the formation of a stable solution should be employed—10 per cent. is suggested as a suitable figure.

(iii) a suitable flavouring agent consists of about 20 per cent. of syrup of orange together with 0·3 per cent. of citric acid.

(iv) if the solution is to be stored protected from light a small proportion of dextrose monohydrate—say 7·5 per cent., should be included. If storage in the light is considered to be safe the proportion of dextrose should be increased to 20 per cent.

Storage protected from light is accompanied by a decrease in ferrous iron content and a corresponsing increase in ferric iron content which becomes greater as the volume of liquid in the bottle is reduced, but the general appearance of the preparation remains satisfactory.

Storage in the light of a suitably formulated product results in a slight increase of ferrous iron content due to the reduction of ferric iron present initially, and the maintenance of the ferrous iron throughout the storage period. It is, however, accompanied by the formation of a product which may be considered undesirable and may be attended by some deposition of solid matter in partially-filled bottles.

The authors are indebted to the Council of the Pharmaceutical Society for permission to publish this work, and to Dr. K. R. Capper, Editor of the Society's Scientific Publications for helpful criticism and advice.

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DISCUSSION

The paper was presented by MR. C. A. JOHNSON.

MR. L. M. SPALTON (London) said that investigations with which he had been connected confirmed the authors' findings. It was claimed that ferrous gluconate was less liable to produce side effects in susceptible people than other iron salts, but a high ferric content would negative this advantage. In his view, the ferric content of ferrous gluconate should be not more than 2 per cent. of the total iron present. He did not regard the possibility of the formation of ferrous oxalate as too serious, because, although there was little information available about its toxicity, the dosage given in the French Codex was $1\frac{1}{2}$ grains. There was, however, a remote possibility that a child could be given a slurry of ferrous oxalate which might be harmful. A general criticism of liquid iron preparations was that being solutions of low *p*H they might cause some damage to the teeth. As tablets of ferrous gluconate were stable, it seemed preferable to administer the drug in tablet form.

DR. W. MITCHELL (London) said that it seemed a sugar-coated tablet was the best method of presentation. He suggested that if a solution was required, ascorbic acid might prove a suitable antioxidant.

DR. J. B. STENLAKE (Glasgow) said the problem required fundamental study. There was a close connection between the oxidation of the gluconate and the fairly well-known oxidation methods for carbohydrates using such agents as peroxides, which were catalysed by light and ferric ions. Was the oxalate produced as a result of a series of degradations, or was it a primary oxidation process?

DR. G. BROWNLEE (London) said that reduced iron salts such as ferrous sulphate and oxidised iron salts such as ferric sulphate are physiologically outside the tolerated limits in which life is possible. Ferrous salts taken orally are immediately oxidised and become tolerable. The proposal to stabilise a reduced preparation at an E_h at or below that of ascorbic acid is dangerous, particularly so in a liquid preparation when given to infants, in whom ferrous salts are sometimes rapidly absorbed. A difficulty which the authors face is in stabilising the iron preparations within the physiological range of *p*H in the absence of suitable E_h buffers. The tocopherols are possibilities and all three isomers are suitable.

DR. G. E. FOSTER (Dartford) said that no one had suggested that there might be some ferrous oxalate present initially. He had found calcium oxalate in a stored sample of calcium borogluconate solution. It seemed to him that, as a result of contamination of the organisms used in the preparation of commercial gluconic acid, some oxalic acid might be produced and thus the ferrous gluconate might initially contain some ferrous oxalate.

MR. C. L. J. COLES (Greenford) said that at the beginning of the paper the formulæ of a number of elixirs were mentioned. What was the *p*H of these products? What was the temperature at which these samples were stored? Was the sample buffered at *p*H 4·5 more or less stable than the elixirs referred to earlier? What was the *p*H of the sample referred to in paragraph (iii) of the conclusion, containing 0·3 per cent. of citric acid? Had the authors any experimental evidence concerning the effect of *p*H on the rate of oxidation?

MR. E. G. SPEAKMAN (Birmingham) said that the use of ascorbic acid did not solve the problem of oxidation of the preparation in half-filled bottles.

MR. G. RAINE (Manchester) said he had found that a formulation containing glycerol 50 per cent. v/v and sucrose 20 per cent. w/v with 2 per cent. dilute hypophosphorous acid considerably retarded the oxidation. When ascorbic acid was added to ferrous gluconate solutions a red colour was produced which disappeared in a day or two, and ascorbic acid was scarcely detectable after a few days; nevertheless, the resulting solution was less readily oxidised than a simple ferrous gluconate solution. A mixture of ferrous gluconate with syrup, citric acid and ascorbic acid appeared to stabilise the ascorbic acid to some extent, and he suspected

the ferrous iron was less rapidly oxidised. He had been unable to devise a method of estimating ferrous iron in the presence of ascorbic acid. The method used to protect the preparation from light was not described. Ferrous gluconate solutions were more rapidly oxidised in amber bottles than in clear glass bottles. Some experiments had shown, however, that storage in complete darkness caused less rapid oxidation than storage in amber bottles, and one sample of ferrous gluconate solution had been as stable when stored in complete darkness as when stored in daylight. The formulation with glycerol, sucrose and dilute hypophosphorous acid had been equally stable under both conditions. No proof had been given that ferrous oxalate was present in solution in any formulation. He had obtained only inconclusive evidence of the absence of oxalate in a mixture containing glycerol and glucose, stored for a month in daylight. His experiments had shown that ferrous oxalate was not appreciably soluble in the mixture. Could animal experiments be undertaken to determine the toxicity of formulations, exposed to air and light, and which had not deposited? The loss of 25 per cent. of ferrous iron content in 10 days was considerable. In his experiments, using partly-filled bottles and removing the corks for a minute or two each day so as to replace the air above the fluid, only in exceptional formulations were there losses of more than 20 per cent. in 4 weeks. The quality of ferrous gluconate available commercially seemed to vary considerably, and the stability in solution varied in consequence. He had prepared a sample of ferrous gluconate which complied with the N.F. requirements and found under all conditions that it was more stable than commercial samples. He hoped that ferrous gluconate of a much higher degree of purity would become available. The various problems of treating iron deficiencies made it desirable that an iron compound should be available in liquid form as well as in tablets. It was also possible that pure ferrous gluconate might be given by intramuscular injection with considerable benefit, instead of the saccharated iron oxide injection solution which was usually given only by the intravenous route.

MR. D. N. GORE (Dorking) said that in tablets of ferrous sulphate the presence of ascorbic acid had a considerable stabilising effect. There was evidence that ascorbic acid potentiated and helped the clinical effect of iron, but he had always assumed that this was through keeping it in the ferrous state and not a function of ascorbic acid *per se*.

MR. F. A. J. TALMAN (Liverpool) asked whether the authors had any information on the effect of sucrose concentrations on the rate of oxidation. He had found some evidence that increasing sucrose content tended in some cases significantly to decrease the rate of oxidation.

MR. C. A. JOHNSON, in reply, said they had examined a number of commercial tablets and had noticed no serious loss in ferrous iron content on storage. The use of ascorbic acid had been considered, but an American paper by Stone had shown the undesirability of this because of the colour formed. The solid ferrous gluconate used had been examined by the method of the U.S. National Formulary for oxalate, and negative results were obtained, but he could not be certain that there was no oxalate

present. The *p*H of the samples made without any acid present were about 5·6 to 5·8. The *p*H of the preparation containing hypophosphorous acid was 4·8 and that containing citric acid was 4·5. All preparations were stored under ordinary laboratory conditions, and most of the work was done after March, 1954, with room temperatures of 50° to 70° F. Some had been stored in the refrigerator, with no effect on oxidation retardation. The effect of *p*H on the rate of oxidation had not been examined in detail. American work showed that at a *p*H of 1·9 the oxidation was apparently very small, whereas with a *p*H of 6·9 there was an 85 per cent. loss of ferrous iron in 48 hours in a solution which was oxygenated. The *p*H there may have been adjusted using mineral acids, but the presence of these was undesirable. The effect of sucrose concentrations had not been examined. All the preparations were stored in white flint glass bottles and "storage in the dark" meant storage in a closed cupboard, but opened daily for a few minutes. There was no evidence of preparations being more stable in these conditions than when stored in amber bottles. They had had a number of samples of ferrous gluconate, one of which had been very pure, containing only about 1·5 per cent. of the total iron in the ferric state. It produced about the same rate of oxidation however. There was clinical evidence that the presence of ascorbic acid, if administered with iron, allowed absorption of ferrous iron to be increased, but he felt that ascorbic acid was not the answer in a liquid preparation of ferrous gluconate.

THE STABILITY OF PENICILLIN IN SUGAR-COATED TABLETS

By F. W. Ashby, P. W. Muggleton, F. Taylor and W. A. Woodard

From Glaxo Laboratories Ltd., Greenford, Middx.

Sodium and potassium benzylpenicillin tablets for oral use have been available for several years. These penicillin salts have an unpleasant bitter taste, difficult to disguise in compressed products; the obvious solution is a sugar-coated product, but this has not until recently made its appearance.

Soluble benzylpenicillin salts are known to be unstable in the presence of moisture: the importance of controlling the free moisture in penicillin tablets has been stressed by the Ministry of Health in presenting data collected from British penicillin tablet manufacturers[1]. It appears to have been assumed that tablets of soluble penicillin could not be sugar-coated by the conventional coating process, and the new British process of compression-coating, an anhydrous method, has been announced as scoring a success by making possible for the first time the manufacture of sugar-coated penicillin tablets[2].

Experiments carried out in these laboratories have demonstrated the feasibility of sugar-coating soluble penicillin tablets by the conventional process and the stability studies being reported here show that such tablets are at least as stable as uncoated ones over a 2-year period. Further, we have evidence that in temperate climates the sugar-coating gives protection to the tablet against careless handling after dispensing from the original container.

Some fears might be expressed that the sugar-coating could, by delaying the disintegration or solution time of the tablet, interfere with the absorption and utilisation of the penicillin. Maximum utilisation of oral penicillin is said to occur in the duodenum[3,4] and Boger and Beatty[5] have stressed the importance of a short solution time for oral penicillin tablets. We have shown that our pan coated penicillin tablets dissolve completely in well under 15 minutes *in vitro*, but we also considered it desirable to show by clinical tests on human volunteers that the penicillin in sugar-coated tablets was as readily absorbed as from uncoated ones.

Part I

Stability studies.

All assays were made by crushing the tablet, dissolving it in sterile solution of standard *p*H 7 and determining the penicillin content biologically with *Bacillus subtilis*. Each result reported is the mean of 3 separate assays.

Free moisture content was determined by finely powdering 5 tablets and determining the weight loss of 1 g. of the well-mixed powder after it had reached constant weight in a vacuum desiccator over phosphorus pentoxide.

In the first series of experiments a number of small batches of 200,000 unit potassium benzylpenicillin tablets (2000 to 5000 per batch) were

sugar-coated in a normal sugar-coating pan. In the first few batches the free moisture content of the tablets was above 1 per cent. and the coating split away from the tablet after a few weeks, but in subsequent experiments, when the free moisture content was low, satisfactory coating was obtained. These tablets were packed in glass screw-capped bottles fitted with a waxed cork shive. Samples were stored in a cupboard in a part of the laboratory reserved for keeping samples at room temperature and also in a cabinet thermostatically controlled at 38° C. The temperature of the laboratory throughout the test varied from 18° to 24° C.

TABLE I

STABILITY OF POTASSIUM PENICILLIN IN SUGAR-COATED TABLETS IN CLOSED CONTAINERS AT 38° C.—1ST SERIES

Units per tablet

Experimental batch	Free moisture, per cent.	Original assay	1 Month	2 Months	3 Months
E	1·2	207,000	183,000	Discontinued,	coating split
F	0·3	194,000	201,000	205,000	200,000
G	0·3	201,000	203,000	212,000	196,000
H	0·4	200,000	202,000	203,000	200,000
I	0·45	190,000	190,000	200,000	188,000

TABLE II

STABILITY OF POTASSIUM PENICILLIN IN SUGAR-COATED TABLETS IN CLOSED CONTAINERS AT ROOM TEMPERATURE (18° TO 24° C.)—1ST SERIES

Units per tablet

Experimental batch	Free moisture, per cent.	Original assay	1 Month	3 Months	2 Years
E	1·2	207,000	185,000	185,000	174,000*
F	0·3	194,000	200,000	201,000	203,000
G	0·3	201,000	212,000	208,000	208,000
H	0·4	200,000	202,000	197,000	199,000
I	0·45	190,000	191,000	208,000	197,000

* Many of the tablets of batch E had split coatings after 3 months at room temperature.

Periodic assays for penicillin were carried out on the tablets. Table I gives the results obtained on 5 batches of tablets stored for 3 months at 38° C. Table II gives the results obtained on the same 5 batches of tablets stored for 2 years at room temperature. Batch E with a free moisture content over 1 per cent. showed signs of instability after a few weeks at 38° C.; even at room temperature the coating on some of the tablets in this batch began to split off after about 3 months. The tablets of the remaining 4 batches remained unchanged throughout the tests.

The successful sugar-coating of these experimental batches of 200,000 unit potassium benzylpenicillin tablets prompted us to extend the investigation to sodium benzylpenicillin, to cover potencies other than 200,000 unit and to scale up the process to batches of production size. Table III gives the results of stability tests on this second series stored for 2 months at 38° C. The coating and stability have so far proved satisfactory.

It is well known that uncoated soluble-penicillin tablets dispensed from

bulk packs into unsuitable containers will deteriorate rapidly under adverse climatic conditions. Exposure to the atmosphere of a room in the Home Counties does not necessarily produce the adverse conditions such tablets might meet in coastal or other humid areas of the British Isles. A standard condition of 25° C. and 75 per cent. relative humidity

TABLE III

STABILITY OF POTASSIUM AND SODIUM PENICILLIN IN SUGAR-COATED TABLETS STORED IN CLOSED CONTAINERS AT 38° C.—2ND SERIES

Units per tablet

Batch and salt	Free-moisture, per cent.	Original assay	1 Month	2 Months
1 Potassium	0·6	49,000	48,000	44,000
2 ,,	0·6	202,000	200,000	199,000
3 ,,	0·7	211,000	213,000	203,000
4 ,,	0·8	420,000	424,000	418,000
5 ,,	0·6	510,000	527,000	535,000
6 Sodium	0·6	50,000	54,000	51,000
7 ,,	0·6	205,000	203,000	202,000
8 ,,	0·8	207,000	200,000	202,000
9 ,,	0·8	529,000	510,000	519,000

was therefore adopted for testing the effect of exposure to the atmosphere under fairly severe conditions of humidity. The tablets were exposed on open Petri dishes in an air-tight cabinet maintaining these conditions over a saturated salt solution. Uncoated tablets of penicillin from production batches were used as controls.

Table IV gives the result of this test and shows that the effective life of uncoated penicillin tablets under these conditions does not exceed some 14 days, whereas the sugar-coated tablets are perfectly stable for at least 2 months. The experiment is continuing.

An experiment exposing the tablet to a relative humidity of 90 per cent.

TABLE IV

STABILITY OF PENICILLIN IN TABLETS EXPOSED IN AN OPEN PETRI DISH TO A RELATIVE HUMIDITY OF 75 PER CENT. AT 25° C.—UNCOATED PENICILLIN TABLETS USED AS CONTROLS

Batch, sugar coated	Initial		After 7 days		After 14 days		After 21 days		After 1 month		After 2 months	
	Assay	Free moisture, per cent.	Assay and appearance	Free moisture, per cent.	Assay and appearance	Free moisture, per cent.	Assay and appearance	Free moisture, per cent.	Assay and appearance	Free moisture, per cent.	Assay and appearance	Free moisture, per cent.
2	202,000	0·6	206,000	—	203,000	0·5	207,000	—	214,000	0·6	189,000	0·6
3	211,000	0·7	209,000	—	199,000	1·0	208,000	—	208,000	0·6	199,500	0·8
4	420,000	0·8	430,000	—	430,000	0·9	418,000	—	430,000	0·8	420,000	0·9
Uncoated penicillin tablets used as controls												
Uncoated X	200,000	0·7	196,000 No change in appearance	2·1	190,000 Off white tablets. Few yellow speckles	3·5	Tablets damp yellow shapeless mass, impossible to assay					
Y	415,000	0·9	401,000 No change in appearance	2·4	366,000 Darker tablets. Speckled surface	3·1	357,000 Speckling heavier. Tablets yellow		Sticky mass, impossible to assay			

at 38° C. (extreme tropical conditions) resulted after 3 days in an unassayable sticky mass from the sugar-coated tablets: a similar state of the uncoated tablets was produced in 16 to 24 hours.

PART II

Penicillin blood levels and urinary excretion in human volunteers.

In view of the claimed need for oral penicillin tablets to dissolve rapidly[4], the disintegration time for our sugar-coated tablets was determined. Batch H had a solution time of 5½ minutes when first prepared; this time was found to be substantially unaltered 2 years later. The

TABLE V

BLOOD LEVELS AND URINARY EXCRETION OF PENICILLIN AFTER ORAL ADMINISTRATION OF 2 × 200,000 I.U. TABLETS TO HUMAN VOLUNTEERS.

Tablets	Volunteer	Penicillin blood levels (I.U./ml.) at: ½ hour	1 hour	2 hours	4 hours	6 hours	Total units excreted in 6 hours
Sugar-coated penicillin tablets	1	0·25	0·55	0·12	<0·03	0	excluded*
	2	0·55	0·51	0·22	<0·03	0	23,700
	3	1·14	0·73	0·30	<0·03	0·08	40,600
	4	2·33	0·97	0·31	0·09	0·09	66,600
	5	0·56	0·32	0·08	<0·03	0	20,000
	6	0·73	0·73	0·28	0·03	0	33,600
	7	0·88	1·20	0·57	0·26	0·02	52,400
	8	2·05	2·11	0·56	0·11	0·02	123,900
	9	1·35	1·28	0·75	0·11	0	58,100
	10	1·27	0·73	0·22	<0·03	0	41,900
	Average	1·11	0·91	0·34	0·06	0·02	51,200
Uncoated penicillin tablets	A	0·50	1·04	0·56	0·12	0·03	58,200
	B	0·85	0·25	0·58	0·10	0·04	91,100
	C	1·25	0·95	0·25	0·06	0	68,200
	D	0·98	1·45	0·42	0·10	0·05	51,300
	E	1·48	1·00	0·53	0·06	0	22,200
	F	1·30	1·00	0·28	<0·03	0	31,200
	G	2·00	0·74	0·18	<0·03	0	80,600
	H	0·87	0·89	0·21	0·03	0	54,300
	I	0·55	0·91	0·28	<0·03	0	31,200
	J	0·94	0·82	0·50	0·13	0·06	91,200
	Average	1·07	0·91	0·38	0·06	0·02	57,960

0 indicates less than 0·02 I.U./ml.
* some loss of sample probable.

solution time for batches 3 to 11 (second series) were found to range from 6 to 11 minutes. The average solution time of production batches of uncoated penicillin tablets is 6 minutes: the results on our sugar-coated tablets indicate no appreciable increase in the time of solution of the tablets due to the sugar-coating.

Besides these *in vitro* solubility tests, an experiment was carried out to study the absorption of penicillin from the sugar-coated tablets by human volunteers, as shown by urinary excretion and blood levels.

10 volunteers were each given 2 × 200,000 unit pan sugar-coated penicillin tablets. Blood samples were taken from each at ½, 1, 2, 4 and 6 hours after administration. These were assayed for penicillin by plate bioassay with *Sarcina lutea* (strain CPI–1001) as test organism.* Each

* Standard methods U.S. Food and Drug Administration Laboratories, Washington, U.S.A.

volunteer collected all urine passed during the 6 hours of the test and the bulked sample from each was titrated for penicillin in a similar manner.

The results, shown in Table V and compared with those of a similar experiment on uncoated tablets, show no significant differences between penicillin blood levels or excretion after administration of the two types of tablet.

Summary

1. Figures have been presented to show the stability for 3 months at 38° C. and at room temperature (18° to 24° C.) for 2 years of penicillin tablets sugar-coated by conventional pan methods.
2. Such sugar-coated penicillin tablets have been shown to withstand a relative humidity of 75 per cent. at 25° C. for at least 2 months.
3. The results presented show that conventional sugar-coating does not interfere with the *in vivo* absorption of the penicillin from the tablets.

The authors are indebted to Mr. L. J. Hamilton, Mr. B. I. Helliwell, and Mr. W. R. Long for analytical assistance and recording of stability figures.

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DISCUSSION

The paper was presented by Mr. F. Taylor.

Dr. D. C. Garratt (Nottingham) suggested that details of the methods of coating should have been given.

Mr. F. Berry (Nottingham) said that the keeping properties and the stability of the tablets depended largely on the method of manufacture. What was the nature of the diluent used? Was the process moist granulation or dry granulation? What was the nature of any subcoating used?

Mr. R. Levin (Liverpool) asked whether there had been any variation from batch to batch in the formulation of the tablets. Were the authors satisfied that their method of estimating free moisture was satisfactory? By crushing 5 tablets and mixing them it seemed that they exposed the tablets to the atmosphere, and changes in the free moisture condition may have taken place. Had they attempted to estimate the moisture in an uncrushed tablet so as to compare the figures with those after crushing? Could they give additional information on the free moisture after a period of storage? It would be interesting to see whether a relationship existed between moisture picked up and loss of penicillin content. From the blood level figures it seemed that an unsatisfactory level existed after four hours. Was that so?

MR. A. W. BULL (Nottingham) said it was an established fact that if penicillin tablets could be prepared in conditions of low humidity and low moisture content, and if they were placed into sealed containers, then stability would be achieved. The solution to the problem seemed to lie in the subcoating of the tablet, and it would have been valuable if details of the subcoating techniques—the moisture protection techniques—had been included in the paper.

MR. D. STEPHENSON (Dartford) said that batches would have to be much more rigorously examined when prepared by this method than by methods where the use of moisture could be entirely avoided. It seemed unlikely that there would ever be a large use for sugar-coated penicillin tablets because children, who were the principal consumers of penicillin oral tablets, could not be persuaded to swallow them whole.

MR. TAYLOR, in reply, said that the tablets were coated by someone skilled in the art of sugar-coating. In regard to batch variation, so far as sugar-coating was concerned there was no variation but there was variation in some batches in the basic formula of the uncoated tablet. This did not seem to affect the problem, which was whether such tablets could be sugar-coated satisfactorily. The method for determining moisture content was the best they could devise and appeared to give reasonably reproducible results, but he agreed it was a difficult problem. With a sugar-coated tablet, a determination of the loss by storing over phosphorus pentoxide meant very little. It was assumed from the humidity studies that the water did not go through the coating to the penicillin tablet, and it therefore seemed unlikely that it would come out of the core through the coating. It was doubtful whether determination on the whole tablet would yield any information. It was true that to sugar-coat tablets which were very sensitive to moisture, rigorous batch control was necessary. In reply to the question about the blood levels, he said that for soluble penicillin the figure of 0·06 I.U./ml. was twice what was considered to be a reasonable therapeutic level, and in any case tablets were taken at four-hourly intervals.

SYMPOSIUM SESSION

REPORT OF A SYMPOSIUM ON TABLETS

AT the Symposium Session the Chairman, Dr. H. Davis, presided, and introductory addresses were given by Mr. H. D. Fitch, Mr. H. Burlinson and Mr. T. C. Denston.

Mr. Fitch, speaking from the dispensing point of view, said that the advantages of tablets included accurate dosage, portability and mass production, with resultant economy, but although it seemed that pharmacists might be developing into "mere tablet-counters" it would still be essential for them to have full knowledge of material used.

A recent report on test prescriptions had shown that some samples of tablets failed to comply with the official disintegration test, and packaging and labelling of tablets from pharmacies had been criticised recently by a public analyst.

The pharmacist should check the condition of the tablets and date stock on receipt. He should ensure that conditions of storage are suitable. Many factors are responsible for deterioration, and often the product is better protected by the manufacturers' original container than in stock bottles. Care should be taken to avoid over-stocking of those drugs which are sensitive to atmospheric moisture and other external conditions. Many tablets should be dispensed in well-closed containers and protected from light or moisture, particularly as the patient might keep them in a steamy kitchen or under similar unsuitable conditions; again, the manufacturer's original packing was often the best, but in many cases the number prescribed did not tally with the original pack. Strip packing in "Cellophane" or metal foil was satisfactory in preserving tablets in good condition, and it also protected the tablets from the attention of young children. On the other hand, this type of packing was costly, and too bulky when a large number of tablets was prescribed.

The variation in the size of tablets from one batch to another had often caused trouble. The Association of British Pharmaceutical Industry recommended sizes for many of the official tablets, but variation still occurred in tablets obtained from the same or different makers which led to technical explanations which were incomprehensible to a querulous patient. Coated and coloured tablets were open to misuse, and had proved too attractive to young children on many occasions, sometimes with fatal results.

The addresses of Mr. Burlinson and Mr. Denston are printed below in abridged form.

THE PREPARATION OF TABLETS

By H. Burlinson, F.P.S.

From the Laboratories of Thomas Kerfoot & Co., Ltd., Ashton-under-Lyne, Lancs.

Tablet history, like the tablet itself, is compressed into a comparatively small space—a mere fraction of the age-old story of pharmacy. There seems little doubt that in 1843 a patent was granted to a North Country chemist, William Brockendon, for a machine designed "for shaping Pills, Lozenges and Black Lead, by pressure in a die", and potassium bicarbonate was the first drug to be compressed into a tablet.

Most drugs in solid form can be compressed, and, in addition, the active ingredients of liquid galenicals such as tinctures and liquid extracts, may be isolated and offered in tablet form.

Tablets can be made in a wide variety of shapes and sizes, and such diverse pharmaceutical products as dental cones, pessaries and bougies can be made by compression. For the purpose of this paper, however, it is proposed to consider only the preparation and manufacture of tablets which are administered orally. The fact that they are used so widely suggests that tablet medication has certain advantages, and these may be briefly stated.

1. *Accuracy of Dosage*

This is of prime importance, and can be achieved by the use of adequate mixing plant and frequent check weighings of the tablets when they are being made. Alternatively, the granules may be assayed for active agent content before they are compressed, and the tablet weight calculated to give the theoretical dose of drug. Whatever the method used it should be possible to obtain uniformity of dosage within $\pm$ 5 per cent. of theory, a factor of particular importance where small doses of potent drugs are required.

2. *Stability*

A tablet should retain its appearance and potency for a reasonable period. Access of moisture to the drug must be prevented, a matter of particular importance for a preparation such as penicillin oral tablets where inactivation may occur without visible signs of deterioration.

3. *Economy*

Few pharmaceutical preparations lend themselves so readily to manufacture by mass production methods, thereby lowering the cost of medication.

The standards of pharmaceutical elegance that apply to older forms of dispensing should be maintained. Tablets should be symmetrical in appearance and free from imperfections. Any colour included in the formula should be uniformly distributed to avoid a mottled appearance. If the drug has a nauseous taste, or is liable to chemical change when exposed to air, it should be protected by a suitable coating. Tablets

should be compressed sufficiently hard to withstand normal hazards of packaging and transport, in order to reach the consumer in as perfect a condition as when they were made.

Whilst tablets have been widely used for many years, the principles underlying their formulation and the methods used in their manufacture were not readily available. The Seventh Addendum to the British Pharmacopœia, 1932, imposed for the first time standards for uniformity of weight, accuracy of dosage and, where necessary, limits for the time of disintegration. This led to a general examination of standards and manufacturing methods, and resulted in an all round improvement in the quality of tablets.

Firth[1] pointed out that the physical form of a drug may have a marked effect on the ease or difficulty with which it can be compressed and Fishburn[2] elaborated this with particular reference to sulphanilamide. The plant chemist may, therefore, be required to produce a drug which is not only chemically pure, but which is in the physical form which has been found to be most satisfactory for tabletting. Aspirin crystals are the best example of this, others include exsiccated ferrous sulphate, calcium lactate and dry extract of cascara.

A tablet is made by compressing a predetermined volume of granules in a die between two punches. For a few substances, usually crystalline and soluble in water, tablet making is a comparatively simple process. The crystals are dried to remove adherent moisture, sieved to uniform size, and then compressed without further admixture. Halides of the alkali metals, potassium chlorate, sodium nitrite, hexamine and urea, can all be treated in this way.

Substances in powder form do not readily pour or flow evenly, but when placed in the hopper of a compressing machine will give an irregular feed into the die, making it impossible to obtain an accurate dose of drug in each tablet. This difficulty is overcome by converting the powder into uniform, free-flowing granules, possessing the essential cohesive property which will enable them to retain a firm hard shape after compression. The manner in which this is achieved is called the granulation process, and it is the most important operation in tablet making, the quality of the granules largely controlling the difficulties encountered in tablet making.

The excipients used to assist in the manufacture and to ensure the efficacy of the tablets may be required to fulfil one or more of the following functions: (1) filling; (2) absorption; (3) adhesion; (4) wetting; (5) disintegration; (6) lubrication.

1. Where small doses of potent drug are ordered, such as hyoscine hydrobromide 1/200 grain, a filler is used to provide bulk and it is customary for the weight of such tablets to be made up to approximately one grain. Substances used for this purpose include dextrose, sucrose, lactose, mannitol, dextrin, starch, kaolin, sodium citrate, sodium chloride, exsiccated sodium sulphate, calcium phosphate and chocolate base. Care is necessary in selecting a filler which is completely inert for that particular formulation[3].

2. An absorbent may be necessary if oils, tinctures or fluid extracts are contained in the formula. For aqueous or alcoholic liquids a filler such as lactose or starch will also act as an absorbent, whilst for oils, magnesium oxide and magnesium carbonate are used. Powdered liquorice root can be employed as an absorbent where its colour is not objectionable.

3. The function of the adhesive is to assist in producing granules which are free from excessive "fines". Drugs having a low bulk density and possessing little cohesive property require the use of a strong adhesive, which is generally more efficient in a solution or suspension than as a dry powder. There is a wide range of substances suitable for this purpose. Should one of the sugars be present as a filler it will also serve as an adhesive when moistened with water or dilute alcohol. Mucilage of starch (10 per cent. w/v) is most useful, but it should be freshly prepared each day. Dilute mucilage of acacia (10 per cent. w/v) can be used either alone or mixed with mucilage of starch. It must be used with caution, however, since it tends to produce hard granules which can adversely affect disintegration. Syrup 50 per cent. w/v and glucose solutions are useful, the latter often being employed to granulate substances which oxidise, such as ferrous salts. Uncoated tablets containing glucose may soften in warm climates, however, rendering the tablets unfit for use.

When preparing compressed lozenges, one aims to produce a hard tablet which will dissolve slowly in the mouth and thereby prolong local medication. This effect can be obtained by including gelatine solution (10 per cent. w/v) as the adhesive, but since its use may encourage mould growth particular care should be taken in packaging and storing the tablets. Dextrin, pectin, tragacanth and quinine mucilages have been used, and, more recently, the sodium salts of algenic acid and carboxymethylcellulose (4 per cent. w/v).

Water cannot be employed for granulating drugs which would decompose, or which are deliquescent. In such cases, organic solvents such as ethanol, methanol, *iso*propanol and acetone, are employed, but granules prepared thus are not usually so robust as those made with aqueous adhesives and they should be handled with care.

4. There are a few drugs which are water repellent and tablets made from them may not break down when administered, although an adequate amount of disintegrant is present. Guaiacol carbonate, guaiacum resin, phenacetin and phenothiazine all share this disadvantage which can be overcome by including in the formula a small proportion of non-toxic wetting agent.

5. Should the drug be sparingly soluble in water, the tablet must be made to disintegrate and this is achieved by the use of substances which readily absorb water and in so doing swell up, causing disruption of the tablet. The tablet should break up in a reasonable time, producing numerous fine particles, the combined surface area of which is greatly in excess of that of the original tablet, thereby increasing the rate at which the drug is exposed to gastric and intestinal action.

Satisfactory disintegration can be obtained by the use of 5 to 15 per cent. w/w of one of a number of starches, and amongst those commonly

used are maize, tapioca, potato, arrowroot and rice. Alginic acid (10 per cent. w/w) and sodium carboxymethylcellulose (2 per cent. w/w) are two of the more recent disintegrants now in regular use, whilst agar, gelatin, pectin and bentonite are other disintegrants worthy of mention.

The effervescence produced by the interaction of sodium bicarbonate and citric or tartaric acids is another means of obtaining quick disintegration, and magnesium peroxide, which liberates oxygen on contact with water, behaves in a similar way.

6. When a tablet is made, it is necessary to introduce a lubricant to prevent adherence of powder to the punches and ensure smooth ejection of the tablet from the die. Inadequate lubrication will cause a film to be built up on the punches resulting in undesirable markings on the tablet. Powdered lubricants should be passed through a fine sieve so as to give the greatest possible covering value. It is assumed that such lubricants act by coating the surface of the granules but the granules are not in fact coated with lubricant, which is mostly located in the granular interspaces[4] and there is, therefore, some doubt as to the exact manner in which powdered lubricants function.

Purified talc (3 to 5 per cent. w/w) is widely used, but objections have been raised because of its complete insolubility and probable retention in the body. This disadvantage is overcome by magnesium or calcium stearate ($\frac{1}{2}$ to 1 per cent. w/w). Tablets thus lubricated have a fine surface polish which enhances their appearance.

Stearic acid gives a similar effect if dissolved in chloroform and added during the moist granulation process. Used thus, it is termed an "internal lubricant" since it is dispersed throughout the tablet mass and tablets containing it may be recompressed without the necessity of relubrication. The lubricating power of talc is much reduced once it has been compressed, and a further addition of lubricant is needed if the tablets should have to be re-made. The stearate group of lubricants should not be used excessively in case "waterproofing" of the tablets should occur and disintegration be seriously retarded. Hydrogenated peanut oil and light liquid paraffin are examples of other lubricants which are often used.

Dried starch has some lubricating value, and aspirin tablets can be made from aspirin crystals with 10 per cent. of dried starch which acts both as disintegrant and lubricant.

All the above-mentioned lubricants are insoluble, but it may be required to prepare tablets which dissolve completely in water to give a clear solution. The number of soluble lubricants available is smaller, and they are generally not so efficient as the insoluble. Powdered boric acid (5 to 10 per cent. w/w) is widely used, but does not readily "wet" and tends to increase the solution time of tablets containing it. Carbowax 4000, a polyethylene glycol, although wax-like in appearance is soluble in water and is effective added as a dry powder (4 per cent. w/w). Tablet makers are still looking for the perfect lubricant. It should be white or colourless, odourless, tasteless, soluble in water, pharmacologically inert and effective in low concentrations.

Having described the types of excipients and the manner in which they are used, it must now be shown how they are employed. Tablet granules may be prepared by one of three methods, the moist granulation process, the dry granulation process and the precompression process.

Each stage of the moist granulation process will be considered in some detail; a fuller account can be found in a useful book by Little and Mitchell[5].

1. *Preparation of the Ingredients in Powder Form*

Except for those crystalline substances which are compressed pure, it is usually advantageous to reduce to a fine powder all the ingredients contained in the formula. For small batches, the powders may be rubbed through a No. 80 sieve by hand. Alternatively, ball mills can be used, but these are not easily cleaned and their use is often reserved for special purposes, when, for example, exposure of the powder to the atmosphere is undesirable because of colour, taste or hygroscopicity. For larger batches, use is made of the comminuting mill, a machine which not only serves to pulverise solids to a very fine powder, but is also used to produce both wet and dry granules.

2. *Mixing of the Powdered Ingredients followed by Granulation*

Upon the efficiency of this operation will depend the accuracy of the active ingredient within the tablet. As was pointed out by Evers[6], absolutely perfect mixing of powders is unattainable, the degree of uniformity depending upon the particle size, air space and relative proportion of ingredients. The use of adequate mixing plant should ensure, however, that the distribution of medicament within the mix complies with specification requirements.

Powder mixing machines are available, and the comminuting mill will also fulfil this function. It is often convenient to mix the powders in a machine used for moist granulation and this may be of the type having a central revolving arm or blades located in the bucket of the machine, or the change-can type where the blades are lowered into the mixing bowl and then raised after use, thus making cleaning of the mixer an easy operation.

Moist granulation is carried out by adding a liquid binder or moistening agent to the mixed powders. For an untried formula, liquid excipients must be added slowly in successive quantities until a damp but firm mass is obtained which can be moulded in the hand. The use of too much liquid in an endeavour to speed up granulation will give an overdamped mass which cannot be sieved until it has dried out. If one is overcautious and does not add sufficient liquid, crumbly granules are obtained which, on drying, produce an undesirably high proportion of "fines."

The damp mass is emptied into a stainless steel container and large granules produced by passing it through a coarse sieve of 4, 6 or 8 mesh. Oscillating or rotary granulators rub the mass through a sieve by means of rollers or blades; the comminuting mill is also much used for this purpose, although the principle employed is different. The hammers

are replaced by knives having a blunt cutting edge, and they are revolved inside the casing at a very much reduced speed. A particular advantage of this machine lies in its ability to handle overdamp granulations which could not normally be sieved by the more conventional type of granulator.

4. *Drying of the Granules*

It is not usually necessary or desirable to remove all traces of moisture from the granules; in fact, granules which are too dry may be as difficult to compress as those which are too moist. The greater part of the moistening agent must, however, be removed.

The modern drying oven is a large unit into which stacks of trays loaded on to trucks can be wheeled in and out. Heat may be provided by steam or electricity and the stove is designed so that warm air enters near the bottom and is directed over the trays by a system of louvers. A drying temperature of 53° to 60° C. is adequate for most drugs, but heat-sensitive substances will need longer drying at a lower temperature. The trays which hold the moist granules may consist of canvas covered wooden frames, but enamelled iron, aluminium or stainless steel trays are preferable, since they are easily washed and eliminate risk of contamination.

Whilst the method described is in common use, experimental work has been done using different sources of heat. Infra-red heat as a means of drying tablet granules has been investigated[7,8]. The heat generated within a non-conducting substance by the rapid alteration of the radio frequency field, has been used to obtain substances free from moisture, and the method has been applied experimentally to dry tablet granules[9,10]. Whilst this work is of interest, one wonders whether it has any practical significance on the manufacturing scale due to probable high operating cost.

5. *Preparation of Uniform Granules and Lubrication*

To produce tablets of accurate and uniform weight necessitates the delivery of a constant volume of granules into the die and their preparation is the next operation in tablet making. When granules are reduced in size by sieving a certain amount of powder is produced, and in fact such "fines" are necessary since by filling the interspaces of the granules, they ensure that a constant weight is delivered into the die at each filling operation. Hard granules will usually produce less "fines" than those which are not so cohesive. The weight of the tablet normally determines the approximate granule size, a small tablet weighing only one grain and of 3/16″ diameter needing finer granules than a larger tablet whose diameter may be up to ½″. A 20's mesh sieve can be used to prepare granules for small tablets and intermediate grades employed up to about 10's mesh for larger ones.

The same machines are employed to dry granulate as were used to sieve the damp mass, but finer screens are inserted. When feeding coarse, dry granules into the hopper, it should not be overloaded since frictional effects will produce more "fines" than may be desired.

The addition of powdered lubricant is the last operation before the granules are ready to be compressed, and it can be introduced manually

or by slowly tumbling in a powder mixer. Lubricants such as liquid paraffin can be sprayed on to the bulk of the granules and gently mixed throughout. Flavours and essential oils dissolved in a volatile solvent are also added at this stage.

The moist granulation process may be modified by excluding all or part of the disintegrant at the time of granulation. For a tablet which does not readily disintegrate, it may be advantageous to add half of the disintegrant during moist granulation and the remainder with the lubricant to the dry granules. This added starch disrupts the tablet into its component granules, whilst the granulated starch, still retaining the capacity to absorb water and swell up, breaks down individual granules into finely dispersed particles. It must be remembered that by adding dry starch the proportion of fine powder may be substantially increased.

(B) The dry granulation process is one not widely used since it is limited to those substances already mentioned which can be compressed pure, or to drugs which require only the addition of disintegrant and lubricant before they are ready for compression.

(C) Granulation by precompression is being increasingly used, and the process has certain advantages over moist granulation. It consists in compressing the mixed powders into large but imperfect tablets or "slugs". These are sieved to produce small uniform granules which may then be recompressed into tablets of the correct weight and size. Robust rotary machines have been developed to make the "slugs" incorporating special feeding devices to ensure uniform filling of the die with powder. This point is important since a variable feed will produce friable "slugs" which readily break down to powder when sieved, instead of producing the hard granules necessary for tabletting. It will be seen that elimination of the drying process not only excludes the need for drying equipment but makes the process of precompression a continuous one, a matter of considerable economic importance. It is also suited for making tablets of incompatible ingredients which would react if moistened, for substances sensitive to heat and for making effervescent tablets.

Since no liquid binder is used, the formula must contain excipients with cohesive properties, otherwise a friable "slug" is obtained which will not readily compress, and repeated recompression may be necessary before satisfactory tablets are produced. The omission of a moistening agent tends to produce a greater proportion of "fines" than is obtained by the moist granulation process, and this may cause "capping" troubles. The process of precompression has been fully described by Peck[11].

The tablet maker from time to time encounters compressing difficulties of which the commonest is that of lamination or capping. It results in the top of the tablet becoming detached and even when this is not immediately apparent, the "cap" can be removed with the thumb nail or it will fall off when a few tablets are shaken in a bottle.

This phenomenon may be due to a number of causes and the compressor must decide whether the trouble lies in faulty granules, imperfect tools, or incorrect speed of compression. Capping is commonly experienced in granules containing a high proportion of "fines". At the

time of compression, air within the granules normally escapes via the narrow clearance between the top punch within the die. Should the interspaces be filled with fine powder, the compressed air cannot escape and much may be retained within the tablet. When the pressure exerted by the top punch is removed, the trapped air expands to attain an equilibrium and escapes from the tablet at its weakest point, which is the periphery, the cap becoming detached at once or when the tablets are handled.

The trouble may be remedied by sieving out some of the "fines" but obstinate cases require regranulation before satisfactory tablets are produced. If the speed of compression is too rapid, a stamp rather than a squeeze occurs, the entrapped air cannot escape before it is compressed and capping occurs. The remedy lies in slowing down the speed of compression and capping due to this cause is usually less frequent on a rotary machine where both top and bottom punches move to compress the granule between them, as compared with a single punch machine where the lower punch is stationary at the moment of compression. The use of excessive pressure will cause capping, as will an insufficiency of lubricant in the formula. Granules which have been overdried sometimes cause this trouble, easily rectified by slight moistening. Certain crystalline forms appear to be responsible for persistent capping, and where this is anticipated, the drug should be finely powdered before granulation.

A die which is in continuous use will begin to show a "ring" at the point of compression, causing a slight distortion in the tablet when it is made and frequently leading to capping when the tablet is ejected from the die. Certain vegetable drugs such as powdered digitalis leaf have an extremely abrasive effect on punches and dies, whose working life is much reduced. The introduction of dies made of tungsten carbide has overcome this hazard to a considerable extent, even the most abrasive substances having little effect on its mirror polish.

"Picking" and "sticking" are problems occurring less frequently, and are more readily corrected. A particle of granule or film of powder adhering to the punch face, gradually builds up until the upper and lower surfaces of the tablet are disfigured. This trouble may be caused by:— (*a*) a punch surface which has been marked or scratched; (*b*) the use of imperfectly polished punches; (*c*) lack of lubricant; (*d*) granules which are too damp. Attention to these points will usually correct any further difficulty.

"Binding" in the die is either caused by shortage of lubricant or by the use of damp granules. Ejection of a tablet is usually accompanied by a "grunting" noise from the machine, a danger signal that must be heeded.

When commencing to compress a batch of granules, a few trial tablets are first made, turning the machine by hand, so that any error in setting up the machine can be adjusted before it operates at speed. Until the correct weight of granules in the die is obtained, all tablets made should be rejected. The pressure is varied until a firm tablet is obtained and the sample should then be shaken in a bottle and examined for capping and the disintegration time must be checked against specification.

The tablets should be examined for friability by determining the amount of powder produced when a known weight of tablets is shaken in a bottle under controlled conditions. The quality control of tablets has been comprehensively described by Nutter Smith[12].

Circumstances may require a tablet to be coated with a protective layer; this often consists of sugar, but it may be a pearl, silver, balsam or gelatin coating. Apart from the aspect of sales appeal, coating may be necessary for the following reasons:— 1. The tablet may have an unpleasant taste. 2. It may contain a drug which is unstable or which will deteriorate on exposure to air. 3. It may consist of drugs of different colour which would appear mottled and unsightly when compressed. 4. The active ingredients may be decomposed by gastric juice and therefore need to be protected with an enteric coat.

The following operations are employed:—

1. The application of a protective subcoat on to the tablet.
2. The gradual building up of the coating by adding concentrated syrup, removing the moisture by drying, and re-applying more syrup until a coat of the required thickness is obtained.
3. Polishing the finished tablet.

Tablets which are to be coated should be firmly compressed on deep concave punches, the compression thus obtained having a comparatively thin edge.

The subcoating consists of a thin shell of dammar resin or shellac, applied as an alcoholic solution, but it must not be used to excess otherwise the tablet will not disintegrate. Alternatively, the subcoat may consist of a mixture of gelatin, sucrose and acacia. The tablets are moistened with a small volume of liquid and allowed to roll in the pan until slightly "tacky." A dusting powder is next added until the moist tablets are covered, warm air is introduced into the pan and the tablets allowed to roll until the subcoating is dry. The process is repeated four or five times, taking care to dry after each wetting before adding more dusting powder. At this stage the edges should be filled in and the tablet sealed off from the effect of adding syrup.

Dusting powder can be made to various formulæ, a satisfactory one consisting of a mixture of precipitated chalk, icing sugar, powdered gum acacia and starch.

The coat is now built up by adding successive quantities of warm syrup in which starch grains are suspended, until just before reaching the final weight when starch syrup is replaced by a plain syrup which gives the desired smooth finish. The total amount of coating added usually equals the weight of the uncoated tablet. For coloured work, an edible dye or pigment is added to the syrup at the later stages of coating.

Before the final process of polishing can be commenced, the tablets must be quite dry, otherwise a dull finish will result. Polishing is carried out by rolling the tablets in a pan lined with a carnauba-beeswax mixture. An alternative method is to use a revolving canvas drum supported on a metal framework, into which a few drops of wax solution are added from time to time. It is convenient to have separate polishing pans for white

and coloured work, but the inside of the pan can be cleaned with a cotton wool pad moistened with chloroform.

When a formula contains ingredients which are incompatible, the uncoated tablets may be made to contain one ingredient whilst the other may be dissolved or suspended in the coating syrup and applied in the coating process[13]. More detailed information on tablet coating is available in a work by Clarkson[14], whilst a full summarisation of coatings and standards was carried out by Stephenson and Smith[15].

A method has recently been described by Whitehouse[16] by which a tablet can now have a layer of sugar compressed around it. The idea is not new, previous attempts having failed on mechanical grounds. Machines have now been developed, however, in which a tablet is compressed on one rotary machine, picked up by a travelling arm and accurately centred in a die of a second rotary machine, the die already containing a charge of lubricated sugar granules, a further amount of which is fed in to cover the tablet and the whole compressed, producing a core surrounded by an even coat of sugar. Whilst this coating differs somewhat from that obtained by the older method, it does in fact do all that is required, and the complete mechanisation and speed of the process has sound economic advantages.

This brief survey of tablet coating would be incomplete without mention of enteric coating. It is sometimes necessary to administer a tablet which must pass unchanged through the stomach, but disintegrate in the duodenum. Drugs such as emetine salts, stilbœstrol and ammonium chloride, produce nausea and the tablet may be rejected by the patient before it can be utilised. Glandular products which decompose in gastric juice may need protection, likewise anthelmintic drugs which are required in a local concentration to be effective, will not be diluted by gastric juice if they are enteric coated.

The problem of applying such a coating is complicated by the fact that the rate at which a tablet may pass through the stomach is a variable factor. Individuals differ both in their gastric secretions and the rate at which food is digested. The efficacy of an enteric coating may depend upon the following factors:—

1. *The difference in* p*H between the stomach and the duodenum.* Amongst the substances which have been used for enteric coating are shellac, dammar, mastic and sandrac, all containing insoluble resin acids which would be converted into soluble salts in the intestinal tract, resulting in a weakening and disruption of the protective coat. It has been shown, however, that the duodenal contents have a mildly acid reaction[17], and other factors may be responsible for the effectiveness of these substances.

2. *The action of lipase enzymes upon the saponification of esters.* This action is employed in the use of *n*-butyl stearate for enteric coating, since it is readily hydrolysed into stearic acid and *n*-butanol[18].

Substances such as stearic acid and cetyl alcohol, either alone or mixed with one of the above-mentioned resins, have been used with success, cetyl alcohol and mastic having been reported to be 98 per cent. efficient[19].

Other substances used in the past are keratin and salol—the former is of little value, but salol is useful for the extemporaneous enteric coating of pills and tablets.

Cellulose acetate phthalate has given very satisfactory results[20] employed as a 5 to 15 per cent. solution in ethyl acetate and ethanol. Because of the volatile nature of the solvents, successive coatings can be quickly applied. Bauer and Massucci[21] state that this enteric envelope is broken down by the hydrolytic effect of the intestinal esterases in pancreatin and not by alkalinity in any part of the duodenum. Whatever the substance used for enteric coating, it must be non-toxic and inert. It is applied at the subcoating stage if the final tablet is to be sugar coated.

The efficacy of these coatings has been studied both *in vitro* and *in vivo*, the former method being most commonly used. The tablet under test is immersed in artificial gastric juice at body temperature and occasionally agitated. Opinions differ on the time considered necessary for the tablets to withstand this treatment without breaking down, periods varying from 3 to 6 hours having been proposed, but 4 to 5 hours seems a reasonable average. The tablet is then removed from the acid digestive fluid and placed in alkaline pancreatin solution, where it should break down within one hour. The artificial fluids used by Abbott and Allport[20] have been found satisfactory for this purpose.

Whilst laboratory digestion tests give an approximate valuation of the efficacy of the enteric coating, a more accurate picture is obtained from *in vivo* tests, although results obtained from one person would not necessarily be closely reproduced in another, for reasons already given. The test consists in swallowing a tablet of barium sulphate coated with the enteric substance to be examined. Its progress through the digestive system and alimentary tract is followed by radiographs taken at intervals, successive pictures showing the exact location of the coated tablet within the body[22]. The authors of the original work consider that an effective enteric coat should be stable for 6 hours, after which it should disintegrate rapidly in whatever part of the digestive tract that it may be found. Since such coatings are now used increasingly, it is probable that standards will be laid down to determine their efficiency.

In this brief survey, the principles and methods used in making oral tablets have been examined. It has been shown that satisfactory tablets can only be produced after careful thought has been given to the manner in which the various excipients are combined in the formula. Whilst for reasons of economy, tablet production is a highly specialised branch of manufacturing pharmacy, it must always be remembered that tablets are a form of dispensing and should comply with the highest standards of accuracy and elegance.

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THE STANDARDISATION OF TABLETS

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Standardisation

The first official monograph describing a drug in tablet form appeared in the British Pharmacopœia 1885 under the title Tabellæ Trinitrini and it remained until 1945 as the only representative of this class of medicament. In comparison with the style of the monographs in the current Pharmacopœia, the original specification appears to be inadequate in that it states only the total weight of a tablet and the nominal content of active ingredient, and makes no reference to the excipient, the method of assay or the permitted limits of glyceryl trinitrate. The Seventh Addendum to the British Pharmacopœia 1932, published in 1945, described 35 tablets, with a general monograph governing the requirements laid down in the individual monographs. This marked a great advance in the control of tablets in that not only did each monograph include a method of assay and state the permitted tolerances for active ingredient but a test for disintegration was applied to all tablets except those intended to be chewed or crushed, or allowed to dissolve slowly in the mouth, or to be dissolved in water before administration. The tablet monographs for the Pharmacopœia increased to 50 in 1948, 6 more were added by the Addendum 1951 and the British Pharmacopœia 1953 has 63. It is proposed to include 72 monographs on tablets in the British Pharmaceutical Codex, 1954. A similar development has occurred with some other pharmacopœias; for example, the United States Pharmacopœia, which had described no tablets until 1936, when one monograph was added (Tablets of Glyceryl Trinitrate), now has 92 monographs. The Danish Pharmacopœia 1948, with Addendum 1952, describes 99 tablets. On the other hand, whilst there is a general monograph, there were no monographs on individual tablets in the pharmacopœias of Argentine (1943), Belgium (1951 Supplement), France (1949) and Turkey (1948).

Before considering details of the specifications given in the tablet monographs of the British Pharmacopœia it must be emphasised that, as stated in the General Notices to the book, the official standard for any particular tablet is not confined to the specified limits for the amount of active constituent. Tablets are not of pharmacopœial quality unless they comply with all the requirements described in the particular monograph or referred to in the General Monograph. These requirements include a method of preparation (which may, however, be permissive and not mandatory), identification, disintegration, limit tests for impurities, uniformity of weight and content of active ingredient when determined by the assay described in the monograph. It should be noted that the Pharmacopœia, in outlining the general methods of preparation, gives latitude to the manufacturer provided that the other ingredients are innocuous and therapeutically inert in the quantities present. Lists of suitable diluents, lubricants and moistening agents are given.

The British Pharmacopœia imposes no requirements for the dimensions of tablets or their total weight, but these aspects of standardisation have engaged, and continue to engage, the attention of the Commission. In so far as the amount of excipient and the die size for a particular tablet vary from one manufacturer to another, there is undoubtedly a source of trouble to the pharmacist and a cause for doubt in the mind of the patient as to the accuracy with which his prescription has been dispensed when he receives a second supply of tablets differing obviously in size from the first.

The trade association of the pharmaceutical manufacturers recognised some years ago the importance of uniformity and prepared and issued to its members, with a recommendation for its adoption, a detailed schedule of die sizes and total weights for some 250 different tablets and covering all the common dose-strengths. It is understood that many tablet makers now work to the recommended schedules, but unfortunately other manufacturers have not accepted their association's recommendations. The Commission continues to receive or learn of complaints which show that disparity in practice still causes embarrassment and trouble. That these conditions should persist must lead the Commission to consider whether a trade recommendation could be adequate to deal with the situation. It may prove that official standards, at least for the diameter of the punch, must be laid down. If the diameter, but not the total weight, is officially specified conspicuous distinctions in size between batches from different manufacturers might well disappear but at the same time some latitude in the amount of material to be included in addition to the medicinal ingredient would remain.

Colouring of Tablets

From the introduction of a series of monographs in the Pharmacopœia by the Seventh Addendum of 1945, the general monograph has always stated that the addition of colouring agents is not official. There are no important medical grounds for the colouring of plain or coated tablets but there are strong objections from both pharmaceutical and medical considerations. The colouring of tablets tends to give dangerous encouragement to reliance on identification by appearance instead of by the reading of the label. Further, experience has shown that some manufacturers are not always able to produce or maintain a particular shade, and variation in appearance between different batches may be conspicuous. Most important is the enhanced danger to infants and young children from brightly coloured tablets. Many references to the poisoning of children by coloured tablets have appeared in the medical journals in recent years[1,2,3].

Disintegration

Simple requirements for the disintegration of tablets began to appear in the pharmacopœias 20 to 30 years ago. Probably the first reference is in the Brazilian Pharmacopœia of 1926 and 4 years later the Belgian Pharmacopœia, in its general monograph, had the statement that tablets must

dissolve or disintegrate within a short time when shaken with tepid water. Within a few years other pharmacopœias adopted the same or a similar broad statement, notably those of Denmark (1933), Switzerland (1934), France (1937), Finland (1937) and Russia (1937). The method of the Swiss Pharmacopœia is typical: "pour 50 c.c. of water at 37° C. on a tablet in an 100-ml. conical flask; shake the flask gently from time to time; the tablet must completely dissolve or disintegrate within 15 minutes". Substantially the same method is used in the current pharmacopœias of Egypt (1953), Japan (6th edition), Denmark and Sweden. Many attempts have been made to find a method which will give a definite end-point whatever medicinal substance, diluent or binding agents may be present. At the 1939 British Pharmaceutical Conference, Brown[4] described a method which applied a shearing force to the tablet and on the same occasion Berry[5] suggested a method depending on a weighted wire cutting through a tablet after it had been softened in water. In a later paper Berry and Smith[6] proposed a method which was essentially a development of that given in the Swiss Pharmacopœia and it was later adopted, in its essentials, in the Seventh Addendum to the British Pharmacopœia 1932.

Other workers, including Hoyle[7], Prance, Stephenson and Taylor[8], Sperandio, Evanson and DeKay[9] and Evanson and DeKay[10], have attempted to achieve a sharp end-point by the use of a wire-gauze screen through which the particles of the disintegrated tablet must pass.

The diversity of methods used in the national pharmacopœias is shown by comparing (i) the number of tablets used in the test, (ii) the temperature and volume of the water, and (iii) the apparatus.

(i) Some pharmacopœias do not state the number of tablets to be used; in others the number ranges from 1 to 6, as shown by the following examples:—

1 tablet	Argentine, Switzerland, Japan.
2 tablets	Yugoslavia.
3 tablets	Denmark.
5 tablets	Britain, France.
6 tablets	United States of America.

There is a difference between the French practice on the one hand and the British and American on the other, in that the former uses 5 tablets in one vessel and the latter direct one tablet to be placed in each tube. Moreover the British Pharmacopœia directs that if one tablet fails to comply, the test may be repeated with 5 tablets from the same batch, when all must comply; a tolerance of 90 per cent. is thus permitted.

(ii) The volume of water varies from about 50 ml. to 1 l. but most pharmacopœias stipulate that the water shall be at about body temperature, e.g., 37° C. (B.P.), 35° to 39° C. (U.S.P.), 38° to 40° C. (Danish Pharmacopœia), 40° C. (Swedish Pharmacopœia). The French Codex has now adopted 20° C. Berry and Smith obtained more consistent results with their method at 37° C. than at 18° C.

(iii) The apparatus may consist of a flask or beaker containing the tablet or tablets and water, or a series of tubes closed with a cork, as in the British Pharmacopœia, or provided with gauze at the lower end, as in the United States Pharmacopœia. The French Codex has a metal cylinder with gauze at the bottom; 20 glass beads are used with 5 tablets and the tube is shaken with a circular movement sufficient to carry round the beads.

In an attempt to improve on the present official test, the Tablets Committee of the British Pharmacopœia Commission has examined a number of methods intended to yield consistent results and an unmistakable end-point especially when applied to tablets which tend to form a gummy mass and to coated tablets. It has also been the intention to keep the test as simple as possible. Arising from this collaborative work, the following test, which is based on that of Prance, Stephenson and Taylor[8] is now under examination and, subject to comments which may be received—and criticisms are invited from those who may try it—will be recommended to the Commission for inclusion in the forthcoming Addendum to the British Pharmacopœia 1953.

Apparatus. A glass tube 80 to 100 mm. long, with an internal diameter of about 28 mm. and an external diameter of 30 to 31 mm., is fitted at the lower end with a disc of rustproof wire gauze complying with the requirements for a *No.* 10 *sieve*, British Pharmacopœia 1953, page 853, and suspended in a volume of *water* having a depth of not less than 15 cm. and at a temperature between 35° and 39° C. in such a way that it can be raised and lowered repeatedly in a uniform manner through a distance of 75 mm.; at the highest position of the tube, the gauze just breaks the surface of the water, and at the lowest position, the upper rim of the tube remains clear of the water. The tube may be manipulated by hand or mechanically.

Method. Place 5 tablets in the tube and raise and lower the tube in such a manner that the complete up and down movement is repeated 30 times a minute. The tablets are disintegrated when no particle remains above the gauze which would not readily pass through it. The time required for the 5 tablets to disintegrate in the manner described is, unless otherwise stated in the monograph, not more than 15 minutes. If the tablets fail to comply, the test may be repeated using a guided disc as described below, inserted in the tube; the tablets must then comply with the test. The guided disc consists of a disc of suitable plastic material, about 26 mm. in diameter and 2 mm. thick, with 3 holes equally spaced and 10 mm. from the centre. In each hole a stainless steel wire of No. 22 Standard Wire Gauge is secured at a right-angle to the plane of the disc and the end of each wire is turned out radially and secured to a guide ring of No. 22 Standard Wire Gauge and 27 mm. in diameter. The guide ring is co-axial with the disc in a parallel plane at a distance of 15 mm. from the upper surface of the disc. The difference between the diameter of the disc and the internal diameter of the tube is not more than 2 mm. The total weight of the guided disc is not less than 1·9 g. and not more than 2·1 g.

Enteric-coated Tablets

Special coatings are applied to tablets when it is intended that the active ingredient shall not come into contact with the acid secretion of the stomach. Official specifications for the composition of such coatings are not provided. The criterion generally accepted is that the tablets should not disintegrate when immersed in an acid pepsin solution but must disintegrate in an alkaline pancreatin solution. A test for enteric coated tablets is proposed for inclusion in the new edition of the British Pharmaceutical Codex. The apparatus to be used is similar to that described above. The test consists of two parts: in the first part, a watery solution is used containing pepsin, potassium chloride, calcium chloride and hydrochloric acid, and immersion continued for 3 hours. No portion of the tablets, other than fragments of any outer coatings, passes through the gauze. The tablets are then rapidly washed with water and, as the second part of the test, they are immersed for one hour in a watery solution containing pancreatin, sodium tauroglycocholate and sodium bicarbonate. The tablets must disintegrate completely.

Durability

The pharmacopœias do not provide standards to ensure that tablets will show resistance to "wear and tear" during storage, packaging and transit. It is important that tablets should not break or crumble easily, or become chipped at the edges, and simple tests are commonly applied during production. The firmness of the tablets may be assessed by noting the amount of pressure needed to break a tablet by hand or by shaking together a number of tablets in a bottle and determining the weight of powder produced. Such simple tests can be carried out whilst the machine is running and any necessary adjustments immediately made. They are scarcely amenable to description as official standards, and no other method has so far proved acceptable for this purpose.

The application of mechanical methods has been described by Smith[11,12]. Tests were made with instruments designed for measuring the hardness of metals, such as the scleroscope which measures the height of rebound when a hammer of fixed weight is allowed to fall on the surface of the tablet; the Vickers diamond hardness testing machine measures the depth of the impression made in the surface of the tablet. Neither instrument appears to be acceptable for the routine testing of tablets. Smith regards the Monsanto pressure tester, a spring-loaded device with which the pressure required to break a tablet may be determined, as the instrument most easily and rapidly operated.

Weight Variation

Those pharmacopœias which provide, in any detail, standards for tablets usually set limits to the variation in total weight within a batch of uncoated tablets. The requirements may be stated as one figure applicable to tablets of all sizes or as graded requirements according to 3 or 4 categories of weight. Thus, the French Codex states that the average

weight, determined on 10 tablets, is not less than or more than 5 per cent. of the stated weight. The British Pharmacopœia controls the uniformity of weight by requiring that in a sample of 20 tablets not less than 18, when weighed singly, shall fall within the limits stated in a Table, and not more than 1 tablet shall deviate by more than double these limits. The pharmacopœias of the United States of America and Egypt have similar requirements with permitted limits as shown in Table I.

The Swiss Pharmacopœia has limits of ± 10 per cent. (less than 250 mg.), ± 8 per cent. (between 250 and 500 mg.) and ± 5 per cent. (more than 500 mg.), when determined on 100 tablets. The Danish Pharmacopœia also employs 100 tablets and with more detailed directions for weighing permits deviations of ± 10 per cent. for tablets weighing less than 80 mg. and ± 4 mg. + 5 per cent. of average weight for tablets weighing 80 mg. or more; the method provides a more gradual narrowing of the limits with increase in weight.

TABLE I

VARIATION IN WEIGHT OF TABLETS

PHARMACOPŒIAL TOLERANCES

Percentage deviation	Average weight		
	B.P. 1953 mg.	U.S.P. XIV mg.	Egypt. Ph. 1953 mg.
± 15	—	13 or less	25 or less
± 10	130 or less	more than 13 and including 130	26 to 150
± 7·5	more than 130 less than 324	more than 130 and including 324	151 to 300
± 5	324 or more	more than 324	more than 300

The pharmacopœias which have been cited refer only to uncoated tablets, except the Swedish Pharmacopœia which states that "the variation in the weight of tablets of the same production batch shall not be greater than that corresponding to a relative standard deviation of 4·5 for uncoated tablets and 6·5 for coated tablets.

CONTENT OF MEDICAMENT

A few of the monographs of the B.P. 1953 provide a formula for one strength of tablet, as for example Codeine Compound Tablets and Sodium Bicarbonate Compound Tablets, but most of the monographs are framed to cover all strengths which may be prepared. Consequently, in laying down a standard for the amount of medicament contained in each tablet, the tolerances are intended to apply to all dosage strengths. In selecting these tolerances, three main factors have been taken in account, namely:— (a) a manufacturing tolerance in recognition of the variation to be expected when reasonable care and skill is exercised in the preparation of the granules and in compression. The figure may be about ± 5 per cent.; (b) any tolerance allowed in the monograph on the medicinal agent itself. Thus, official acetylsalicylic acid may contain 99·5 per cent. of $C_9H_8O_4$ and the lower figure for the content in the tablets is 94·5 per cent., not 95·0 per cent. It has not been considered necessary to observe

this factor in all tablets and indeed the principle may need reconsideration, due to the growing practice among manufacturers of assaying the granules before compression, and adjusting the total weight in accordance with the result; (c) the error inherent in the assay, due either to the very small amount of the substance present in each tablet or to the nature of the assay method. Ergometrine maleate tablets provide an example of tablets with a small dose (0·5 mg.) and a colorimetric method of assay leading to the specification of a wider tolerance, namely 85·0 to 110·0 per cent. In the British Pharmacopœia 1948, aneurine hydrochloride tablets were assayed by a fluorimetric method and it was necessary to allow a tolerance of 85·5 to 119·0 per cent. The same tablets are assayed by a silicotungstate method in the British Pharmacopœia 1953 and the tolerances are narrowed to 92·5 to 107·5 per cent.

Apart from any allowance for the purity of the medicament, official tolerances fall into three main groups, 95·0 to 105·0 per cent., 92·5 to 107·5 per cent. and 90·0 to 110·0 per cent., with a minority in the last group. It has been the practice to narrow the tolerances on revision of the Pharmacopœia when data has become available to show that it was reasonable to do so. The following are examples of such changes.

Tablet	*Tolerances (per cent.)*	
	B.P. 1948	*B.P.* 1953
Acetomenaphthone	89·0 to 110·0	92·5 to 107·5
Atropine Sulphate	89·5 to 112·5	90·0 to 110·0
Ephedrine Hydrochloride ..	89·5 to 110·0	90·0 to 107·5
Mepacrine Hydrochloride ..	88·0 to 111·0	92·5 to 107·5
Nicotinamide	88·0 to 110·0	92·5 to 107·5
Nicotinic Acid	88·5 to 110·0	92·5 to 107·5
Phenobarbitone	90·0 to 110·0	92·5 to 107·5
Phenobarbitone Sodium ..	85·5 to 110·0	90·0 to 110·0
Sodium Citrate	89·0 to 112·0	92·5 to 107·5

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DISCUSSION

MR. D. N. GORE (Dorking) suggested a test for durability of tablets based on rotating them in a horizontal fluted cylinder at a standard speed for a certain number of revolutions, separating the resulting powder from the tablets and weighing it. He did not see why it was not as accurate a measure of durability as the disintegration test was a measure of disintegration. He had simulated the traditional hardness test of exerting pressure on the tablet between the finger and thumb, by the use

of a bridged anvil and a weight travelling along a scale. There was no correlation between durability and resistance to snapping.

MR. M. TEEMAN (Leeds) asked the authors to comment on the various shapes used for tablets and their possible advantages.

MR. W. TRILLWOOD (Oxford) drew attention to the difficulty of rapidly identifying tablets in hospital when they had been dispensed under the National Health Service in a container labelled only with the name of the patient and the pharmacist. He suggested that N.H.S. prescriptions might be in duplicate, one copy to be retained by the pharmacist. He was glad that Mr. Denston disapproved of coloured tablets. Did manufacturers realise that 0·4 per cent. of the female population and about 4 per cent. of the male population were colour blind? Shape, colour, smell, appearance or any other device which detracted from label reading should be deplored.

DR. J. G. DARE (Leeds) referred to the accidents occurring to children and adults by the consumption of tablets in excessive doses or for purposes for which they were not intended. More should be done, in co-operation with the medical profession, to emphasise to the public that any medicine was potentially dangerous. For instance, in antenatal clinics in small towns and villages iron tablets were often supplied without adequate supervision of dispensing. It might be better if people were compelled to go to a pharmacy for all their tablets.

MR. J. B. LLOYD (Manchester) protested against the colouring of tablets, for which he said no adequate pharmaceutical reason had been given. He asked whether there was evidence that the *in vitro* tests of enteric-coated tablets gave an indication of what happened to the tablets *in vivo*. Would following the passage of an enteric-coated barium sulphate tablet through the body radiographically show what would happen to any other enteric-coated tablet? There was some evidence that certain proprietary enteric-coated tablets which had passed these tests in fact passed through the body unchanged.

MR. T. D. WHITTET (London) criticised the practice of stamping initials on tablets. He described the use of relatively cheap kitchen equipment for making tablets. The standard cake mixer made an excellent granulation machine. Powders, after mixing in a bowl and adding a moistening agent were put through a soup strainer, which gave moist granules, and then the coffee bean grinder attachment was used, giving granules ready for tabletting. Another excipient, which had not been mentioned, was bentonite, which had been found excellent where an entirely inorganic tablet, such as potassium perchlorate, was required. Magnesium trisilicate was a satisfactory disintegrant where its pharmacological properties were not contra-indicated. It seemed impossible to make a good tablet of aminophylline by the moist granulation process recommended in the B.P. It became very dark after 2 or 3 months' storage and smelled of ammonia and acetamide.

MR. S. DURHAM (Sheffield) asked whether it would be possible to specify in the official monographs the exact punch size for each tablet.

MR. A. F. CALDWELL (Singapore) spoke of the packing of tablets for tropical countries. Transparent cellulose sheeting collected dust and also grew moulds, although there was nothing wrong with the tablets. An excess of filling agents and excipients which were hydrophilic colloids might cause difficulty in that the tablets, whilst not becoming damp, swelled until it was impossible to get them out of the bottle.

MR. E. SHOTTON (London) thought it right to resist the use of coloured coatings. He asked for a clear definition of the properties which were being measured in durability. Engineers had already defined what they meant by hardness, which involved more of a surface property by indentation than breaking or crushing.

MR. C. W. ROBINSON (Liverpool) said that the conscience of the profession had been disturbed by the many deaths of infants due to eating tablets, in particular tablets containing ferrous sulphate. Tablets of ferrous sulphate and compound tablets of ferrous sulphate, which looked like ordinary white uncoated tablets, could be made by the compression coating technique. The June issue of the *Practitioner* contained an admirable review by Dr. Fraser, lecturer in child health in the University of Aberdeen, and it was clear from his analysis of many cases of accidental poisoning in children that it was by no means certain that colouring and sugar-coating played a decisive part in accidental poisoning. Therefore the question of whether tablets should be coloured ought not at this stage to be related to the causation of accidents until there was clearer evidence that it was the colour or the sugar which caused this fatal attractiveness. With the co-operation of the chief pharmacist of a large children's hospital, the organisation with which he was associated was in the process of organising some planned experiments with inert white sugar-coated, coloured sugar-coated and plain uncoated tablets to try to determine differences in attractiveness to infants. Plastic strip packs were easy for adults to undo and difficult for infants' fingers to open, but cost a little more.

MR. G. RAINE (Manchester) said that he had recently drawn attention to the unsatisfactory nature of many soluble aspirin tablets. There had been some improvement, but many, although labelled soluble, in fact were not. Manufacturers of some antibiotics would not supply the pure materials, and soluble tablets had to be used in making mixtures. Experience showed that these so-called soluble tablets, in water, gave inelegant liquids with a good deal of suspended matter. If manufacturers labelled anything as soluble, it should be soluble; or any insoluble ingredient should be in such a form that it readily dispersed and produced an elegant suspension in water.

PROFESSOR H. BRINDLE (Manchester) said that some years ago, at Manchester University, an enteric-coated capsule was devised which resisted a test similar to that mentioned by Mr. Denston. By using different gelatin mixtures and various formalin treatments a capsule was devised which resisted acid pepsin solution at body temperature for

3 hours and dissolved in alkaline pancreatin solution in 1 hour. A number of capsules were filled with barium sulphate, administered to volunteers, and the passage of the capsules was followed through the body. To their surprise only about one capsule out of 50 behaved as it should have done, i.e., resisted the action of the stomach juices and dissolved in the upper intestine—which suggested that the tests mentioned were not an accurate guide to what happened in the body.

MR. F. BERRY (Nottingham) emphasised the need for careful storage of tablets. Was liquid paraffin ever used nowadays in tablet manufacture? He thought a great need was for a water-soluble lubricant, and he agreed that coating by the new pressure technique was of value for incorporating medicaments in tablet coatings. Mr. Burlinson had mentioned the use of gelatin in a coating process. Had the tendency to mould growth in such tablets been overcome? He had found that incorporating gelatin made the product rather brittle. Commenting on the size of tablets he said that some tablets containing, for example, only 5 mg. of medicament weighed 4 grains, which seemed unnecessarily bulky. The general principle he followed was to use as little added diluent as possible, a 5 mg. tablet weighing about 1 grain. He had found the Monsanto hardness tester useful for comparative experiments.

MR. A. R. G. CHAMINGS (Horsham) said that a large proportion of British pharmaceutical products were exported, and in discussing tablet diameters the profession could not restrict its outlook to British pharmacy. The originator of the product determined in the first instance not merely the ingredients but also the physical characters of the tablet.

MR. A. W. BULL (Nottingham) said that two different processes of coating by compression were already being operated in this country. Mr. Burlinson had said that coating by compression did all that was required of it, but one process which had been described fell down when compared with the traditional pan coating. There was no opportunity to put on a sub-coat, which was an important coat in giving protection, particularly for tablets for export to tropical countries. He had tested tablets of cascara extract stored under hot, humid conditions, where temperatures were fluctuating, and found that the traditionally coated tablet was better than that coated by one particular compression process. Referring to cellulose sheeting, he pointed out that even the so-called moisture-proof film had an appreciable transmission rate for water vapour. Better foils were available for strip packaging of tablets where complete moisture protection was required. He had had occasion to investigate the tabletting of a quaternary ammonium compound where bactericidal effect was important, and, in that case, tablet lubricants which were normally regarded as innocuous had a material effect on the biological efficiency of the resulting tablets. The choice of suitable lubricants in such cases was a difficult problem. Mr. Burlinson had said that certain chemical substances could be compressed without a granulation process and had given aspirin as a typical example. He had examined many specimens of aspirin and had found that the crystal form

varied considerably. He felt that more work was required on crystal shape of many chemicals and that this might greatly assist tablet making. Commenting on Mr. Whittet's remarks on aminophylline, he said that a great deal could be done by attention to the excipients. Sugar should not be used and it was also important to observe official requirements for storage in a well-closed container protected from light.

MR. R. HENRIKSEN (Epsom) asked for statistics showing the number of infant fatalities which had been caused by non-coloured tablets. Referring to the standardisation of tablets, he emphasised that manufacturers had to consider the export market, where conditions might be very different from those in this country, but where 50 per cent. of the output might be sent. It was not feasible to make a tablet of one size and shape for this country and a tablet of a different size and shape for export. A good deal of money had been spent on tablet punches and dies, and these could not simply be thrown away.

MR. D. F. SMITH (Bournemouth) said he had been struck by the anomalous therapeutic results from enteric-coated tablets. A satisfactory test for disintegration was needed, but a test which satisfied an *in vitro* specification and which could be checked *in vivo* with volunteers might not necessarily mean that the product would give similar results with sick patients, for the secretions of the alimentary tract of a sick patient were not necessarily the same as those of a healthy volunteer.

MR. J. R. ELLIOTT (London) dealing with enteric-coated tablets, said that pancreatin was given in a number of cases where pancreatic deficiency existed in the patient. There should be another method of testing enteric-coated pancreatin tablets so as to ensure that they would disintegrate in the alimentary tract where pancreatic deficiency existed.

DR. G. BROWNLEE (London) said that anyone who considered the problems of enteric-coating would recognise that the physiological aspect had the last word. What might justifiably be asked of an enteric-coated product? Contrary to what might have been suggested, it was reasonably easy to coat a tablet so that it passed intact through the stomach, even when allowance was made for the enormous variation in emptying times of stomachs. But what of the requirement implied by the *in vitro* test to restrict the contact time with the pancreatic juices in the upper third of the small intestine to 1 hour? There were those who emptied their gut in as little as 6 hours and those who emptied it in 48 hours. That was a rough and ready measurement of the speed with which products passed through the upper third. Anybody who had studied blood levels with such preparations as penicillin, the sulphonamides or aspirin would appreciate the wide normal range which existed. The manufacturer's problem was therefore physiological. All that should be expected of him was that he should make an enteric-coated tablet. He should not be blamed if the tablet sometimes passed through.

MR. H. HOYLE (Leeds) said he thought the suggested new disintegration test was more satisfactory than the present test. A more definite dividing

line was drawn between tablets which passed and those which failed, and the test would give similar results with different operatives. Referring to coated tablets, he urged the B.P. Commission to continue to use caution in connection with the disintegration time of coated tablets. The best solution to the problem of child fatalities was education of the public, but a degree of success had been obtained with a tablet having a harmless and colourless bitter in the coating. Some of the standards needed revision, for example the requirements for aneurine hydrochloride tablets seemed to be a little more stringent than those for nicotinic acid tablets. In the case of ferrous sulphate, this drug was assayed by the permanganate method, but the tablets by the iodate method. It would be better if the same method were adopted for the raw material and for the finished product.

MR. H. E. BROOKES (Nottingham) said he was concerned about the chronic toxicity of some tablet ingredients. Mr. Burlinson had suggested that the excipients used in tablet manufacture should be pharmacologically inert. Boric acid had been used as a tablet lubricant, although its use as a preservative of foods was not permitted. He was concerned with the considerable number of new wetting, suspending and lubricating agents which were coming into use, and he thought that some of these products might later be found to show chronic toxicity. Thorough pharmacological tests should be carried out on these agents.

MR. D. STEPHENSON (Dartford) said that the suggested new test for enteric-coated tablets in the B.P.C. was better than no test at all, and would probably help to remove from the market entirely unsatisfactory tablets. Barium sulphate tablets had been treated with a coating similar to that which complied with the suggested B.P.C. test, and the results on volunteers had been excellent. After $2\frac{1}{2}$ hours the tablets could be seen in the stomach. Later they could be seen breaking up. Part of a batch of similarly coated tablets was sent to Africa and part to South America. Most of the Africans passed the majority of the tablets entire, and many of the Brazilians vomited!

MR. A. BRAGG (Liverpool) suggested that one system only should be adopted for recording the content of active principle in tablets, either the metric or the Imperial system.

MR. E. LOCKER (Reading), speaking as a retail pharmacist, said that the colouring of tablets was an assistance, and did not lead to carelessness. It had been suggested that certain types of drugs should have certain colours so that people would know immediately what type of drug had been used.

DR. G. E. FOSTER (Dartford) said that there was something to be said for the manufacturer being allowed to put a mark on the tablet so that people might know by whom it had been manufactured.

MR. D. STEPHENSON (Dartford) disagreed with Mr. Locker on the question of colouring. If they were to adopt the practice of trying to

identify tablets by colour there would soon be difficulty in distinguishing between the slight variation of successive batches of one tablet.

MR. R. GILLHAM (Leeds) said his Company placed in every box of tablets a circular reading "These tablets, being in a form likely to attract children, should be kept beyond their reach." A simple test for durability was to place the tablets inside a bottle and rotate them in a revolving drum. Another test was to transport them under severe conditions.

MR. L. H. BOARDMAN (Manchester) said he was convinced that the colouring of tablets was to be deprecated, but he would not be dogmatic. The question of fixing standards for colouring was difficult. From the point of view of dispensing in this country he was convinced that B.P. and B.P.C. tablets should be of fixed diameter.

MR. W. A. PARK (Aberdeen) said that his firm micro-photographed all prescriptions, which gave a ready means of tracing them. Some of the more potent drugs were dispensed, by his firm, in tubes with screw caps, which were more difficult for children to open, but the real problem was in the homes and it was a question of education. Many pharmacies and surgeries in Aberdeen carried cards emphasising that medicine should be kept away from children, and 20,000 copies of a booklet on Home Safety, containing a feature on child poisoning, were to be distributed.

MISS M. C. ISLIP (Harrow), speaking as a children's hospital pharmacist, agreed that publicity and education were the answer to the question of child poisoning. Colour and taste had little effect, for a small child would put anything into its mouth.

MR. T. JAMES (London) said he was perturbed about the containers in which many tablets were dispensed. Containers which could be well sealed should be used.

MR. H. WILLIAMS (Reading) complained of the tendency to supply smaller and smaller tablets, which were difficult to handle.

MR. FITCH, in reply, said that relying on a sign or a colour in identifying tablets was to be deplored: the only way to identify them was by reference to the person who dispensed them.

MR. BURLINSON, replying to Mr. Teeman on the shapes of tablets, said they should distinguish between the shapes of the official B.P. or B.P.C. tablets and those offered as proprietaries, where the shape could be anything the manufacturer wished. Mr. Whittet had raised an interesting point on the use of culinary equipment. He had come across bentonite only as a disintegrant and not as an adhesive. Mr. Caldwell had mentioned excipients which caused tablets to swell in the tropics. When tablets were to be exported, special packaging conditions were required. Dealing with hardness tests, he said that the ultimate test was how well the tablets travelled. Actual hardness did not convey very much. Replying to Mr. Berry, he said that liquid paraffin was still used, but it was important to incorporate a solubiliser to prevent an oily film appearing on the surface of the solution. He was still looking for a satisfactory

water-soluble lubricant. Reference had been made to gelatin and mould growth. Possibly, in the case in question, too much was being used. He thought gelatin was intended to give a certain elasticity, and he had not experienced cracking due to its use. His experience of coating by compression was no more than that of Mr. Bull. He did not think that a tablet which had a sugar coating pressed round it differed greatly in properties from the original tablet. There were physical forms of material more suitable for tablet making than others, and chemical manufacturers should bear that in mind when making drugs which were primarily used for tabletting. Mr. Brookes should know that boric acid would not be used internally, but only for making solution-tablets. As for the newer excipients, manufacturers should satisfy themselves that the substance was not harmful in the proportions used.

MR. DENSTON, in reply, dealing with the introduction of the metric system, said that the B.P. had simplified the position in a logical manner by deleting all reference to Imperial dosage from tablets and medicaments which had never been presented in Imperial quantities, e.g., the sulphonamides and sex hormones. Dr. Capper could rightly claim that the enteric-coating test included in the B.P.C. was the best available at present. Replying to Mr. Whittet on aminophylline tablets, he said that he thought the moist granulation process could be used satisfactorily, but careful drying was necessary. To label a package of tablets to the effect that they were dangerous for children and should be kept out of their reach might create the danger that other tablets would be regarded as not dangerous at all, and would be treated with less care than otherwise would be the case. He agreed with Mr. Whittet that the surface of a tablet should not be used as an advertising medium. He said that regarding tablet identification, the B.P. tried to assist by including a simple identification test which required nothing more than reagents and test tubes. In 1946, Mr. Hoyle had published a useful paper on tablet disintegration, and was one of the first to advocate the use of the wire screen to determine the end-point. In fixing limits for the percentage of medicaments, the figures had to take into consideration the degree of accuracy obtained by the assay method used. He agreed that where appropriate they attempted to adopt the same method for the assay of the tablet as that applied to the medicament in the tablet.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Morphine, Isolation and Purification of. L. B. Achor and E. M. K. Geiling. (*Analyt. Chem.*, 1954, **26**, 1061.) A method is given for the isolation of morphine (and particularly ^{14}C labelled morphine) from the opium poppy which is satisfactory for the recovery of quantities of the order of 1 to 10 mg. The opium is extracted with aqueous sodium carbonate solution from which, after adjustment to *p*H 8·6, the morphine is removed by shaking with a 1-butanol-benzene mixture. After purification dilute sulphuric acid is used to remove the morphine from the organic phase, the aqueous solution being treated with barium hydroxide in potassium hydroxide before being passed down a column of Nalcite SAR. Elution of morphine from the column is accomplished with 0·1N hydrochloric acid. The Nalcite effluent containing morphine is adjusted to *p*H 7·0 with 10 per cent. sodium hydroxide and passed through a column of Amberlite IRC–50 buffered at *p*H 7·0 with 0·5M dihydrogen potassium phosphate-disodium monohydrogen phosphate buffer. The final effluent is evaporated to dryness over phosphorus pentoxide; the solid material remaining is morphine suitable for crystallisation. R. E. S.

ANALYTICAL

***Digitalis purpurea*, Chromatography of Glycosides and Aglycones from.** K. B. Jensen. (*Acta pharm. tox. Kbh.*, 1954, **10**, 69.) Details are given for the paper chromatographic separation and fluorimetric determination of the cardioactive glycosides and aglycones purpurea glycoside A, digitoxin, digitoxigenin, purpurea glycoside B, gitoxin, and gitoxigenin. Separation was effected by descending, one-dimensional chromatography on formamide-impregnated filter paper with chloroform or benzene-chloroform as the mobile phase. For the purpurea glycosides A and B the chromatograms were developed for 3 to 4 days with chloroform, for gitoxin and gitoxigenin for 3 to 5 hours with chloroform, and for digitoxin and digitoxigenin for 3 to 4 hours with benzene-chloroform (6:4). Localisation of the substances on the chromatogram was obtained by means of parallel chromatograms on known substances. Quantitative determinations were made by the simultaneous chromatography of known amounts of standard substances at different levels. The substances of the A series were located with trichloroacetic acid-chloramine-R and those of the B series with the same reagent or trichloroacetic acid-R (*Acta pharm. tox. Kbh.*, 1953, **9**, 99). The cut out paper strips with test substance were eluted directly with the fluorescence producing test solution. The fluorescence curves were linear for quantities ranging from 2 to 15 μg. of digitoxin and 1 to 10 μg. of gitoxin, or equimolecular amounts of the corresponding glycosides and aglycones, per 15 ml. of test solution. For the estimation of both glycosides the standard deviations varied from about 6 per cent. at the lowest concentrations to about 2 per cent. at the highest concentrations employed. R. E. S.

Diphenan and Urethane, Assay of. P. Ekeblad. (*Svensk. farm. Tidskr.*, 1954, **23**, 557.) Heating a carbamic ester with perchloric acid in acetic acid solution gives the corresponding ester of acetic acid, and ammonia. The method

may be applied to the assay of diphenan and urethane, and is simpler than the usual one of heating with sulphuric acid. For diphenan, one millimole (0·2273 g.) is dissolved in 10 ml. of anhydrous acetic acid neutralised with 0·1N perchloric acid in acetic acid, using Blue BZL as indicator, treated with a further 25·0 ml. of the standard acid, and heated on the water bath for 30 minutes. A silica gel tube is used to keep steam out of the mixture. After cooling and the addition of indicator, the solution is titrated with a standard 0·1N solution of trimethylamine in acetic acid. One ml. of acid corresponds to 0·02273 g. of diphenan. For urethane 60 minutes' heating with 3 equivalents of the acid is required. The addition of acetic anhydride to remove traces of water is not permissible before heating, but may be employed afterwards. G. M.

Penicillin, Determination of, by Hydroxylamine. P. Mørch. (*Dansk. Tidsskr. Farm.*, 1954, **28**, 157.) A detailed study was made of the Boxer and Ewerett (*Analyt. chem.*, 1949, **21**, 670) method of determination of penicillin, in which the penicillin is converted to a hydroxamic acid which is determined colorimetrically after the addition of ferric iron. The colour is due to a mono-complex having an absorption maximum at 490 mμ. No higher complex is formed with penicillin. Fading may result from reduction of the ferric salt by excess of hydroxylamine. In applying the method of Boxer and Ewerett to penicillin cultures, a preliminary extraction is necessary. About 10 ml. of the filtered liquid (10,000 to 30,000 units) is mixed with 20 ml. of water and 40 ml. of amyl acetate. After cooling to 0° to 5° C., 20 ml. of ice-cold glycine buffer (*p*H 1·1) is added and the mixture is shaken for 1 minute. The aqueous layer is removed, the amyl acetate solution is dried with sodium sulphate and filtered: 30 ml. of the filtrate is shaken with 5 ml. of 2 per cent. sodium bicarbonate solution and the aqueous phase is used for the colorimetric determination. The determination is repeated with a corresponding amount of culture to which has been added 15,000 to 25,000 units of sodium penicillin. In the case of penicillin ointments, the material is dissolved in chloroform and shaken out into 2 per cent. sodium bicarbonate solution. Another portion of the ointment is treated similarly, but shaken with bicarbonate solution containing a known amount of penicillin standard. Combination preparations may be treated as follows. Total penicillin is determined after hydrolysis of the penicillin esters by allowing to stand for 2 hours in 2 per cent. bicarbonate solution. Sodium penicillin + procaine penicillin are determined after removal of penicillin esters by shaking with amyl acetate at *p*H 2, the esters remaining in the aqueous phase. Finally, procaine penicillin is determined from the absorption of the procaine at 290 mμ. G. M.

Pyrethrum Extracts, Determination of Pyrethrins in. J. A. Cornelius. (*Analyst*, 1954, **79**, 458.) A quantitative chromatographic method is given for the separation of pyrethrum extracts in *n*-hexane. Alumina is used as the adsorbent and it is modified by exposure to a humid atmosphere or by drying until standardisation with Sudan yellow/Sudan red solution shows it to be of the required activity. The pyrethrins are eluted from the alumina with *n*-hexane containing 10 per cent. and 20 per cent. diethyl ether. The eluates are evaporated to dryness, dissolved in ethanol and the ultra-violet absorption determined at 224 mμ for the "pyrethrin-I" fraction and at 229 mμ for the "pyrethrin-II" fraction; empirical conversion factors are used based on parallel analyses of a pyrethrum extract by the current A.O.A.C. mercury reduction method and the present chromatographic method. For pyrethrum extracts in mineral oil, the solvent is first removed by distillation at a low pressure (less than 0·001 mm. of

mercury) not exceeding 40° C., the residue being dissolved in *n*-hexane. A table is given showing a comparison of results obtained by chromatography with those obtained by the A.O.A.C. mercury reduction method. R. E. S.

Vitamin B_1, Fluorimetric Determination of, by the Thiochrome Method. G. Pruner. (*Rendiconti Ist. Sup. Sanit.*, 1954, **17**, 129.) The method of Jansen is modified by reducing the amount of potassium ferricyanide and carrying out the oxidation of the vitamin B_1 to thiochrome at a higher temperature. To 1 ml. of solution containing 1 to 10 μg. of vitamin B_1 1 ml. of 0·01N hydrochloric acid is added, followed by 1 ml. of 3 per cent. potassium ferricyanide and 2 ml. of buffer solution [prepared by mixing: (1) anhydrous monopotassium phosphate 0·283 g., disodium phosphate dodecahydrate 1·665 g., distilled water to 100 ml., and (2) anhydrous sodium carbonate 0·857 g., water to 100 ml.]. The tube is covered with a Kjeldahl bulb and placed in a boiling water bath for 5 minutes; a standard solution containing a known amount of vitamin is treated in the same way. The tubes are removed and cooled for 10 minutes, and 8 ml. of *iso*butanol is added to each. The tubes are shaken for 2 minutes and the *iso*butanol removed by means of a pipette and dried over sodium sulphate. The fluorescence is then compared in a suitable instrument. The fluorescence is proportional to the vitamin content. For quantities of vitamin B_1 of 1 μg. or less, 1 mg. of potassium ferricyanide is used; 3 mg. of ferricyanide oxidises 5 μg. of vitamin B_1 even in the presence of 200 μg. of ascorbic acid, but larger quantities of ascorbic acid cause complete decolorisation at the heating stage and a reduction in the fluorescence intensity. E. H.

ESSENTIAL OILS

Essential Oils, Chromatography of. R. H. Reitsema. (*Analyt. Chem.*, 1954 **26**, 960.) Chromatoplates prepared by coating glass plates with silicic acid using starch as a binder were used for the rapid analysis of the constituents of essential oils. Following the application of 1 to 2 μg. of material, development was accomplished in a covered jar using 10 to 15 per cent. ethyl acetate in hexane as developing agent. The plates were first inspected under ultra-violet light and were then sprayed with an acidic solution of 2 : 4-dinitrophenylhydrazine and inspected under visible and ultra-violet light to detect ketones. After heating, the plate was again inspected under visible and ultra-violet light to detect heat- and acid-sensitive materials. Results are given of a chromatographic comparison of spearmint-type oils, and various compounds including common constituents of essential oils were run on chromato-plates. The R_F values of carvone varied widely but the relative positions of any two materials on a path were nearly constant, and a fairly consistent ratio of R_F values could be calculated. R. E. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Digitalis, Relation between Digitoxigenin and Gitoxigenin Glycosides in. F. H. L. van Os, C. H. Galenkamp and A. R. Kliphuis. (*Pharm. Weekbl.*, 1954, **89**, 429.) The method used for the assay of the relative proportions of the two groups of glycosides was that of Tattje (*J. Pharm. Pharmacol.*, 1954, **6**, 476.) In commercial Dutch samples of digitalis, 40 to 50 per cent. of the glycosides were of the gitoxigenin group. This explains the low yields of digitoxin obtained commercially, especially in view of the fact that of the "digitoxin" a large proportion is present as purpurea glycoside A. In French samples the glycosides contained about 20 per cent. of the B series. The difference in these results

appears to be due to hereditary factors, since wild plants from Belgium had the same composition as the Dutch ones but other plants, known to have been descended from specimens from the Botanical Gardens at Cambridge, resembled the French specimens. With regard to the method of assay, it is known that gitoxin is practically insoluble in both water and chloroform. The authors' experiments show, however, that even with high gitoxin content, the extraction with chloroform is practically complete. It is known that the solubility of gitoxin in water and in chloroform is influenced by the presence of digitoxin. Objections to the shaking out of gitoxin into chloroform must therefore be regarded as merely theoretical. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Cholinesterase, Selective Inhibitors of. M. P. Fulton and G. A. Mogey. (*Brit. J. Pharmacol.*, 1954, **9**, 138.) A series of bis-quaternary ammonium salts is described which have in common a bis-[*p*-(trialkyl ammonium)-phenyl] structure joined by a four carbon-atom chain. Alterations of the molecule were confined to the second carbon atom of the chain and the quaternary nitrogens. The series had a highly selective reversible action on true cholinesterase, activity being measured by the Warburg method using acetylcholine as substrate for true cholinesterase activity and benzoylcholine for pseudocholinesterase activity. The compounds increased the twitch height of the isolated rat diaphragm indirectly stimulated and had an anticurare action, and in high doses a neuromuscular blocking action, on the same preparation. Adrenaline increased this blocking action. One of the compounds contracted the isolated frog rectus; the others had no effect other than as anticholinesterases. The effect on the blood pressure of the cat varied from compound to compound. Death with toxic doses in mice appeared to be due to respiratory failure. *d*-Tubocurarine, dibenamine, hexamethonium, nicotine tartrate or a combination of nicotine and atropine had no effect on the toxicity. G. P.

Cholinesterases of the Central Nervous System, Inhibition of. L. Austin and D. R. Davies. (*Brit. J. Pharmacol.*, 1954, **9**, 145.) To establish whether cholinesterase inhibition was responsible for chronic paralysis in chickens caused by some phosphorus-containing anticholinesterases, dyflos, sarin, tabun, soman and ethyl-sarin were administered, in doses within the lethal range, to chickens treated with atropine. Only dyflos produced any signs of chronic paralysis, although all five were powerful anticholinesterases. Determination, in the birds treated with dyflos and sarin, of both true- and pseudo-cholinesterase levels in the blood and central nervous system showed that with both drugs these levels fell soon after administration, but most recovered their original ranges by the time chronic paralysis set in. The exception was the pseudocholinesterases of the spinal cord after dyflos. Their level only returned to 50 to 75 per cent of the original and remained there. To repeat the state of cholinesterase inhibition found with dyflos, sarin was given in small repeated doses. Under these conditions no paralysis was obtained with sarin. Plasma pseudocholinesterase levels were markedly lowered by each of the inhibitors, but recovery was rapid and in all cases rose higher than normal. With dyflos this rise was particularly marked. Paralysis in chickens with dyflos therefore did not appear to be due to inactivation of the cholinesterases of the central nervous system or of the blood. G. P.

BIOCHEMICAL ANALYSIS

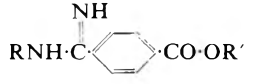
Adrenaline and Noradrenaline on Paper Chromatograms, Detection of. M. E. Pitkänen. (*Scand. J. clin. Lab. Invest.*, 1954, **6**, 78.) The sensitivity of the usual paper chromatographic detection of adrenaline and noradrenaline using either potassium ferricyanide or iodine solution as an oxidative agent can be considerably increased by subsequently spraying the paper with a solution of *p*-dimethylaminobenzaldehyde. This reduces the limit of sensitivity from 1 to 2 μg. of adrenaline or noradrenaline to 0·2 μg. of either. M. M.

Bilirubin, A New Tablet Test for. J. A. Tallack and S. Sherlock. (*Brit. med. J.*, 1954, **2**, 212.) A simple method is described for the detection of bilirubin in urine. Five drops of the urine is placed on a test mat composed of a mixture of asbestos and cellulose fibres. A tablet, containing a stable diazo dye (*p*-nitrobenzene diazonium *p*-toluene sulphonate), sulphosalicylic acid, sodium bicarbonate and boric acid, is placed on the centre of the mat. Two drops of water are allowed to flow on the tablet and the colour developing on the mat is recorded within 30 seconds. If bilirubin is present the mat around the tablet turns purple, and the amount of bilirubin is roughly proportional to the speed of development and intensity of the colour. Any colour developing after 30 seconds is ignored. The method is specific, as sensitive as the Fouchet test, and superior to the iodine test. No false positives were obtained with 100 urines from normal, non-jaundiced patients, receiving a variety of drug treatments. The presence of urobilinogen also gave no false positives. Between 0·1 and 0·15 mg. of bilirubin per 100 ml. can be detected. G. F. S.

Chloramphenicol Esters, Assay of. C. Trolle-Lassen. (*Arch. Pharm. Chemi.*, 1954, **61**, 435.) For the assay of chloramphenicol esters it is first necessary to hydrolyse them, and as chemical hydrolysis leads to destruction of the chloramphenicol, the author uses a commercial bacterial lipase (Lipase A. Rohm and Haas Co.). A study of the effect of different conditions led to the following method. A suitable quantity of the ester is dissolved in ethanol and diluted with buffer solution (*p*H 6) to give a suspension containing about 0·1 mg. of ester per ml. To this is added 1 mg. of the lipase per ml., and after vigorous shaking, the mixture is kept at 37° C. for 4 hours. The chloramphenicol is then assayed in this solution microbiologically in the usual way. G. M.

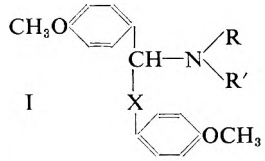
CHEMOTHERAPY

Amidines, Anaesthetic Activity of. O. Gisvold. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 372.) The duration of anaesthesia caused by application of a 1 per cent. solution of several *p*-carboxybenzamidines to the eyes and skin of guinea-pigs was determined.



The compounds tested included R = H, methyl, ethyl, propyl and butyl, R′ = 2-chloroethyl, benzyl, *cyclo*hexyl and *cyclo*pentyl. Some of the compounds showed a high anæsthetic activity, but in some cases irritation of the skin was reported. Most of the substances were bactericidal at a concentration of 0·1 per cent. against *Staphylococcus aureus* and *Salmonella typhosa*. G. B.

Antispasmodics, New Synthetic. J. Cymerman-Craig, K. V. Martin, P. C. Wailes, R. H. Thorp, R. Ladd and G. Thorburn. (*Nature, Lond.*, 1954, **174**, 231.) A series of *N*-alkyl-1 : 2-di-(*p*-methoxyphenyl)-ethylamines (I; X = CH_2), N-alkyl-α-aminodeoxyanisoins (I; X = CO), and *N*-alkyl-2-amino-1 : 2-di-(*p*-methoxyphenyl)-ethanols (I; X = CHOH) were prepared and, in view of the promising activity of certain 1-phenyl*iso*quinoline compounds, a



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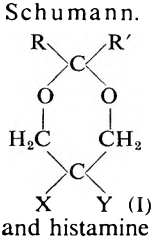
number of *N*-alkyl-di-(*p*-methoxy-phenyl)-methylamines (I; X = —) were also synthesised. All compounds exhibited spasmolytic activity when tested on isolated guinea-pig ileum against spasms produced by barium chloride and carbachol, using papaverine and atropine as standards. Some of the compounds were more active than papaverine. Relative neurotropic activity was between 0·005 and 0·2 of atropine, and acute toxicities (intravenous in mice) were uniformly between 60 and 80 mg./kg. for the compounds of the series. A. H. B.

Benzotriazines, Compounds having Antimalarial Activity. F. J. Wolf, K. Pfister, 3rd, R. M. Wilson, Jr. and C. A. Robinson. (*J. Amer. chem. Soc.*, 1954, **76**, 3551.) In an attempt to obtain compounds having the desirable therapeutic properties of sulphaquinoxaline, without the undesirable effect of forming a highly insoluble 3-hydroxy compound which may cause the formation of kidney stones when high levels of the drug are administered, the preparation of a nitrogen isostere, 3-sulphanilamido-1 : 2 : 4-benzotriazine was undertaken. The substitution of nitrogen for carbon eliminates the possibility of hydroxylation. The method of synthesis is described. A series of chloro substituted compounds was prepared because of the antimalarial properties exhibited in the intermediate bases. 7-Chloro-3-amino-1 : 2 : 4-benzotriazine-1-oxide and 7-chloro-3-amino-1 : 2 : 4-benzotriazine had excellent activity as suppressive agents in avian malaria. On a weight basis, these compounds are about 4 times as potent as quinine and 5 times as potent as sulphadiazine, or about equivalent to sulphaquinoxaline. Effectiveness was limited to the series having a halogen in the 7-position and maximum activity was obtained with an amino group in position 3. Activity could not be detected when the halogen was in other positions, or when other groups were substituted in the 7-position. Replacement of the amino group by hydroxyl, or acylation of the amino group, gives a great reduction in the activity. A. H. B.

1 : 3-Dioxanes, Basic, as Antispasmodics. F. F. Blicke and E. L. Schumann. (*J. Amer. chem. Soc.*, 1954, **76**, 3153.) A series of substituted 1 : 3-dioxanes of type I: where R = H and C_6H_5–; R′ = $-CH_2N(CH_3)_2$, $-CH_2N(CH_3)_3I$, and C_6H_5; X = CH_3, C_2H_5, C_6H_5, H, and CH_2OH; Y = $CH_2N(CH_3)_2$, $-CH_2N(CH_3)_3I$, CH_2OH, C_6H_5, NH_2, $N(CH_3)_2$, $-CH_2N(C_2H_5)_2$, $-CH_2CH_2NH_2$ were prepared. The pharmacological activity is recorded against acetylcholine and barium chloride induced spasm of isolated rabbit jejunum and histamine induced spasm of isolated guinea-pig intestine. A. H. B.



(I)

3-Indolecarboxaldehyde Thiosemicarbazone, a New Antitubercular Compound. L. E. Weller, H. M. Sell and R. Y. Gottshall. (*J. Amer. chem. Soc.*, 1954, **76**, 1959.) The synthesis of 3-indolecarboxaldehyde thiosemicarbazone is described. It has been shown to have high bacteriostatic activity *in vitro* and to suppress tuberculosis in mice after injection of virulent tubercle bacilli. A. H. B.

Local Anæsthetics Derived from *p*-Cymene. B. Samdahl, G. Gjerstad and E. Rydström. (*Ann. pharm. franç.*, 1954, **12**, 125.) Diethylaminoacetylcarvacrylamine, the *p*-cymene derivative analogous to lidocaine (lignocaine) was prepared by reaction of carvacrylamine with chloracetyl chloride and treatment of the product with diethylamine. The substance was anæsthetic to the tongue and rabbit cornea, but the hydrochloride, sulphate and nitrate were too acid for injection. Certain organic acid salts are being investigated, and the possibility of making a long-acting anæsthetic solution of the base in a mixture of propylene and polyethylene glycols and water is being examined. Carvacryloxyethanol and chlorcarvacryloxyethanol were obtained by reaction of glycol monohydrin with the corresponding phenolate. The substances, analogous to phenoxyethanol, were not sufficiently soluble in water to show antibacterial properties. A feeble local anæsthetic activity was observed in phenoxyethanol, carvacryloxyethanol and chlorcarvacryloxyethanol. The *p*-aminobenzoic esters were prepared with the object of intensifying this property, and the products may be useful as local anæsthetics for use in propylene/polyethylene glycol solution. G. B.

PHARMACY

NOTES AND FORMULÆ

Cetrimide and Acriflavine in Ointments, Bactericidal Action of. R. Frank and G. Stark. (*Pharm. Acta Helvet.*, 1954, **29**, 81.) The action of cetrimide and acriflavine was tested in a number of ointment bases. In water in oil emulsions the action of both of these compounds was poor. Oil in water emulsions (e.g., emulsifying ointment B.P.) gave much better results. The diffusion of acriflavine from pure soft paraffin was good, at least in fairly high concentrations. The best results were obtained with a glycerine ointment and with sodium alginate jelly. It was observed that there was no incompatibility between cetrimide and sodium alginate, in spite of opposite electrical charges. There is a marked incompatibility between acriflavine and cetrimide on the one hand and hydrous emulsifying ointment B.P. on the other hand. The two antiseptics in question have no synergistic action together. Optimum concentration of the antiseptics is between 1 and 1.5 per cent. G. M.

Steroids, Preparation of, in Microcrystalline Form by Freeze-Sublimation. A. P. Lemberger, T. Higuchi, L. W. Busse, J. V. Swintosky and D. E. Wurster. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 338.) A solution of the steroid in chloroform or carbon tetrachloride was allowed to fall from a funnel into liquid air or liquid nitrogen, so that droplets were formed before they came into contact with the frozen liquid. The frozen solution was transferred to a chilled sample tube, which was then connected to a condensing vessel and vacuum pump. The sample tube and condenser were surrounded with liquid air and the apparatus evacuated. The cooling material around the sample tube was replaced by acetone/solid carbon dioxide mixture and sublimation allowed to proceed, about 24 hours being required to dry 50 ml. of the frozen solution.

The particle size of the resulting powder was determined by making measurements of the specific surface area, using the low temperature nitrogen absorption method. The process appeared to be well suited to the production of submicroscopical particles, a diameter as low as 0·25 μ being achieved under optimum conditions. Differences were observed with the various steroids and solvents employed, but generally an optimum concentration of solution could be found which would yield the smallest particles. Larger particles were produced when the temperature of the subliming sample approached the melting point of the solvent. The addition of ethanol, methanol or tween mixture increased the particle size. When caffeine or polyvinylpyrrolidone were introduced as protective agents, the powders were readily wetted and the steroid particles tended to aggregate. There was however no tendency towards aggregation with powders prepared with tween mixture and polyvinylpyrrolidone, and dispersions of almost colloidal characteristics could be obtained. G. B.

Water, Demineralisation of, by a Single Column. J. Büchi and M. Soliva. (*Pharm. Acta Helvet.*, 1954, **29**, 221.) A column of 60 cm. height and 5 cm. diameter was layered first with 250 ml. of cation exchange resin (Amberlite IR 120) upon which rested the widened end (2 cm. diameter) of a 50 cm. length of glass tubing of 1 cm. diameter. 500 ml. of anion exchange resin (Amberlite IRA 410) was then added around the tubing giving a column of resin 40 cm. in height. 300 ml. of 15 per cent. sodium hydroxide solution was run through the column which was afterwards rinsed with tap water to nearly neutral reaction. The cation exchange resin was then treated with 300 ml. of 10 per cent. hydrochloric acid run in through the glass tubing. After rinsing, the two beds of resin were mixed by bubbling air through the column. Water was run at the rate of about 12 to 15 l. per hour until the column was exhausted, as shown by the presence of chloride in the issuing fluid. For bacteriological reasons it is best to run the apparatus so that all the water required is produced in one operation. The quality of the product corresponds in every way to Swiss Pharmacopœial requirements and the authors consider that demineralised water should be permitted for all preparations except eye-drops and solutions for injection. G. M.

PHARMACOGNOSY

Alkaloid Production in *Datura*, Influence of Shoot and Root on. E. Steinegger. (*Pharm. Acta Helvet.*, 1954, **29**, 141.) The influence of root and shoot on alkaloidal production was followed by experiments in grafting the diploid *Datura tatula* with the tetraploid *Datura tatula* var. *inermis*. It was found that the 4n root produces a somewhat lower proportion of alkaloids as compared with the 2n root. On the other hand, a 4n shoot graft gives a considerably higher yield than a 2n graft. The highest alkaloidal production was obtained with a 4n shoot and 2n root, but calculated on a basis of alkaloid per sq. m. of soil area the highest yield is given by the wholly 4n plant. The increase in the size of the plant in the latter case is however undesirable in practice. The composition of the alkaloids was not influenced by the chromosome number. The results appear to indicate a balance between two opposing factors: a slightly increased production with the 2n root, and a decisive increase in the 4n shoot. Although it has been observed that the alkaloids are produced in the root of *Datura*, it is now shown that the shoot can influence the production, e.g., by influencing the root growth. G. M.

***Cinchona succirubra*, Biosynthesis of Alkaloids in.** P. de Moerloose. (*Pharm. Weekbl.*, 1954, **89**, 541.) Plants of *Cinchona succirubra* were grown under laboratory conditions in an atmosphere containing radio-active carbon dioxide, which after a varying time was replaced by ordinary carbon dioxide. The results show that the view that the alkaloids are formed in the leaf and transported to other parts of the plant is incorrect. Study of the time factor showed that synthesis occurs both in the leaf and in the bark. The leaf produces mostly quinine and quinidine, while cinchonine and cinchonidine are formed mainly in the bark. It does not appear probable that one of the alkaloids is a precursor of the others. Apparently all of the alkaloids are derived from a common precursor, the syntheses being independent of one another. Biosynthesis in the leaves is inhibited by illumination, but not in the bark. G. M.

***Digitalis purpurea* L. and *Digitalis lutea* L., Carbohydrate and Chlorophyll Content of Leaves of, after Freeze-drying and Oven-drying.** F. P. Cosgrove and E. P. Guth. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 268.) A comparison was made between leaves dried at 50° C. and freeze-dried. An ethanolic extract was made, diluted with water and the ethanol removed on a water bath. Phenols, tannins, etc., were removed with lead acetate, the excess of which was precipitated with sodium carbonate. After hydrolysis with hydrochloric acid, followed by neutralisation, the product was analysed for sugars (as glucose) and for chlorophylls A and B by the A.O.A.C. method. No significant difference was found in sugar content between the freeze-dried and oven-dried leaves of *D. purpurea*, whereas oven-dried *D. lutea* contained appreciably more sugars than freeze-dried. No correlation was established between sugar content and total glycosides, as estimated by a colorimetric method; therefore it is unlikely that there is a greater yield of total glycosides from oven-dried leaves. A more likely explanation is that the heat renders the glycosides more soluble in ethanol. Freeze-drying appeared to preserve more chlorophyll A, but there was little difference in the yield of chlorophyll B after freeze- or oven-drying. G. B.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and Noradrenaline in Urine after Adrenalectomy. U. S. von Euler, C. Franksson and J. Hellström. (*Acta physiol. scand.*, 1954, **31**, 1.) The adrenaline and noradrenaline content of the urine of patients, suffering either from hypertension or from cancer of the prostate or mammary gland, was estimated both before and after either unilateral or bilateral adrenalectomy. Unilateral adrenalectomy did not significantly alter the output of adrenaline or noradrenaline but after bilateral removal the adrenaline output fell considerably while the noradrenaline output was either maintained or increased. These results indicate that most of the adrenaline excreted in the urine is derived from the adrenals while the noradrenaline comes from other sources, probably the adrenergic nerves. M. M.

***N*-Allylnormorphine, Effect of, on the Antidiuretic Action of Morphine.** C. Winter, C. Gaffney and L. Flataker. (*J. Pharmacol.*, 1954, **111**, 360.) *N*-Allylnormorphine (nalorphine) effectively blocks the antidiuretic effect of morphine in rats. The drug itself has neither a diuretic nor an antidiuretic action nor does it antagonise the antidiuretic effect of vasopressin. It is suggested that morphine stimulates the release of antidiuretic hormone from the

posterior lobe of the pituitary gland and that nalorphine blocks this action, the site of action being the hypothalamus. M. M.

Autonomic Ganglia, Relation of Chemical Structure to Action on. R. Wien. (*Arch. int. Pharmacodyn.*, 1954, **97**, 395.) Spatial configuration of the molecule appears to play a greater part in determining the ganglionic blocking activity of the bisquaternary ammonium series than does the interquaternary chain length. In the methonium series the maximum potency (blockade of the superior cervical ganglion) for chain lengths of 5 and 6 carbon atoms was obtained where the "onium" radical was dimethylethyl and for a chain length of 4, where the radical was diethylmethyl. In hexamethonium, replacement of the nitrogen by sulphur, or a primary amino group for one of the quaternary nitrogens, or an amino group for one of the methyl groups on each nitrogen, or tertiary nitrogens for the quaternary nitrogens all reduced the activity on the superior cervical ganglion. Phenylethane *p*-ω-bis(trimethyl ammonium) iodide was 3 times more active than hexamethonium iodide. The fact that the interquaternary distance was intermediate between penta- and hexa-methonium might be significant. Also in this compound saturation of the phenyl radical gave two geometric isomers, one of which was as potent as the unsaturated compound while the other had only one-tenth the activity. With the two isomers the interquaternary distance differed only slightly. Other polymethylene series, where the "onium" radicals were the nitrogens of quaternary heterocyclic nuclei, confirmed these results. The peak activity in a polymethylene bis(1-methylpyrrolidinium) series was very sharp with the pentane member, while in similar morpholinium and piperidinium series the peaks were comparatively flat. To extend these results to parasympathetic ganglia in the cat several procedures were used: (i) mydriasis; in the presence of light the sympathetically denervated eye is reduced to a slit. The compounds when injected dilated the pupil quantitatively; (ii) salivary flow on stimulation of the chorda-lingual nerves; (iii) vagal ganglionic-blocking activity—the fall in blood pressure being the response measured; (iv) the bladder-pelvic nerve preparation. Further results were obtained with the peristaltic reflex of the isolated guinea-pig ileum and in the mouse with the standard mydriasis test for atropine-like agents. There was good agreement of activity between the superior cervical ganglion and the mydriasis and salivary flow tests in cat for hexamethonium, penta- and hexamethylene pyrrolidinium, and phenylethane (bis-trialkyl) ammonium derivatives. For various reasons discrepant results were obtained on the guinea-pig ileum, cat blood pressure, cat bladder-pelvic nerve preparation and mouse mydriasis test. G. P.

Cortisone Acetate *v.* Cortisol in Rheumatoid Disease. H. F. West and G. R. Newns. (*Lancet*, 1954, **267**, 168.) Cortisol (hydrocortisone "free alcohol"), the natural adrenocortical hormone, has been administered to 22 patients who had received cortisone acetate daily for from 1 to 3 years and had not improved. The patients were observed over three months. The physical ability of 6 patients was considerably improved, 15 showed no change and one was worse. The erythrocyte sedimentation rate fell on an average from 27 to 21·5 mm. Increased effectiveness was however paralleled by an increase in side effects—increased deposition of fat, increased leucocytosis and a very definite rise in blood pressure. Cortisol is a more potent antirheumatic than cortisone acetate but because of the increased side effects the authors do not advocate its use in place of cortisone acetate. G. F. S.

Cortisone, a Chloro-derivative of, with Enhanced Activity. R. K. Callow, J. Lloyd, and D. A. Long. (*Lancet*, 1954, **267**, 20.) The biological activity of a new chloro derivative of cortisone (9-α-chloro-17-α-hydroxy-corticosterone) has been studied in mice, rats and guinea-pigs. A dose of 50 mg./kg. injected intramuscularly daily for ten days killed 7 out of 10 mice, compared with 3 out of 10 with cortisone. There was a greater fall in body weight and an increase in kidney size. In nestling rats 9α-chlorohydrocortisone (2·5 and 1·25 mg./kg.), and cortisone at the same doses, inhibited growth to a comparable degree; but 9α-chlorohydrocortisone was 3·4 times as active as cortisone in causing thymus involution, and 4·76 times as active in producing liver hypertrophy. In the tuberculin sensitivity test in guinea-pigs, 2 mg./kg. of 9α-chlorohydrocortisone had approximately the same desensitising activity as 10 mg./kg. of cortisone. In all tests therefore 9α-chlorohydrocortisone was more active than cortisone, and it is suggested that a high therapeutic activity in man may be expected.

G. F. S.

Curarising Agents, Central Action of Some. R. Hazard, J. Cheymol, P. Chabrier, Y. Gay and P. Muller. (*Thérapie*, 1954, **9**, 314.) Considerable sedative action, in addition to curariform action, was found in some members of a series of bisquaternary ammonium compounds with a piperazine nucleus in the interquaternary chain. Activity was measured directly, and by the prolongation of the sedative action of hexobarbitone, in mice. There was no correlation with curarising and central depressant activity. The quaternary nitrogens could be replaced by corresponding tertiary groups, but with loss of central depressant potency. On the "onium" radical, ethyl was more effective than either methyl or benzyl substitution. The piperazine nucleus in the interquaternary chain appeared essential for sedative action since a similar series where this nucleus was replaced by an ethylene oxide ($-O-CH_2-CH_2-O-$) grouping was without activity.

G. P.

β-Diethylaminoethyldiphenylpropylacetate Hydrochloride, Pharmacological Effect of. L. Cook, J. T. Toner and E. J. Fellows. (*J. Pharmacol.*, 1954, **3**, 131.) This paper describes the effects of the compound on the duration of hypnosis (loss of righting reflex) induced by hexobarbitone sodium in mice and rats. Premedication with 25 mg./kg. i.p. enhanced a subhypnotic dose of hexobarbitone (50 mg./kg. i.p.) in rats, which slept for an average time of 35·2 minutes. In mice oral or i.p. doses of 50 mg./kg. administered simultaneously with 100 mg./kg. of hexobarbitone i.p., significantly prolonged the duration of hypnosis, indicating quick absorption and onset of action. The optimal premedication time was 40 to 60 minutes, but the duration of action was long, the compound being still effective after 15 to 20 hours. There was a linear relationship between the log. sleeping time and the log. dose. The compound had a wide margin of safety, 1/500th of the oral LD50 (= 538 mg./kg.) in mice enhancing the duration of hexobarbital hypnosis. Normally the duration of hypnosis with hexobarbitone is limited because of toxic effects. Premedication with the compound prolonged hypnosis with very little effect on the toxicity of hexobarbitone. Neither kidneys nor adrenals were essential for the compound to produce its effects. Mice did not become tolerant but some rats did. When it was administered to rats immediately after recovery from hexobarbitone hypnosis, further hypnosis did not occur. Diphenylpropylacetic acid, which has been supposed to be a metabolite of the compound, showed only a weak activity. The paper also describes other pharmacological actions of this compound.

G. F. S.

Diethyl-*p*-nitrophenyl Phosphate (Paraoxon), Acetylcholine Production in Animals Poisoned by. J. M. Barnes and J. I. Duff. (*Brit. J. Pharmacol.*, 1954, **9**, 153.) Anæsthetised fully atropinised rabbits, cats and dogs recovered from a lethal dose of paraoxon (E600) if artificial respiration were administered. The animals were then insensitive to further injections of the same dose of the anticholinesterase. Concomitant determinations of the venous blood level of acetylcholine showed that in the cat and the rabbit this rose with each dose of paraoxon, but in the dog the rise was not as great and doses subsequent to the first had no further effect. Evisceration of the cat resulted in a slower acetylcholine blood level rise after the anticholinesterase. Acetylcholine production by the isolated rat diaphragm stimulated at a rate of 50 per second through the phrenic nerve for twenty minutes, was assayed on the cat blood pressure and found to be constant over a long period, in presence of either eserine or paraoxon. An explanation is advanced for this tachyphylaxis to paraoxon. G. P.

Isoniazid and *p*-Aminosalicylic Acid, Study of a Combination of. R. Kourilsky, S. Kourilsky and S. Micoulaud. (*Thérapie*, 1954, **9**, 273.) The compound formed by the interaction between *p*-aminosalicylic acid and isoniazid was well tolerated by guinea-pigs when given in doses 8 times those used in isoniazid therapy. Tolerance in man was good at an oral dose level of 10 mg./kg. Increasing the dose to 15 mg./kg. also increased the incidence of side effects, mainly of an intestinal nature. In experimental tuberculosis in the guinea-pig and in pulmonary tuberculosis in man the activity of the compound was comparable with those of isoniazid, *p*-aminosalicylic acid and dihydrostreptomycin. Experimetally its activity was similar to that of isonizid, but was more rapid and more regular with the same dose. *In vitro* studies on H37Rv sensitive, H37Rv resistant and "D" strains of *Myco. tuberculosis* showed the drug to be more active and more constant in activity over period of time than was isoniazid. Also, isoniazid-resistant strain "D" organisms were not resistant to the compound. G. P.

Isoniazid and Weight Gain. I. S. Mudie, N. W. Horne and J. W. Crofton. (*Brit. med. J.*, 1954, **1**, 1304.) In groups of patients with pulmonary tuberculosis treated with isoniazid a remarkable gain in weight has been one of the principal effects reported. 8 healthy males, aged between 25 and 40, none of whom had had tuberculosis, were divided into a treatment group and a control group. The control group received capsules containing lactose and the treatment group capsules containing 100 mg. isoniazid twice daily. The total period of trial was 14 weeks, including an initial observation period of 2 weeks before administration of any drugs and a final 2 weeks period after stopping administration. The control group received lactose for the whole 10 weeks. During the 10 weeks of administration the treatment group received isoniazid for 8 weeks and lactose for 2 weeks. The average weekly weight gain in the 4 subjects receiving isoniazid for 8 weeks was similar to the gain in the same group treated for 2 weeks with capsules containing lactose and to the gain during 4 weeks on observation alone. The average weekly gain was less than that of the group treated for 10 weeks with lactose alone. The authors conclude that there is no evidence from this trial to suggest that isoniazid in tuberculosis patients has any effect on weight gain other than that due to its effect on the disease. S. L. W.

Mephenesin and Gallamine Triethiodide in Tetanus. C. M. Parkes. (*Brit. med. J.*, 1954, **2**, 445.) The case under review deals with the treatment of tetanus in a young man of 22. In spite of the administration of 100,000 units of tetanus antitoxin intravenously, soluble penicillin 500,000 units 4-hourly, and 14 ml. of paraldehyde in 100 ml. of saline rectally, painful muscle spasms began, increasing in frequency and severity. An intravenous infusion of normal saline was set up and run in at the rate of 1 pint in 8 hours (5000 units of heparin to each pint of saline); this was kept going for 9 days. To control the spasms a 10 per cent. solution of mephenesin was injected into the drip tubing in doses of 5 to 10 ml.; this was followed by immediate relief of the spasm. To reduce the general muscular rigidity between spasms the patient was also given mephenesin elixir by mouth, 1 fl. oz. (1 g.) 5 times daily, and 30 ml. of the 10 per cent. solution was added to each bottle of normal saline. The dosage of mephenesin needed rose daily and on the 5th day hæmoglobinuria occurred and the mephenesin was replaced by gallamine triethiodide, given in a maximum single intravenous dose of 0·5 ml. (10 mg.), when immediate loss of spasm and pain followed. With the diminution in the frequency and intensity of spasms and the reduction in the amount of gallamine required to control them the respiratory condition improved and the patient made an uneventful recovery. The author concludes that the absence of respiratory depression makes mephenesin a highly useful drug in the treatment of tetanus, but it has the disadvantage that increasing dosage is required which may result in hæmoglobinuria. Gallamine triethodide should be reserved for those cases which fail or cease to respond to mephenesin since it carries a risk of respiratory depression. S. L. W.

Mercurial Diuresis, Observations on the Character of. R. A. Dale and P. H. Sanderson. (*Brit. J. Pharmacol.*, 1954, **9**, 210.) 0·2 g. mersalyl injected intravenously into normal male human subjects caused a fall in potassium excretion where normal diet was being taken; on salt-poor diets the potassium excretion rate rose. With normal diet the mersalyl increased urinary *p*H, but had no constant effect on inorganic phosphate excretion. There was a rise in ammonia excretion and a fall in bicarbonate excretion where the subjects were in normal acid-base balance. These results were reversed where the mersalyl was given after acidosis was induced by ingestion of ammonium chloride, although in both normal and acidotic subjects a fall in urinary *p*H occurred. In subjects made alkalotic with sodium bicarbonate, mersalyl diuresis was inhibited. The creatinine excretion fell transiently between 30 and 90 minutes of the injection, but the fall was not marked. Uric acid excretion rose within 30 minutes of injection whereas changes in the rates of water, sodium and chloride excretion generally occurred only after 60 minutes. The significance of the results in relation to the sites of excretion of the urinary constituents and to the site of action of mersalyl is discussed. Four points are put forward as worthy of further attention: the changes in potassium excretion; the delay in onset of diuresis; the reversal of the usual changes in ammonia and bicarbonate excretion in acidosis; and the abolition of diuresis in alkalosis.
G. P.

Nitrogen Mustard in Treatment of Systemic Lupus Erythematosus. E. L. Dubois. (*Arch. intern. Med.*, 1954, **93**, 667.) 20 patients with active systemic lupus erythematosus on maintenance cortisone dosage were treated with mustine hydrochloride or triethylene melamine. The mustine hydrochloride was administered in a single dose of 20 mg. injected into the tubing of an intravenous infusion

of 5 per cent. dextrose in water. The drug was given during the evening after pre-medication with 0·1 g. of phenobarbitone and 0·1 g. of pentobarbitone sodium. The triethylene melamine was given in 5 mg. tablets, taken with water on an empty stomach an hour before breakfast. Initial courses consisted of a total dose of 10 to 15 mg. over a 2- to 3-day period and the interval between courses was not less than 2 weeks. 4 of 5 very œdematous patients with the nephrotic syndrome of lupus nephropathy had good results, diuresis appearing in 3 of the patients within 3 to 14 days after administration of the drug. 5 of 6 relatively dry nephrotic patients had improvement in their renal and in their general condition. 2 patients with hypertension and nephropathy and no œdema were not benefited. None of 7 patients without evidence of renal damage were benefited. Triethylene melamine was administered by mouth to 5 patients in 11 courses. Agranulocytosis occurred in 1 case and fatal aplastic anæmia in another despite the usual precautions. No serious toxicity was noted from the 34 courses of intravenously administered nitrogen mustard. S. L. W.

Octylamine and Compound 48/80—Comparison of Histamine Release by. W. Feldberg and J. L. Mongar. (*Brit. J. Pharmacol.*, 1954, **9**, 197.) Octylamine is a more potent liberator of histamine from minced guinea-pig lung than is 48/80. The situation reverses when the two are compared by the triple response of human skin *in vivo*. To investigate the comparison further the activities of the two agents on isolated perfused tissues were determined. Throughout the range of preparations used 48/80 was in most cases much more active than octylamine. The ratios of potency (48/80 : octylamine) on the preparations were:—cat skin flaps and gastrocnemius muscle, 200 : 1; perfused hind-quarters—rat 1000 : 1,—guinea-pig 60 : 1; perfused lung, 1 : 1 to 20 : 1 depending on species. With the lung preparations large doses of the drugs had to be used to liberate the histamine. G. P.

Pilocarpine, as an Antagonist to the Undesired Effects of Ganglion-blocking Agents. J. A. Gunn and A. M. Cooke. (*Brit. med. J.*, 1954, **1**, 1473.) A hypertensive patient, under treatment first with hexamethonium and later with pentolinium tartrate, was treated successfully with pilocarpine to abolish the dryness of the mouth and eyes, constipation, difficulty with micturition and loss of accommodation consequent to the parasympathetic ganglionic blockade caused by the blocking agents. With 6·6 mg. of pilocarpine nitrate given orally, salivation was excessive and the dose was reduced to 5 mg. which appeared adequate. The side effects of the ganglionic blockade disappeared within half an hour of taking the pilocarpine and returned in 6 to 10 hours, depending on the dose. G. P.

Serotonin, (5-Hydroxytryptamine), Species Difference in the Respiratory and Cardiovascular Response to. J. A. Schneider and F. F. Yonkman. (*J. Pharmacol.*, 1954, **111**, 84.) The authors have, by pharmacological and surgical means, attempted to localise species differences in respiratory and cardiovascular responses to 5-hydroxytryptamine. The study was conducted on dogs, cats and rabbits under barbiturate anæsthesia. Blood pressure was recorded by a glass membrane manometer, respiration by body plethysmography, afferent nervous activity was recorded from the intact vagus and heart rate by an electronic frequency recorder. In all 3 species a period of apnœa and bradycardia was observed after 5-hydroxytryptamine, but in the dog this apnœa was preceded by a short period of hyperpnœa. The blood pressure

response in the dog was mainly pressor, preceded in some instances by a brief fall. In cats and rabbits only a sustained vasodepressor response was obtained. On the isolated perfused Langendorff heart preparations of the 3 species 5-hydroxytryptamine had the same action, any difference being quantitative. Heart rate, rate of coronary flow and amplitude of contraction were all increased. Activation of pulmonary stretch receptors as evidenced by afferent nerve activity in the vagal fibres subserving this function was demonstrable after 5-hydroxytryptamine intravenous injection in the cat and dog, but not in rabbit. The bradycardia and initial blood pressure fall were considered to be due to such a reflex mechanism, since in all 3 species these effects could be abolished by procaine (in a dose which had been found to abolish similar effects by the veratrum alkaloids), by cutting the vagi, by ganglionic blockade with pendiomide, and by atropine. This reflex effect of 5-hydroxytryptamine was also responsible for the respiratory arrest in expiratory position in the cat; both bilateral vagotomy and intravenous procaine abolished the response. In the rabbit respiratory stimulation could be abolished only by combining spinal cord section at C6 and bilateral vagotomy. Neither of these measures alone completely eliminated the stimulation, nor did procainisation. The initial respiratory stimulation in the dog, however, was easily blocked by either cord section at C6 or bilateral vagotomy. In the dog the rise in blood pressure was due to a direct vasoconstriction and probably also to a direct effect on the heart. Regitine effectively blocks this action, but ganglionic blockade by Pendiomide potentiates the rise. The prolonged fall in blood pressure in rabbits produced by 5-hydroxytryptamine was reversed by cord section at C6, but not by ganglionic blockade. It would thus appear to be reflex in origin, but of a different nature from that seen in the cat. It was concluded that reflexes originating from the heart and lungs were responsible for the differences in cardiovascular and respiratory responses to 5-hydroxytryptamine in the species studied. A direct stimulation of the carotid body or of the brain is unlikely to be the cause of these effects. G. P.

Stanolone (Dihydrotestosterone) in the Treatment of Mammary Cancer. A. Gellhorn, J. Holland, J. B. Herrmann, J. Moss and A. Smelin (*J. Amer. med. Ass.*, 1954, **154**, 1274.) This is a report on the evaluation of stanolone in the treatment of 26 patients with carcinoma of the breast, in all of whom the extent of metastatic disease was such that radiotherapy was not feasible. The disease had been present for 1 to 5 years in 18 of the patients, for more than 5 years in 4, and for less than a year in the remaining 4. The stanolone was given as a suspension in isotonic sodium chloride solution in a dose of 100 mg. intramuscularly either daily or 6 times weekly. 3 patients received less than 3 g. of the hormone, 3 were given more than 10 g., and the remainder received between 3 and 10 g. Treatment was continued for as long as improvement was maintained. 11 of the patients experienced subjective relief for periods of 14 to 183 days, and 4 of these showed objective evidence of temporary tumour regression; 19 of the patients died within 1 to 11 months. There was no indication that the therapy achieved any significant prolongation of life. Of the 26 patients, 24 had side-reactions which were predominantly those of virilisation. From the results obtained in this study the authors conclude that stanolone has no qualitative or quantitative advantages over testosterone propionate in the therapy of metastatic cancer of the female breast. A much larger group of similarly studied cases will be required before it can be determined whether its therapeutic efficacy equals that of testosterone propionate. S. L. W.

LETTER TO THE EDITOR

The Leucocyte Response in the Rabbit to Pyrogen from *Proteus vulgaris*

SIR,—In the discussion on this paper at the British Pharmaceutical Conference at Oxford Dr. Dare said that, of the three papers from this Department dealing with pyrogen and small lymphocyte percentage fall, the first had claimed that there was no correlation, the second that there was partial and the third complete correlation. Careful perusal of what Dr. Dare calls the first paper (*J. Pharm. Pharmacol.*, 1952, **4**, 972) will show his remarks to be misleading, because they betray an inaccurate interpretation of the paper, admittedly preliminary in nature, which dealt with only one dose level and which referred to the possibility of finding anomalous lack of correlation between two responses in any isolated rabbit and not the absence of correlation between the two responses (temperature and small lymphocyte percentage fall) in rabbits generally. To say that "partial correlation" was claimed in the second paper (*ibid.*, 1954, **6**, 317) is quite wrong, since this term nowhere appears in the paper to which he refers. I would gladly refer him to standard texts for a description of this statistic even though, in my view, he used the term carelessly. In the third paper (*ibid.*, 1954, **6**, 962) a completely different index (percentage fall in the average number of lobes per neutrophil) was assessed and correlation found to exist over the range defined by the 4 dose levels. The index which was the subject of the previous papers (small lymphocyte percentage fall) was reinvestigated by a different worker (Mr. Anderson) over 4 dose levels, with the accumulated experience of the Department, and correlation was shown to exist. It was, therefore, with considerable astonishment that I heard Dr. Dare employ reasoning, not at all concerned with the new index presented, in criticism of a new suggestion presented by a young worker.

The School of Pharmacy, J. P. TODD.
The Royal Technical College,
Glasgow, C.1.

October 11, 1954.

ABSTRACTS (*Continued from page* 1095.)

Thiopentone Sodium, Absorption, Excretion and Distribution of, in the Organs and Tissues of the Rat. L. I. Grebennik and Z. Solob'eva. (*Farmakologiya i Toksikologiya*, 1954, **17**, No. 1, 22.) Healthy male rats were given thiopentone sodium labelled with ^{35}S in doses of 5 mg./100 g. into the abdominal cavity, and the urine was examined for radio-active sulphur over the following 4 days. On the first day 53 per cent. of the total sulphur was excreted, and a total of 62 per cent. after 4 days; 30 per cent. was excreted in the oxidised form and 9·4 per cent. in the form of unchanged thiopentone or partial break-down products (extractable with dichlorethane at *p*H 4). In rats suffering from fatty dystrophy of the liver (induced by carbon tetrachloride), the initial rate of excretion was slower, but the total amount excreted in 4 days was unchanged. After either intravenous or intra-abdominal administration the greatest amount of radio-active sulphur was found in the liver; lesser quantities were found in the blood, lungs, heart and brain, in that order. E. H.

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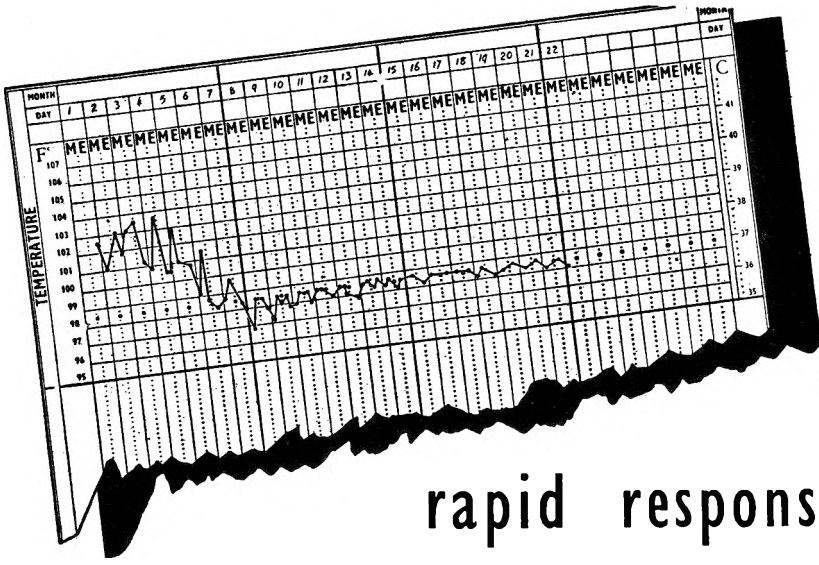

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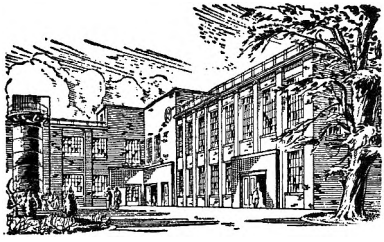

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* Ann. Int. Med. *35 : 1028.* (*1951*)

* South African M.J. *25 : 394.* (*1951*)

* Neurology. *2 : 131.* (*1952*)

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