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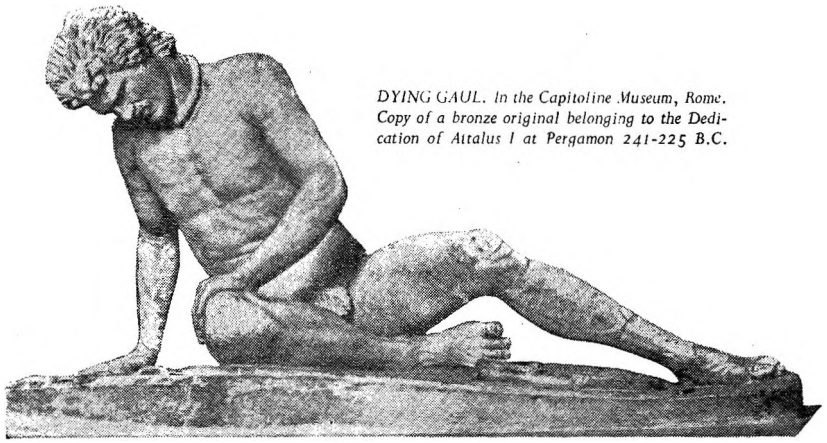
CONTENTS

Continued on page ii

CONTENTS



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REVIEW ARTICLE

SOME RECENT DEVELOPMENTS IN THE PHARMACOLOGY OF THE ANTI-THYROID COMPOUNDS

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It was established in 1943 that thiouracil and similar drugs inhibit the synthesis of thyroid hormone; that they can control human thyrotoxicosis; and that a necessary corollary of complete inhibition of thyroid hormone synthesis is a compensatory hyperplasia and hypertrophy of the thyroid gland, mediated through the pituitary. It was obvious at that date that these drugs were likely to become both a useful instrument of research into thyroid physiology, and effective therapeutic agents; and such they proved to be. This review will attempt to survey some of the major advances in thyroid physiology, in which the thiouracil derivatives have been implicated, and will also indicate the effect of these advances, coupled with the effect of further clinical experience, upon practical therapeutics.

What has chiefly been gained, so far as thyroid physiology is concerned, is a clearer picture of the course pursued by the iodine atom, from the time when it presents itself as inorganic iodide in the thyroid capillaries, to the time when it is incorporated into the thyroxine molecule. The process occurs in two main stages: entry into the gland, and combination with protein. It is convenient in this review to deal first with the factors governing the entry of iodide into the thyroid, and later with those governing the actual synthesis of thyroxine. There follows a discussion of the relative properties of the anti-thyroid drugs and a consideration of their relative merits in clinical practice. No attempt is made here to assess the value of the long-term treatment of thyrotoxicosis with the thiouracil derivatives, nor to compare it with sub-total thyroidectomy. Finally, recent evidence bearing on the part played by naturally-occurring anti-thyroid substances in the causation of simple goitre is briefly reviewed.

The Effect of Thiouracil upon the Entry of Iodine into the Thyroid

Astwood and Bissell[1] described the rapid and almost complete disappearance of iodine from the thyroids of rats treated with thiouracil. The iodine content of the gland appeared to be such a sensitive and specific index of thiouracil action that it was adopted as the chief method of assessing the relative potency of the anti-thyroid drugs. This observation suggested that either thiouracil acted by preventing the entry of iodide into the gland; or that entry of iodide was determined by the rate at which it was being converted into thyroxine and that thiouracil

prevented this conversion occurring. The former suggestion appeared to be contradicted by the observations of Chaikoff and his collaborators[2] on the formation of radio-diiodotyrosine and radio-thyroxine in isolated slices of thyroid, for they found that thiouracil, thiourea and the sulphonamides inhibited this formation, while in no way hindering the uptake of iodide by the slices from the fluid medium in which they lay. However, this was not convincing proof of the mode of action of anti-thyroid drugs in the intact animal, for it could be argued that the transference of iodide from an artificial fluid into the thyroid cell was a very different process from the transference of iodide from capillary to cell in the living animal. Thus while it was clear enough from Chaikoff's work that the anti-thyroid drugs could prevent the formation of diiodotyrosine and thyroxine, it remained possible that in the intact animal they also prevented the thyroid from collecting inorganic iodide from the bloodstream.

However, it presently became apparent from the work of McGinty and Sharp[3], Astwood[4] and Vanderlaan and Bissell[5] that the iodine content of the thyroid, under the influence of anti-thyroid drugs, could still vary with the iodine content of the diet. Astwood[4] showed that when rats, who were receiving adequate amounts of thiouracil in the diet, were given a single dose of iodide by injection, the total iodine content of the thyroid rose sharply as soon as 1 hour after the injection. Vanderlaan and Bissell showed that the iodine thus taken up disappears again from the thyroid a short time later. Since it is unlikely that iodide could have become bound to protein and left the gland in so short a time it seemed probable that the temporary increase in thyroid iodine was all in the inorganic form. The three groups of workers also provided direct evidence that the iodine was, in fact, not bound to protein, and was therefore probably inorganic. Further confirmation came from the detailed studies of Vanderlaan and Vanderlaan[6] and of Taurog, Chaikoff and Feller[7], who showed, by a variety of methods, that the iodine which enters the thyroids of rats treated with propylthiouracil is indisputably in the form of inorganic iodide. They showed that under these conditions the non-hyperplastic thyroid (of rats given a single injection of propylthiouracil 1 hour previously) concentrated iodide to about 25 times the concentration present in plasma. The hyperplastic thyroid of rats chronically treated with propylthiouracil concentrated iodide to about 250 times the concentration present in plasma, unless the latter was inordinately high.

This work shows that the thiouracil compounds do not prevent the thyroid from concentrating iodide to a high degree. But though iodide can enter the gland, it cannot become linked to protein. Therefore it cannot be retained in the gland. Thus Vanderlaan and Vanderlaan[6] showed that the normal rat thyroid can retain 20 times as large a proportion of a dose of radioactive iodine as can the thyroid of a rat which has had a single injection of propylthiouracil. The early observation of Astwood and Bissell[1], that treatment with thiouracil lowers the iodine

content of the thyroid, can now be explained. Thiouracil does not stop iodine getting into the thyroid; it prevents it from being retained there.

Vanderlaan and Vanderlaan[6] also showed that thiocyanate prevented the concentration of iodide by the thyroids of rats treated with propyl-thiouracil and, moreover, caused any iodide which was present to be discharged. When thiocyanate is given, the concentration of iodide in the thyroid becomes approximately equal to that in the blood. Thiocyanates can cause goitre, by preventing the thyroid from concentrating iodide. But if there is an excess of iodide in the diet—and hence in the blood—enough iodide can enter the gland even if the concentrating mechanism is out of action. Hence thiocyanates do not cause goitre in the presence of an excess of dietary iodide (Astwood[8]).

The work of Vanderlaan and Vanderlaan[6], and others, has thus shown very clearly the existence of two separate mechanisms in the thyroid: the mechanism which concentrates iodide from the blood, which is inhibited by thiocyanate; and the mechanism which combines iodide with protein, and stores it in the gland, which is inhibited by the thiouracil derivatives. This new conception has been made use of by Stanley and Astwood[9] in a study of the uptake and discharge of radioactive iodide by the thyroid in normal and thyrotoxic human subjects. Under the influence of a thiouracil derivative the proportion of a tracer dose of iodide taken up by the thyroid is reduced, and the peak of the uptake curve is lower and reached much earlier. The work of Vanderlaan and Vanderlaan[6] makes it clear that what is being observed under these conditions is the uptake of inorganic iodide by the thyroid, and its rapid subsequent dilution by non-radioactive iodide, since the thyroid (under the influence of thiouracil) is incapable of binding it to protein. The ability of thiocyanates to discharge iodide from the thyroid made it possible to estimate the proportion of the radioactive atoms which are built up into thyroid hormone under any given conditions. Thus if a thyrotoxic patient, under treatment with a thiouracil derivative, is given a tracer dose of radioactive iodide, and then (after an interval) an adequate dose of thiocyanate, the amount of radioactivity remaining in the thyroid region is an index of the proportion of the administered iodide which has been converted into thyroxine and is bound to protein. In this way an estimate is obtained of the efficacy of the anti-thyroid drug being administered. The practical value of this method is excellent evidence that the conclusions of Vanderlaan and Vanderlaan[6] apply also to the human thyroid, whether normal or thyrotoxic.

The Effect of Thiouracil upon the Combination of Iodine with Protein

It may be assumed that iodine enters the thyroid in the form of inorganic iodide. But the normal thyroid contains little iodine in this inorganic form. It follows that very soon after its entry into the gland the iodide must enter into combination with protein.

The exact form which this iodine-protein complex takes can at present only be surmised from indirect evidence. Taurog and Chaikoff[10] have

shown that from the blood of normal animals there can be extracted—without the use of any destructive measures—a substance whose properties correspond with those of thyroxine. The same method of extraction failed to remove a similar substance from the thyroid gland. Since this suggests that there is no free thyroxine in the gland, it may be supposed that the tyrosine which becomes iodinated to form thyroxine is already part of a protein molecule when the process takes place. Release of free thyroxine into the circulation would take place when this protein is broken down by enzymic action.

This conception of the form in which the thyroid hormone is stored may be over-simplified. The question of the relative biological potency of thyroglobulin from the thyroid gland, and free thyroxine, is still unsettled. The most recent contribution to this question comes from Frieden and Winzler[11]. They compared the effect of various natural and artificial iodo-proteins in preventing goitrogenesis in rats treated with thiouracil. The biological potency of the natural thyroglobulin preparations was found to be decidedly higher than would be expected from their thyroxine content, as chemically determined. The implications of these findings, if they are to be accepted in spite of other contradictory observations (which the authors review), are far from clear. They are presented here only to remind the reader that the idea that thyroxine (the circulating form of the hormone) is stored in the thyroid by being incorporated into the molecule of a protein (thyroglobin), may not be a wholly adequate account of the situation. It is, however, a highly convenient working hypothesis, and is accepted here for purposes of further discussion.

The analogy of the artificial iodoproteins suggests that iodine in the thyroid combines directly with part of a protein, so that the resulting thyroxine molecule is from its inception built into the structure of the parent protein molecule. The part of the protein with which the iodine combines is presumably the amino-acid tyrosine. Harington[12] pictures the synthesis of thyroxine as occurring in two stages: first the iodination of tyrosine to form diiodotyrosine, then the condensation of two diiodotyrosine molecules to form thyroxine. Oxidation is required for both stages, the first step being the conversion of iodide to iodine. Since, in the presence of thiouracil, iodide can no longer be brought into organic combination in the thyroid, it follows that thiouracil prevents the first step in the process, the iodination of tyrosine. However, Dempsey and Astwood[13] provided evidence that, even if this first step could occur, thiouracil would prevent the second, for diiodotyrosine was extremely ineffective in preventing thyroid enlargement in rats treated with thiouracil. This finding has been confirmed by Frieden and Winzler[11].

It may be assumed that thiouracil prevents the iodination of protein. It could do this by preventing the oxidation of iodide to iodine, or by itself combining with the free iodine after oxidation had occurred. The nature of the enzyme system which effects this oxidation is not known. Schachner, Franklin and Chaikoff[14] showed that the conversion of radio-

active iodine by isolated thyroid slices to diiodotyrosine and thyroxine is prevented by cyanide, azide, sulphide and carbon monoxide. Since all these agents inhibit the cytochrome-oxidase system, they concluded that this system was essential for the production of thyroxine, and suggested that its function was the oxidation of iodide to iodine. However, this enzyme system is of such general importance to the cell that its inhibition might well prevent thyroxine formation indirectly, even if some other enzyme were responsible for the actual oxidation of iodide.

Another system which could oxidise iodide is the hydrogen peroxide—peroxidase system (Westerfield and Lowe[15]). Keston[16] showed that hydrogen peroxide and peroxidase accelerate the iodination of casein *in vitro*. Although there is histochemical evidence for the presence of this enzyme in the rat thyroid[17], Glock[18] and Astwood[4] were quite unable to demonstrate it by chemical means.

Whatever the nature of the enzyme system concerned there are at least three ways in which the anti-thyroid drugs might interfere with its action: they might poison the enzyme itself, they might compete with iodide as substrate or they might themselves combine directly with iodine and thus prevent its combination with protein. If cytochrome-cytochrome oxidase is the effective oxidative system, then the evidence that the anti-thyroid drugs act by enzyme-poisoning is not good. It is true that Paschkis and others[19] claimed that thiouracil inhibits the cytochrome oxidase system of the thyroid, both when added to isolated thyroid slices and when given to the intact animal. However, this enzyme system is responsible for such a large proportion of normal tissue respiration that this finding conflicts with Lerner and Chaikoff's[20] observation that thiourea, thiouracil and sulphonamides have no effect on the oxygen consumption of isolated thyroid slices. Indeed, the thyroids of rats fed on thiouracil have been found to consume more oxygen than normal glands (Jandorff and Williams[21]), presumably as a result of their relatively greater cell mass. It is scarcely likely that this could have occurred if the cytochrome oxidase of the thyroid had been inhibited to any serious extent. McShan and others[22] and Dempsey[17] were unable to confirm the inhibition of cytochrome oxidase by thiouracil.

Dempsey[17] has claimed that peroxidase activity in the follicular cells of the thyroid (as demonstrated by the benzidine reaction) is inhibited by thiouracil. Glock[18] found that thiouracil and thiourea inhibited the actions of peroxidase and hydrogen peroxide on pyrogallol; but, as has been mentioned before, neither she nor Astwood[4] could find any evidence that peroxidase exists in the thyroid gland. Randall[23] examined the behaviour of anti-thyroid compounds *in vitro* in the presence of hydrogen peroxide and peroxidase. He showed that the effect of these compounds on peroxidase cannot be studied by methods involving the oxidation of dyes (*para*-aminobenzoic acid red, 2:6-dichlorophenolindophenol and benzidine blue), because they are such strong reducing agents that they decolourise the dyestuffs. Dempsey's[17] histochemical evidence of

peroxidase inhibition by thiouracil is therefore invalid. Furthermore, if the anti-thyroid compounds are added to hydrogen peroxide and peroxidase it is found that the hydrogen peroxide rapidly disappears. Under these conditions the anti-thyroid compounds do not inhibit the peroxide-peroxidase system; they act as substrate for it. Randall suggests, as a possible mode of action of these compounds *in vivo*, that they may act as reducing agents by competing for hydrogen peroxide as it is formed, and thus prevent it from taking part in the oxidation of iodide. It must, however, be remembered that there is no good evidence that the peroxide-peroxidase system is concerned in thyroxine formation *in vivo*.

Thus the hypothesis that thiouracil and its derivatives act by poisoning oxidative enzymes is unnecessary, and the evidence in its favour can no longer be regarded as substantial. The reducing powers of these substances are such that they could easily act as substrate in competition with iodide. Equally, they could act by combining directly with free iodine as it is formed. The difference between these last two theories is not, from the physiological point of view, very great, for both state that the ability of the thiol compounds to prevent the synthesis of thyroxine is attributable to their reducing properties. However, since it has not been demonstrated that any peroxidase exists in the thyroid, the theory of direct combination with iodine seems the more acceptable.

Direct evidence in favour of this last theory was first presented by Campbell, Landgrebe and Morgan[24], who showed that thiourea reacted with iodine to give formamidine disulphide. If this reaction occurs in the thyroid it would have the effect of keeping the iodine there in the reduced form. Later, Miller, Roblin and Astwood[25] showed that thiouracil was similarly oxidised by iodine to its disulphide. They examined a series of other compounds in a similar way and found that there was a general correlation (with a few exceptions) between the speed of the reaction and the number of molecular equivalents of iodine with which a compound would react, and its goitrogenic power. Some reducing substances, however, such as glutathione, had a marked power of reducing iodine, but no goitrogenic activity. The same authors showed that thiouracil could inhibit the iodination of casein and tyrosine *in vitro*.

More recently, Pitt-Rivers[26] has shown that thiouracil and similar compounds can inhibit the *in vitro* formation of acetylthyroxine from acetyldiiodotyrosine. She demonstrated that when one of these compounds (tetramethylthiourea) is incubated with acetyldiiodotyrosine, iodine is slowly liberated and oxidizes the thiourea compound. The iodine is thus prevented from oxidising the acetyldiiodotyrosine, and no acetylthyroxine is formed. The ability of various compounds to prevent the formation of acetylthyroxine ran roughly parallel to their ability to prevent the synthesis of thyroxine *in vivo*. Thus the iodine-combining power of thiouracil and related compounds provides a satisfactory explanation of their action in preventing the formation of thyroxine in the living animal, even when diiodotyrosine is provided.

The Relative Effectiveness of the Anti-Thyroid Drugs

Two motives have prompted the various surveys of large numbers of chemical compounds for their ability to inhibit thyroxine synthesis: on the one hand, the desire to define the chemical grouping responsible for the physiological effect; and on the other hand, to find the most suitable substance for the control of thyrotoxicosis. The first of these objectives has not been attained; indeed, it is not likely that it will be attained, at least in terms of conventional organic chemistry. The search for useful therapeutic substances has met with more success.

The methods which have been evolved to compare the various substances belong to three broad types: the purely chemical, the use of slices of thyroid tissue *in vitro,* and tests on intact animals. Miller, Roblin and Astwood[25] showed that thiouracil could inhibit the iodination of casein *in vitro,* by itself reacting with the iodine present, and examined the ability of a number of anti-thyroid compounds to react with iodine. Pitt-Rivers[26] compared the ability of various anti-thyroid compounds to prevent the conversion of acetyldiiodotyrosine to acetylthyroxine. The results of these two methods are in reasonably good agreement with each other and with tests on animals. Thus the thiourea derivatives are a great deal more active in both tests than are the sulphonamide derivatives. Thiouracil is decidedly more active than thiourea. The principal discrepancy is in the case of 6-aminothiouracil. According to Pitt-Rivers, this substance is much less active than thiouracil, but Miller, Roblin and Astwood found it equally reactive with iodine. In the rat[27] and in man[28] it showed no detectable anti-thyroid activity.

The reducing action of a number of substances in the presence of a hydrogen peroxide-peroxidase system was examined by Randall[23]. It was again evident that there was a rough correlation with the results of animal experiments. The sulphonamides were weak reducing agents, and thiouracil was more active than thiourea.

Chaikoff and his collaborators[29,2] have studied the effect of various substances on the conversion of radiodide into radio-diiodotyrosine and radio-thyroxine by isolated slices of thyroid. These studies were very valuable as providing the first evidence that the thiouracil group acted by preventing the synthesis of thyroxine and not the entry of iodide into the thyroid cell. For purposes of comparison of various anti-thyroid substances they are less useful, since for the most part substances were only tested at a single concentration. At the concentration generally used by these workers (10^{-3} M) both thiouracil and thiourea inhibited the formation of radio-thyroxine virtually completely; in the case of the sulphonamides and para-aminobenzoic acid the degree of inhibition was a little less.

For tests on intact animals, rats and chicks have been most commonly used. The results obtained in laboratory animals have been extended by studies of the effects of anti-thyroid compounds on radio-iodine up-

take by the thyroid in the normal human subject, and by clinical trials. Using the rat as test animal, Astwood and his colleagues alone have examined more than 300 substances[8,27]. The method used has been to administer the substance under investigation either in the food or the drinking water for 10 days. The animals are then killed, and the thyroids weighed and either examined histologically or kept for estimation of the iodine content. These methods have fully justified themselves as a means of rapid examination of a large number of possible anti-thyroid substances. The results obtained, however, should not be accepted uncritically as giving a quantitative measure of the ability of a substance to prevent the synthesis of thyroxine. For thyroid weight, histological appearances and iodine content in this test depend not only on the completeness with which thyroxine synthesis is inhibited, but also on the rate at which preformed thyroxine disappears from the gland. This is in turn determined by such factors as temperature and perhaps food intake[13]. The presence of many of the anti-thyroid substances in the diet diminishes food intake and slows growth. Astwood's[27] figures show that even a substance as little toxic as propylthiouracil causes a marked fall in growth-rate at the higher dose levels. This fall in growth-rate is associated with a less marked increase in thyroid weight and a less marked fall in thyroid iodine. Determination of total thyroid iodine is also open to fallacy because it depends on dietary iodide, which may change unexpectedly. When these known sources of error can be excluded there still remains the unexplained phenomenon (discussed by Astwood[4]) of the variations in the shape of the dosage curve from one substance to another. The thiobarbiturates, for instance, give an increased thyroid weight and decreased thyroid iodine at a low dosage; with increasing dosage, however, these effects do not increase proportionately, so that it is never possible to produce a really large goitre with these drugs. This result does not seem to be due to decreased food intake[27].

For the reasons given above, this type of test in rats is not suitable for exact quantitative work. Observation of the effect of anti-thyroid compounds on the uptake of radio-iodine by the thyroid is in some ways more satisfactory. The results of such tests are at present available for the normal human subject. The method employed by Stanley and Astwood[28] was to give tracer doses of I^{131} (without carrier) and then measure the increase in radio-activity over the neck. By plotting the count against the square root of time an approximation to a straight line could be obtained, for a sufficiently long period. The drug to be tested was then administered and the amount of downward deflection of the straight line noted. The responses were graded by degree and duration into 5 classes of increasing inhibition. The method is open to the criticism that the final measurement is not a quantitative one. Nevertheless it has yielded interesting results, and is a much more useful test for clinical purposes in that the experimental animal used is man.

The results obtained in this way show the sulphonamides to be even weaker, relative to the thiourea group, than in other tests; in fact,

sulphadiazine had no detectable anti-thyroid action in a 500 mg. dose. This agrees well with the experience gained in the late war, when large numbers of American troops were given sulphonamides for long periods, as a prophylaxis against streptococcal infections; no goitre was reported. This finding is in contrast with tests in the rat[27], which show sulphonamides to be about as active as thiourea.

Thiourea, on the other hand, showed a much greater activity than would have been expected on the basis of its action *in vitro* and in the rat. In Stanley and Astwood's tests in man thiourea proved as potent as thiouracil. The smaller effect in the rat may perhaps be explained by the great loss of appetite which thiourea causes in this animal. Since thiourea is more soluble than thiouracil, it may be that it penetrates more easily into the thyroid, and thus has a greater potency in Stanley and Astwood's tests than would have been predicted from its behaviour *in vitro.*

The results obtained with 6-substituted thiouracils are also of interest. In the rat, thiouracil, 6-methylthiouracil and 6-*n*-propylthiouracil have relative potencies in the order 1 : 1 : 11. In man, the order is 1 : 2 : 0·75. The reason for the discrepancy is not apparent. Propylthiouracil causes an initial loss of appetite and failure to grow in the rat to about the same extent as the other two compounds. The results of Stanley and Astwood's tests on these three compounds are in fairly good agreement with clinical experience; although the minimal doses necessary to give a full response have never been accurately determined, they are of the order of 200 mg. daily for methylthiouracil, and 300 to 400 mg. daily for the other two compounds.

The most potent substance tested by Stanley and Astwood was 2-mercapto-imidazole. This substance had 1·5 times the potency of thiouracil in the rat, but 10 times the potency in man. There are a number of less striking discrepancies between the values obtained for man and the rat, for which the original paper[28] should be consulted. It is sufficient here to say that the existence of such discrepancies must discourage any attempt to correlate anti-thyroid activity with detailed chemical structure.

The significance of these findings to the clinician is not very apparent. The mere fact that minute doses of a compound, such as 25 mg. of mercapto-imidazole, can cause prolonged inhibition of thyroxine synthesis, does not of itself mean that this is the ideal drug for clinical purposes. It might well be, for instance, that such a drug would produce more toxic reactions than another which had to be used in 10 or 20 times the dose, in order to inhibit thyroxine synthesis. Clinical reports on the practical utility of this drug are not yet available.

In fact, the number of *effective* anti-thyroid drugs available to the clinician is embarassingly large. If a test could be devised which would estimate the proportion of " toxic reactions " to be expected clinically, it would be of great practical value. Unfortunately, no such test exists

at present. All our knowledge of toxic effects comes from clinical experience and is almost useless for purposes of comparison of one drug with another. The only common toxic reactions are drug fever, and rashes; the only dangerous toxic reaction is agranulocytosis. All three of these effects are classed as "idiosyncrasies"; that is to say, they only occur in certain subjects who are said to be "sensitive" to the drug in question. The only way we have of estimating the toxic properties of these drugs is to give them to a large number of subjects, and note the proportion who get these reactions. The accumulated clinical experience up to the present date shows that thiobarbital and aminothiazole are too toxic for routine use, and that thiouracil is more toxic than methylthiouracil or propylthiouracil. These are crude statements of clinical experience, and it is very desirable that they should be amplified by further pharmacological research. In this connection, the observations of Lehr[30] on toxic reactions with sulphonamides are of considerable interest. With these drugs also the common toxic reactions are drug fever and rashes. Lehr presents evidence to show that, although these reactions fall into the category of sensitisation phenomena—i.e., they only occur in susceptible subjects—their incidence still shows a relation to dosage. Since there appears to be a critical dosage, below which no reactions occur, he suggests that combinations of two or three different sulphonamides should be used. Since each sulphonamide would be given in a dose less than the critical one, no toxic reactions should occur. The use of such combinations has, he claims, reduced the incidence of toxic reactions in practice. He suggests that the same line of reasoning might well be tried with the anti-thyroid compounds.

It is always assumed that similar toxic reactions do not occur in laboratory animals. The present writer is not aware, however, that there has been any large-scale attempt to discover whether or not a *small proportion* of animals have, say, a transient bout of fever during the administration of thiouracil. It may be that we are too apt to assume that rats cannot display as much individuality as human beings. However, the stimulus to such investigations has to a large extent passed, since in the doses used at present methylthiouracil and propylthiouracil seldom cause alarming reactions.

The Significance of Naturally Occurring Anti-Thyroid Substances

It is established beyond all reasonable doubt that an extreme deficiency of iodine in the diet can cause goitre, and that the mechanism by which such goitre is produced is similar to that of the thiouracil goitre; that is to say, in the absence of iodine no thyroxine can be formed, hence a compensatory overactivity of the pituitary occurs, with consequent thyroid enlargement. It is also established that, even where iodine lack is not extreme, there is a general inverse relationship between iodine content of the soil and water, and the incidence of goitre. But this inverse

relationship does not always hold in detail. Hence, if the occurrence of simple goitre is to be fully explained, some other cause must be sought for which is sufficient to produce goitre in areas where the dietary iodine is not low enough to be goitrogenic by itself.

Since Chesney, Clawson and Webster[31] first described, in 1928, the production of goitre in rabbits by feeding cabbage, a number of different foodstuffs have been found to cause goitre in animals. Among such foodstuffs are soya-beans, rape-seeds, turnips and peanuts. Some of the evidence is, however, curiously conflicting. Thus Chesney, Clawson and Webster's results were confirmed by McCarrison[32] in India and Spence, Walker and Scowen[33] in this country, but Hercus and Purves[34] in New Zealand and Zeckwer[35] in U.S.A. found the goitrogenic properties of cabbage to be weak and uncertain. Webster, Marine and Cipra[36] explained these anomalies by showing that there were marked seasonal and year-to-year variations in the potency of cabbage. 1928-9 was a vintage year, for cabbage maturing in that winter produced palpable goitres in 7 to 10 days. Similarly, Hercus and Purves[34] found a great variation in the goitrogenic activity of turnips when tested on rabbits. Turnips from an area where outbreaks of congenital goitre in lambs had occurred caused large goitres in rabbits in 1933, but not in 1934.

Recently Greer and Astwood[38] have tested some 60 different foodstuffs for their ability to check the uptake of radio-iodine by the thyroids of normal human subjects. The technique was the same as that previously used by Stanley and Astwood[28] in their survey of the effect of various anti-thyroid substances in man. The activity of several members of the *Brassica* group of vegetables was confirmed. Cabbage and turnip were both active, but swedes (rutabaga) even more so. However, several members of other vegetable groups were also active, such as peaches, pears, strawberries, spinach, lettuce, peas, walnuts and carrots. In nearly all these cases much larger quantities were consumed in the test than would ever be eaten in normal circumstances. However, peanuts, filberts and swedes showed some effect in relatively small quantities. Of the animal products tested there was some indication of anti-thyroid activity in milk, liver and oysters. As with previous investigations there was a wide variation in the response to the same foodstuffs in different trials. A significant depression of radio-iodine uptake followed a mixed meal, consisting of raw carrots, swedes, lettuce, pears and milk.

The importance of these results is two-fold. In the first place they show that many foodstuffs can have an anti-thyroid effect in man as well as in laboratory animals. The response to anti-thyroid substances varies greatly from one species to another, so that this evidence is indispensable if a case is to be made out for foodstuffs as a cause of human goitre. Secondly, Greer and Astwood have shown that the range of foodstuffs in which anti-thyroid substances may be found is much greater than had previously been suspected.

Greer and Astwood have no further data to present on the nature of the naturally occurring anti-thyroid substance or substances. Such substances could belong either to the thiocyanate group, which prevents the uptake of iodide by the thyroid, or the thiouracil group, which prevent the synthesis of thyroxine. In previous animal experiments it had been found that cabbage leaves and soya-beans resembled the thiocyanates in that their effects were easily preventable by iodide, whereas those of rape-seeds, like thiouracil, were not. The mustard oils are derived from several members of the *Brassica* group, which contain a glycoside (sinigrin) and an enzyme, myrosin; in the presence of water these interact to form allyl isothiocyanate. However, isothiocyanates do not cause goitre in the rat[27].

In the presence of ammonia allyl isothiocyanate is readily converted to allylthiourea. It was for this reason that Kennedy[37] originally suspected that allylthiourea was the active principle in rape-seed. He demonstrated that allylthiourea could cause goitre in the rat. However, neither this nor any other active goitrogenic substance has actually been isolated from any foodstuff. The nature of the substance or substances which cause the effects noted by Greer and Astwood therefore remains unknown; in the absence of direct evidence the balance of probabilities seems to favour a member of the thiourea group, but the thiocyanates may also make a contribution.

It is difficult to assess the practical importance of these findings. In the case of many of the foodstuffs noted as giving a positive result the deflection of the iodine uptake curve was a very minor one, and was, moreover, often inconstant in different trials. It does not necessarily follow that such minor deflections are of any significance to the human organism. In the normal thyroid, with its ample reserves of stored hormone, partial inhibition of thyroxine synthesis would not be reflected by any change in blood thyroxine levels unless such inhibition had persisted continuously for long periods. Clinicians who have tried to produce myxœdema in subjects with normal thyroids for therapeutic reasons are well aware that many months of intensive treatment with anti-thyroid drugs are necessary before goitre results.

If foodstuffs are an important cause of goitre (apart from their iodine content) they are likely to be so when the iodine intake is already low, and when a highly abnormal diet is being consumed. Greer and Astwood[38] have noted the occurrence of goitre in individuals on vegetarian diets, or who developed a craving for certain foods, such as lettuce. Bastenie [39] has brought forward evidence which suggests that there was an increase in simple goitre in occupied Belgium during the last war. The population at that time lived on a largely vegetarian diet, in which *Brassica* roots were prominent. It is conceivable, as Bastenie suggested, that this diet was responsible both for the increase in simple goitre, and also for the apparent decrease in the severity of thyrotoxicosis, which he considered was occurring at the same time.

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RESEARCH PAPERS

ASSAY OF THE CURATIVE ACTION OF NEOARSPHENAMINE BY TIME-MORTALITY DATA

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The commonest current methods employed for testing the action of neoarsphenamine are those in which the drug is injected into mice or rats already lightly or heavily infected with *Trypanosoma equiperdum* on a previous day. Blood specimens removed from every animal are examined daily, involving the counting of many squares of the counting-chamber, before deciding whether or not the animals are cured. These methods are laborious and time-consuming, and for this reason, and because of other disadvantages, Bülbring and Burn[1] proposed that the activity of a preparation should be estimated from the survival times of mice infected and treated on the same day. In the present work, this method has been extended and examined statistically.

Method

Blood taken from rats which had been infected 2 days earlier was diluted with 1 per cent. sodium citrate solution till it contained 7,000 trypanosomes in 1 microlitre. Mice weighing about 16 to 18 g. were infected by intraperitoneal injection with 0·5 ml. of this trypanosome suspension. Neoarsphenamine was injected intravenously in 0·2 per cent. solution within 2 hours from the time of infection. The doses were calculated in proportion to the body weight. The usual precautions to prevent the oxidation of the neoarsphenamine were taken.

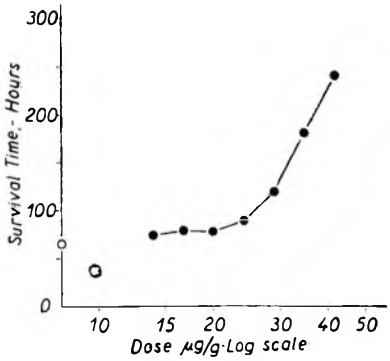

Fig. 1. Survival in hours from the time of infection for the median in every group.

Untreated. ● Treated.

Results

The results of a preliminary pilot-experiment provided a curve relating dosage with survival time. Seven groups each of 15 infected mice were injected with graded doses of neoarsphenamine ranging from 13·9 µg./g. of body weight upwards by steps of 20 per cent. From 2 days after the infection the mortality was noted every hour for 36 hours. After that, observations at night were discontinued. For the two highest doses the mortality was noted only once a day. A control group of 40 mice was followed at the same time. The survival time-

dosage curve was drawn from the medians of the survival times in every group (see Fig. 1). All animals, except those receiving the highest dose, died, the last mouse surviving until the 15th day. In the highest dose group, i.e., those receiving 41·5 μg./g., 3 animals survived, the last one dying on the 18th day. Since we rarely found any animals dying after this time it was decided that any mouse living beyond the 18th day should be counted as definitely cured.

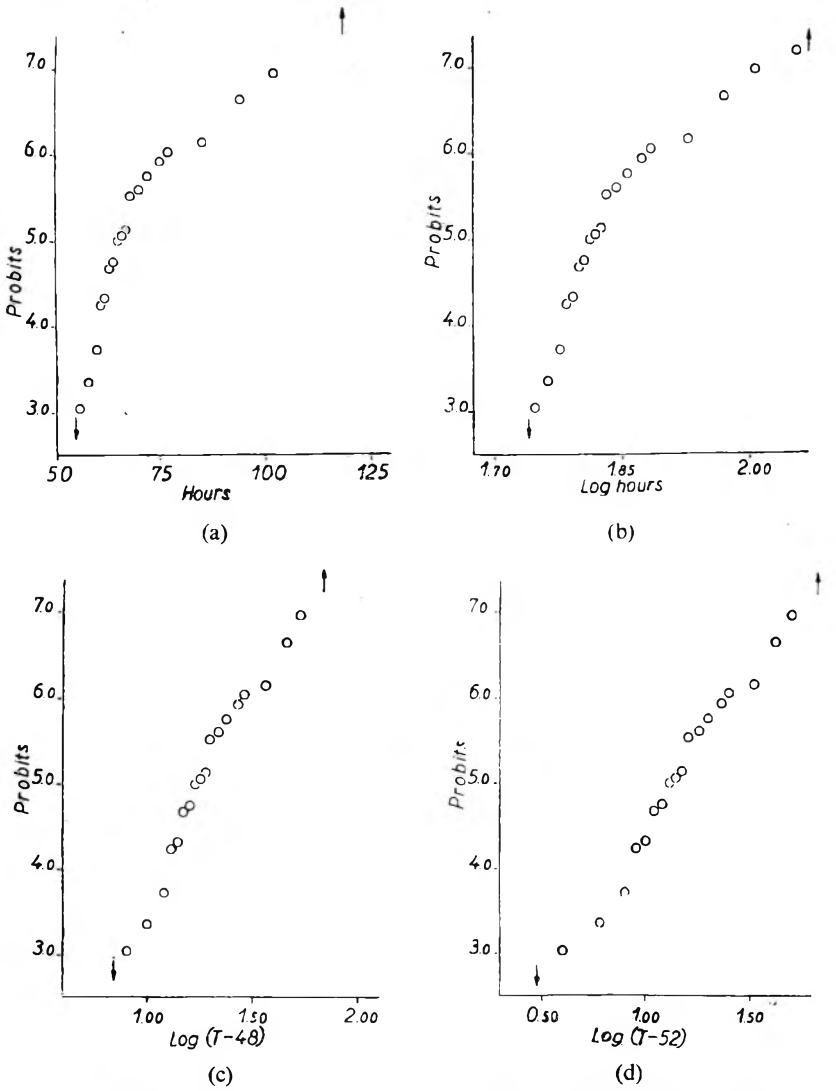

FIG. 2. Probit transformations for the survival times from the time of infection of the 40 infected control mice. (a) Survival time in hours; (b) Log survival time in hours; (c) Log (survival time in hours − 48); (d) Log (survival time in hours −52). The times of the last observation with all living animals and those of the first observation with all dead animals are marked with arrows.

Using this basis we observed which function of survival time had a normal distribution. In toxicity tests in which the lethal time is delayed, some function of the time elapsing between the time of injecting the animal and its death can be found to be normally distributed, and the standard deviation of this function becomes a suitable measure of the varying sensitivity of the animals to the drug. Since the untreated infected controls show differences in their survival times, these were also determined and their limits of variation ascertained.

Of the 40 controls mentioned above, 4 animals died during periods when observations were more infrequent. For the other 36, the time of death could be stated to within an hour. The percentage of animals that had died up to the various times was transformed into probits according to Bliss[2]. These values were then plotted on graph paper with the probits along one axis and the time or the logarithm of the time along the other, the time being measured from the moment when the animals were infected (*see* Fig. 2 a and b). If the plotted function of time had been normally distributed, the points should have fallen mainly along a straight line. In neither case did this happen, however, but the points fell along curved lines. That this was not accidental could be seen from similarly curved lines with corresponding probit transformations for the lower neoarsphenamine doses, in spite of the small number of animals in each one of these groups.

Every mouse was inoculated with about 3·5 million trypanosomes. In this way it received such a large number of trypanosomes that one hardly needs to take into account any differences in virulence between the infecting material of the different mice due to random variation. The dispersion of survival times will thus be mainly due to the host animals, i.e., the possibilities of growth for the trypanosomes in the different mice and the varying resistance of these to the fully developed infection. With intraperitoneal infection the conditions of growth may be regarded as nearly optimal, and so no great differences should exist between the different mice on this ground. This line of reasoning is confirmed by the results from the trypanosome counting method. In this the animals are used when the infection is very strong in the blood, that is, 2 days after being infected. Relatively few animals, however, need to be rejected on account of badly developed infection. In other words, full development of the infection is reached at approximately the same time by the majority of the infected animals. Consequently the differences in survival times would chiefly be due to the varying resistance of the mice against the fully developed infection. If such is the case some function of the time between the point when full infection is reached and the time of death might have a normal distribution.

The first control mouse died 55 hours after being infected. Evidently full infection must have been reached some time earlier, after which the remaining time of survival was influenced only by the resistance of the mouse. Times of 48 and 52 hours after infection were, therefore, chosen, as it was considered that full infection might have been attained

in the control group at one of these periods. New probit diagrams were made with the logarithms of the times from these new starting points along one axis, that is, the logarithm of the survival time in hours minus 48 and hours minus 52 respectively. In both cases good agreement with a normal distribution was obtained (*see* Fig. 2 c and d). In view of the small amount of material the points on both figures appear to lie reasonably near a straight line.

Also for the mice treated with neoarsphenamine the logarithms of (survival time in hours − 48) seemed to be normally distributed for every dose. As the groups were rather small, on a later occasion 2 groups with 40 infected mice in each were treated with doses of 20 and 31 g./g. of body weight respectively. In these groups, too, no certain deviation from the formula just mentioned could be found, the points lying fairly well along a straight line in the probit diagram. On this occasion a certain change in sensitivity to the neoarsphenamine was observed as compared to that shown in Figure 1 since half of the animals in these two groups died after 108 and 218 hours.

If this transformation of the primary values, i.e., the logarithm of (survival time in hours—48) proves to be generally useful for experiments of this kind in different laboratories, the mice of a group in different tests or at different times should as a rule be approximately normally distributed when their times of survival are transformed in this manner. The values given by Bülbring and Burn and also those found in earlier experiments from this laboratory, lend support to this contention. These figures confirm, among other things, that the maximum mortality of the controls as well as that of the animals treated with small neoarsphenamine doses occurs during the night between the second and third day after the infection.

TABLE I

Reading Time: Day after Infection	Reading Time: Time of Day	Time in Hours After the Infection (T)	Class Limit Log (T-48)	Class Width	Class Middle
2	22 o'clock	61	1·114	0·248	1·238
3	8 ,, ...	71	1·362	0·104	1·414
3	14^{15} ,, ...	77·25	1·466	0·102	1·517
3	22 ,, ...	85	1·568	0·104	1·620
4	8 ,, ...	95	1·672	0·104	1·724
4	20^{45} ,, ...	107·75	1·776	0·105	1·828
5	13 ,, ...	124	1·881	0·104	1·933
6	9^{30} ,, ...	144·5	1·985	0·098	2·034
7	10 ,, ...	169	2·083	0·099	2·132
8	17 ,, ...	200	2·182	0·104	2·234
10	10 ,, ...	241	2·286	0·103	2·337
12	14 ,, ...	293	2·389	0·105	2·442
15	9 ,, ...	360	2·494	0·099	2·544
18	17 ,, ...	440	2·593	0·103	*2·645

*Calculated with the class width taken as 0·103, which is the mean of all class widths except the first

As it thus seems that the logarithm of (survival time in hours − 48) in practice may be taken as normally distributed in the different dosage groups, due regard should be given to this fact in the spacing of the reading times. The following example shows how this may be performed so that the intervals between the observations are as far as possible equally large when expressed in the normally distributed function, i.e., log. (time in hours between the time of infection and the observation −48). Even allowing for this, the readings can be so arranged that most of them are made during the normal working day.

Example

Groups of 20 newly infected mice, which had been kept in the laboratory for over a week before this experiment were injected intravenously with doses of 24 and 30 μg./g. of body weight of the International Standard and of a commercial preparation respectively, both in 0·3 per cent. solution. The mice were infected at 9 o'clock in the morning and the injections of neoarsphenamine were made within the following 2 hours. The readings were performed according to Table I. The times when the mice were found dead are given in Table IIa and the corresponding class middle for each animal in Table IIb. Two methods of analysis of the data are now available.

TABLE IIA

RESULTS. NUMBER OF DEAD MICE

Reading Time		Standard		Test Preparation	
Day	Time of day	24 μg./g.(S_L)	30 μg./g.(S_H)	24 μg./g.(U_L)	30 μg./g.(U_H)
2	22 o'clock				
3	8 ,, ...			4	
3	14^{15} ,, ...			7	2
3	22 ,, ...	1		2	4
4	8 ,, ...	4		5	5
4	20^{45} ,, ...	8	3	2	6
5	13 ,, ...	4	6		2
6	9^{30} ,, ...	2	1		
7	10 ,, ...		2		
8	17 ,, ...	1	5		1
10	10 ,, ...		2		
12	14 ,, ...				
15	9 ,, ...				
18	17 ,, ...				
Surviving after the 18th day			1		

The simplest method is to calculate the means, standard deviations, and standard errors of the means in the usual manner for each group as in Table IIB t-analysis for the differences between the means of S_H and S_L and between U_H and U_L shows in the first case $P < 0.001$ and in the second case $P = 0.001$. The differences are therefore not attributable to random variation alone. This shows that the test really is sensitive to a difference in dosage of 20 per cent. For the difference between the means of S_L and U_H $P = 0{\cdot}05$, i.e. the larger test dose has smaller effect than the smaller standard dose.

TABLE IIB

RESULTS TRANSFORMED

CLASS MEANS FROM TABLE I FOR THE DEATH OF EACH MOUSE

Mouse	Standard		Test Preparation	
Number	24 μg./g.(S_L)	30 μg./g.(S_H)	24 μg./g.(S_L)	30 μg./g.(U_H)
1	1·517	1·724	1·238	1·414
2	1·620	1·724	1·238	1·414
3	1·620	1·724	1·238	1·517
4	1·620	1·828	1·238	1·517
5	1·620	1·828	1·414	1·517
6	1·724	1·828	1·414	1·517
7	1·724	1·828	1·414	1·620
8	1·724	1·828	1·414	1·620
9	1·724	1·828	1·414	1·620
10	1·724	1·933	1·414	1·620
11	1·724	2·034	1·414	1·620
12	1·724	2·034	1·517	1·724
13	1·724	2·132	1·517	1·724
14	1·828	2·132	1·620	1·724
15	1·828	2·132	1·620	1·724
16	1·828	2·132	1·620	1·724
17	1·828	2·132	1·620	1·724
18	1·933	2·234	1·620	1·828
19	1·933	2·234	1·724	1·828
20	2·132	2·645	1·724	2·132
Total	35·099	39·914	29·432	33·128
Mean	1·75495	1·9957	1·4716	1·6564
Standard deviation	0·138	0·232	0·159	0·164
Standard error of the mean	0·031	0·052	0·036	0·037
Total S	75·013		Total U	62·560

More information, however, may be extracted from the material if it is subjected to variance analysis (Fisher[3]), the results of which are shown in Table III.

TABLE III

ANALYSIS OF VARIANCE. DATA OF TABLE IIB

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between samples	1	1·9385	1·93850	61·74	<0·001
Between doses of same substance	2	0·9211	0·46055	14·67	<0·001
Random sampling	76	2·3867	0·03140	—	—
Total	79	5·2463	—	—	—

Slope, 2·18 Potency, 0·72 Fiducial limits, 0·580 to 0·809 (P = 0·05).
Percentage limits, 80·5—112·0

The slopes of the dosage-response lines were calculated and found to be homogeneous. It was therefore legitimate to calculate a combined slope and to use this value in order to obtain the potency of the unknown in terms of the standard from the equation provided by Gaddum[4], and the fiducial limits of error of the estimated potency from the equation provided by Fieller[5].

Summarising the results, the unknown preparation had a potency of 0·72 of the standard with fiducial limits of error of 0·580 to 0·809 for P = 0·05.

When material is grouped it is desirable to group it within narrow class limits, i.e. to group it in many classes, in order to extract as much

information as possible. In this case the number of classes directly depends on the frequency of the readings; many times of observations allow many classes with narrow class limits, whereas few readings allow only a few classes and widely spaced class limits. The number of the observations, however, is limited by the fact that in practice it is hardly feasible to make observations during the night. An increase of the number of readings during the day but none during the night implies alternating small and large intervals between the observations, with varying class widths, and this naturally gives no increased precision.

This could also be verified in the material from our example in which more readings were made during the days than are recorded in the tables. The number of readings in the example is near the maximum possible in practice with approximately equally spaced intervals. The fact that the first class has a much larger width than the rest has no great importance as in any case the doses of neoarsphenamine must be so large that only single animals die here.

One objection that has been raised against using survival times for estimating the curative action is that one does not know if the death of the animal is due to the infection. A certain control of this, however, exists. No deaths are caused by the infection on the day when the animals are infected or on the first or even on the greater part of the second day after infection. If the animals die during this time, it is due either to technical faults, which should be few with proper technique, or to non-specific deaths. Mice dying during this time must therefore be excluded from the analysis. When the groups are not too small and the times of survival not too long, the death of one or two mice during this period does not appreciably alter the accuracy of the test. If more mice die, it shows that they were in bad condition and the assay must be rejected. It has been found convenient to keep the mice in the laboratory for some days before they are used in order to make sure that they are in good condition.

When it is desired accurately to determine the curative potency of a preparation, the calculations will be performed as indicated in the example quoted above. Often, however, it is only necessary to ascertain that the potency of the test preparation is not less than that of the standard or of a certain proportion of it. The doses to be used should give a clearly prolonged survival time without making the test unwieldly. In our experiments a dose of 24 to 30 μg/g. of body weight fulfilled these conditions. As a control of the sensitivity of the method a weaker dose of the standard was given as well, by way of example say 20 per cent. weaker. In a successful experiment a difference between the two should be evident.

Practical Performance of a Test

Guided by these principles and by the aforementioned results, the test is performed as follows: 60 mice which have been kept in the laboratory for a week are infected intraperitoneally with 0·5 ml. per mouse of a trypanosome suspension containing 7,000 trypanosomes per

microlitre. This is obtained from the blood of rats that have been infected 2 days earlier. The trypanosomes are counted in a counting chamber and the blood is diluted with 1 per cent. sodium citrate solution to the desired concentration. The infection is performed at 9 o'clock in the morning. Within the next hour the mice are injected intravenously with the different doses of the 0·3 per cent. neoarsphenamine solutions. During the preparation of the solutions the usual precautions against oxidation are observed (*see* Burn[6]). 20 mice receive 24 μg. and 20 mice 30 μg./g. of body weight of the standard and 20 mice receive the dose of the test preparation that is to be compared with the higher standard dose, and which must not be less potent than this standard dose if the preparation is to pass the test. The mice are observed at 16 o'clock on the second day after the infection. Those which have died are rejected and excluded from the calculations. After that the readings of the mortality are spaced according to the times in Table 1. The mean and the standard error of the mean are calculated for every group, the value for every animal being that of the corresponding class middle seen in the table. Thus, if an animal has died only on the fourth day at 8 o'clock its value is 1·620. The preparation passes the test if t-analysis shows that it is stronger or not weaker than the larger standard dose. In the latter case, however, a significant difference between the standard doses must exist, otherwise the test must be repeated. If t-analysis shows that the test preparation is significantly weaker than the larger standard dose it is rejected.

Discussion

When testing substances on animals, it can be shown in many cases that the logarithm of the duration of the effect or the logarithm of the time till the effect appears is approximately normally distributed (Bliss[2], Goodwin and Marshall[7], Goldberg[8] and others). A close study of this question, however, may reveal that a more complicated function of the time has a normal distribution (Ipsen[9]). On the other hand, it is sufficient to have an approximate knowledge of the kind of function that is normally distributed when grouping the observations as the laws for calculating the means, standard errors and t-values are also applicable to a number of different distributions, more or less deviating from the normal. The grouping of the observations advocated in this paper seems to be more rational than making one or two readings every day for a limited number of days as proposed by Bülbring and Burn[1] and Goodwin[10] or for a longer times according to the method of Chen, Geiling and MacHatton[11]. A further advantage is that an estimate of the error may be obtained for every dose, so extracting all information inherent in the material. A comparison with the results of Hawking[12] shows that, whereas in the trypanosome counting method every animal gives only a qualitative expression for the strength of the preparation in this method, the survival times are quantitative estimates of the strength of the drug, and thus furnish more detailed information.

SUMMARY

The basis for using time-mortality data in estimating the curative effect of neoarsphenamine is examined. It is shown how the survival times may be transformed so that they become approximately normally distributed. A routine test has been designed on these lines.

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THE DETERMINATION OF ALKALOIDS BY EXCHANGE OF IONS

By A. Jindra

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The development of research on substances serving for the exchange of ions has been going on for about a century. Research workers were at first dependent only on substances of natural origin, but in the years 1910 to 1930 many experiments were made towards preparing suitable synthetic products. In 1935, Adams and Holmes[1] prepared a new type of artificial resin, and thus, with this new group of substances, started development along new lines. Griessbach[2] examined in detail the composition, action and theory of ion-exchange substances, also their preparation and the determination of their activity, and explained their wide use for metallurgical and chemical purposes, especially as water softeners and salt-removers. In principle we are concerned with two groups of artificial resins. The first includes the condensation products of formaldehyde with aromatic acids, chiefly polyhydric phenols, and natural products of the tannin type, these condensation products serving as exchangers of cations, or eventually of hydrogen ions. The second group is formed by the condensation products of aromatic amines with aldehydes, these products serving for the exchange of anions, including hydroxyl ions. Hesse[3] deals, under the heading of chromatographic determination, with the exchange of ions in some detail, from both theoretical and practical standpoints. Similarly, Myers[4] sees in the exchange of ions only one of the methods of chromatographic determination (see also Zechmeister[5]).

Samuelson[6] has contributed much to our knowledge of the use of these resins for the exchange of ions, especially in inorganic analysis. The Encyclopædia of Chemical Technology 1947 gives information of the use of synthetic resins in obtaining alkaloids of natural origin, such as quinine and nicotine, and details of the literature are given.

Ungerer[7] has studied the adsorption of salts of organic bases, and to some extent alkaloids, on calcium permutite, and has ascertained that certain alkaloids, which are not easily soluble in water, are strongly adsorbed from aqueous solutions of their salts, due to the action of the slightly alkaline calcium permutite in precipitating the free base. In an alcoholic medium the adsorption proceeds in the normal way.

The idea of using the chromatographic adsorption method for the quantitative determination of alkaloids was realised soon after the revival of Tswett's method by Valentin, Franck and Merz[8,9,10,11], who describe the technique of adsorption on a column of aluminium oxide, and the working methods for determining the strength of various galenicals containing alkaloids. They have continued their work for several years, and give preference to the chromatographic method over

those used as standard in the pharmacopœias, the advantages lying chiefly in the smaller quantities of material required for analysis, the simpler technique, and the shorter time required for the analysis.

The most recent workers to direct their attention to chromatographic analysis of alkaloidal salts and to the suitability of aluminium oxide for such analyses are Reimers, Gottlieb and Christensen[12]. They passed an ethyl alcohol solution of alkaloidal salts through a column of 10 g. of aluminium oxide, in a tube of 10 mm. diameter and 22 cm. height. The alkaloids were then washed out of the adsorbent with 25 ml. of ethyl alcohol (86 per cent.), and determined by direct titration in ethyl alcohol, using a suitable indicator in accordance with the experience of Kolthoff[13] and Baggesgaard-Rasmussen and Reimers[14].

The object of the work now to be described was first to ascertain whether artificial resins can be used as adsorbents for the determination of alkaloids, and then, if successful results were obtained, to investigate whether they could be applied to the determination of the alkaloidal content of galenical preparations. In the experimental part these questions are answered, the first very definitely in the affirmative, and the second with certain reservations. Nevertheless, even here certain definite results have been obtained, and the way indicated for further work.

Experimental

As an ion-exchanging adsorbent material, Amberlite IR-4B, manufactured by the Resinous Products and Chemical Company of Philadelphia, U.S.A., was selected, this being suitable for anion exchange. Attempts to find a basic exchanger suitable for quantitative work proved a failure, though such adsorbents gave excellent results when used for the isolation of alkaloids. The reactions in the adsorbing column were as follows:

$$\text{Res. OH} + \text{Alk. HCl} = \text{Res. Cl} + H_2O + \text{Alk. base}$$
$$\text{Res. Cl} + \text{OH} = \text{Res. O.H.} + \text{Cl}'$$

The technique of the method has already been described in detail. Here only a brief description of the preparation of the adsorption apparatus is necessary since the whole was of the simplest type.

A tube of ordinary glass of 0·5 cm. diameter was used, which, at some 20 cm. from its end, was drawn out into a short narrow outlet tube, which was fitted into a rubber stopper, and the whole tube was thus attached to a suction flask.

It was necessary to crush the adsorption material, and it was found best to crush it and then leave it standing under water in a beaker for a day or two. During this period it was several times stirred up, and, when it had settled again, the water was changed. The light yellow powder was thus removed, and a material obtained of constant grain size, which plays a certain role in obtaining a perfect exchange of ions.

This prepared material was poured, together with a little water, into the adsorption tube, whose lower end was closed with a small wad of cotton wool, so as to ensure that the adsorbent material settled firmly in place, and did not tend to form layers when subjected to a steady flow of water. When a sufficient amount of material was in the tube, it was further washed with water, and secured by a second plug of cotton wool. The adsorbent was thus evenly distributed in a column about 5 cm. high, water flowing through it at a speed of about 100 to 120 drops per minute, under its own pressure.

Such a column has to be regenerated before and after each analysis. With Amberlite IR-4B this is carried out by means of a 4 per cent. solution of sodium carbonate. In determining alkaloids about 50 ml. of such a solution was sufficient for each regeneration. The excess of sodium carbonate was removed by washing with boiled distilled water until the washings gave no reaction with phenolphthalein. Usually about 300 to 400 ml. of water was sufficient. It was necessary always to maintain a layer of a few ml. of water above the adsorbent column in order to avoid the drying and packing of the material. When ceasing work the upper end of the tube was closed with a rubber stopper. If working with adsorption in a medium other than water, e.g. in alcohol, the adsorption tube and column should be washed out before the experiment with a few ml. of the solvent used.

The principal condition for success in the analysis of alkaloidal salts is the use of a solvent in which both the alkaloidal salt and the free base are readily soluble. Numerous experiments showed that it was not possible to obtain satisfactory results under any other conditions. Thus, for example, when working with strychnine nitrate in aqueous solution, the free base is precipitated on the adsorbent, and although strychnine is sufficiently soluble in ethyl alcohol, it was never possible, even using hot ethyl alcohol, to wash it out of the column quantitatively; and the results were always some 10 per cent. too low, even when using a considerable excess of solvent. This case is interesting since workers using aluminium oxide as adsorbent obtained quantitative extraction of the base from the adsorbent using comparatively small quantities of alcohol. For the examples used in the course of the present work, ethyl alcohol was found to be suitable, and in the case of morphine, methyl alcohol. In most cases the speed of flow of the liquid through the column is not important, and good results may be obtained with a speed of flow of 90 to 120 drops per minute. Only in the analysis of atropine sulphate did we obtain results rather lower than according to theory, and by reducing the speed of flow to 30 to 40 drops per minute we obtained satisfactory results. The speed of flow may be readily controlled by means of a rubber stopper in the adsorption tube in the neck of the Erlenmeyer flask in which the liquid is collected. After the adsorption is completed the tube and vessel are washed out with about 50 ml. of warm solvent (ethyl or methyl alcohol). This quantity of solvent may be regarded as sufficient for the alkaloids analysed, and

it was not thought necessary to ascertain the minimum quantity of solvent needed, since we were using a quantity appropriate to the amount of substance being determined.

Adsorption from galenicals was carried out in exactly the same manner, since the first experiments showed that it was unnecessary to add inorganic acids in advance, in order to prepare salts of the alkaloids with strong acids. The question of a suitable solvent again plays the same important role. The alcoholic solution of the galenicals lost a small amount of its colour in passing through the adsorbing column together with some of the accompanying substances. In regenerating the adsorbent material after each analysis we remove the colouring matter and accompanying matter retained by the adsorbent, so that it is not necessary to change the contents of the column after each analysis.

The actual determination of the alkaloidal bases was carried out by direct titration with hydrochloric acid. In determining alkaloidal salts, i.e., in colourless solutions, the indicator used was a mixture of methyl red and methylene blue, giving at the appropriate dilution with water (about 100 ml.) a sharp change from green to violet. When working with galenicals it was necessary to ascertain the equivalence point electrometrically, using very sensitive measuring instruments. The potential drop is masked in these cases by the accompanying substances (the more intensely coloured the solution, the more difficult is the determination of the equivalence point). It is interesting to compare the titration curve of strychnine in ethyl alcohol with that of the same quantity of strychnine in tincture of nux vomica, in which the masking effect under the conditions prevailing in the tincture is clearly evident. There are possibilities here for working with minimum quantities, and also of using micromethods for the determination of alkaloids in various galenicals, which are being further investigated by the present author. The cause of the double result obtained with tincture of nux vomica as compared with the theoretical result (the equivalent used was the mean of the molecular weights of the two alkaloids) is also being investigated.

The time required to carry out the analysis is about the following:— filling of apparatus, 15 minutes; regeneration of adsorbent, 30 to 45 minutes; adsorption and washing out, 15 minutes. The total time required is from 1 hour to 1¼ hours, in addition to the time required for the titration.

(a) Determination of Alkaloidal Salts

An accurately weighed quantity of the alkaloidal salt (0·1 to 0·2 g.) was dissolved in 20 ml. of ethyl alcohol (96 per cent.), and adsorbed on the prepared column of Amberlite IR-4B synthetic resin. The vessel was then washed out several times with a total of 50 ml. of warm (50°C.) ethyl alcohol (96 per cent.), and the column washed out with the same solvent. The dissolved base in the alcoholic solution was then determined by titration with N/10 hydrochloric acid (F = 1·029), using

as indicator a mixture of 10 drops of solution of methyl red with 2 drops of solution of methylene blue. To ascertain the equivalence point the mixture was diluted to 100 ml. with distilled water. The colour change from green through blue to violet is sharp.

For quinine and cinchonine the alkaloidal base was used for analyses, being dissolved by the addition of an equivalent quantity of hydrochloric acid and 10 ml. of water, the solution being made up to 100 ml. with ethyl alcohol. For the determination of morphine and quinine methyl alcohol solutions were used. Strychnine nitrate was dissolved in ethyl alcohol (75 per cent.). The percentage content of alkaloids in the salts was ascertained experimentally by the Danish Pharmacopœia methods. This was necessary because in many cases impure compounds were used whose content differed considerably from theory. The results are shown in Table I.

TABLE I

DETERMINATIONS OF ALKALOIDAL SALTS

Alkaloidal Salt	Weighed Out	N/10 Hydrochloric Acid (f=1·029)	Found by Adsorption Method	Found by Danish Pharmacopœia Method
	g.	ml.	per cent.	per cent.
Strychnine nitrate	0·0950	2·17	83·36	84·14
	0·0950	2·16	82·92	
	0·1420	3·27	84·04	
Atropine sulphate	0·1691	2·23	41·74	40·40
	0·2184	2·73	39·48	
	0·2267	3·00	41·79	
Morphine hydrochloride ...	0·1395	3·37	75·24	75·91
	0·1395	3·38	75·46	
	0·1395	3·35	74·79	
Brucine hydrochloride ...	0·1261	2·47	84·37	84·29
	0·1261	2·48	84·71	
	0·0966	1·89	84·23	
Ephedrine sulphate	0·1556	5·05	58·52	58·86
	0·1556	4·98	57·70	
Quinine	0·1344	3·74	98·52	
	0·1344	3·78	99·57	
Cinchonine	0·0976	3·14	98·46	
	0·0976	3·13	98·15	

(b) DETERMINATION OF ALKALOIDAL CONTENT OF GALENICALS

The determination was carried out according to the same principles as for the alkaloidal salts. Tincture of ipecacuanha and tincture of nux vomica were adsorbed direct, while fluid extract of cinchona and tincture of opium had the weighed quantity diluted with 25 ml. of ethyl alcohol (96 per cent.). The further procedure was the same as that detailed above. The colouring matter is adsorbed in the column only in small measure, and the solutions of the alkaloidal bases obtained are coloured. The equivalence point must be determined electrometrically with the aid of the glass and calomel electrodes. With tinctures of nux vomica and ipecacuanha the potential change is, however, masked by the accom-

panying substances, but is none the less perceptible, provided that sensitive apparatus is used. Amounts corresponding to theory have been found in all experiments, except in the case of tincture of nux vomica when the result obtained was invariably double the theoretical figure. Figures 1, 2, 3 and 4 show the course of the titrations. In carrying out the titrations the ethyl alcohol solutions were diluted with water to 100 ml. In working the fluid extract of cinchona much depends upon the careful washing out of the adsorbed column, since cinchonine is far less soluble than quinine. In the case of opium a larger alkaloidal content was found that would correspond to morphine alone since the total content of morphine and other alkaloids was determined. The results are shown in Table II.

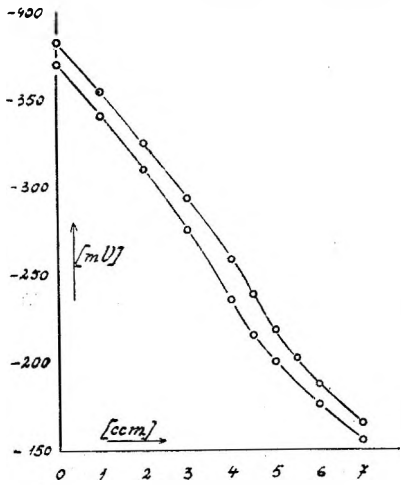

FIG. 1. Tincture of Ipecacuanha

SUMMARY

1. A method has been worked out for the determination of salts of alkaloids by an adsorption method involving exchange of ions, on the synthetic resin known as Amberlite IR-4B which was used with the substances specified in Table I.

2. It is shown that this method is also suitable for determining the alkaloidal content of the galenicals specified in Table II.

TABLE II

DETERMINATIONS OF ALKALOIDS IN GALENICALS

Preparation Used	Weighed Out	N/50 Hydrochloric Acid (f=1·092)	Found by Adsorption Method	Found by Czechoslovak Pharmacopœia Method
	g.	ml.	per cent.	per cent.
Tincture of Ipecacuanha ...	48·80	4·00	0·107	0·095
	48·80	4·50	0·120	
	48·80	4·25	0·114	
	30·00	3·50	0·109	
Fluid Extract of Cinchona...	0·9452	2·65	4·733	4·450
	0·9756	2·65	4·586	
	0·9692	2·65	4·586	
	0·8315	2·25	4·568	
Tincture of Opium	2·9170	3·45	1·842	1·11
	1·3434	1·65	1·912	
	1·9841	2·20	1·727	
Tincture of Nux Vomica ...	35·27	10·00	0·563	0·247
	35·27	9·40	0·530	
	30·00	7·00	0·464	

3. The advantage of the method is its rapidity, simplicity and the use of only small quantities of solvents, together with the determination of all the alkaloids present without any of the possibilities of loss arising from more complicated experimental procedures.

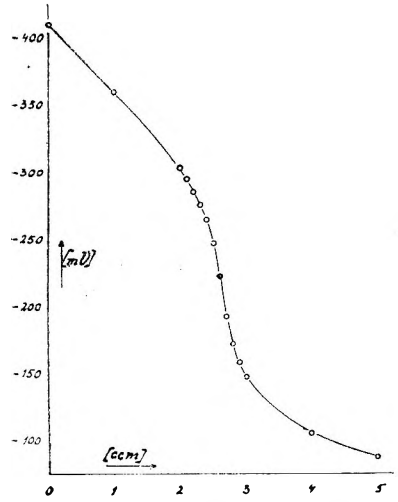

FIG. 2 Fluid Extract of Cinchona

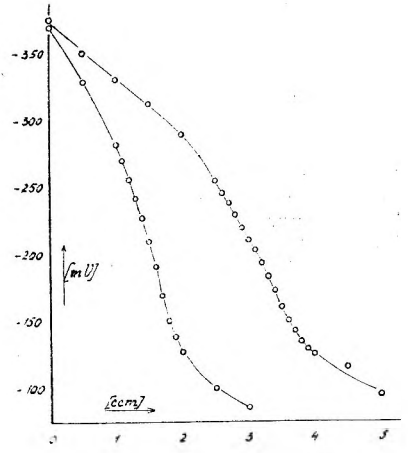

FIG. 3. Tincture of Opium

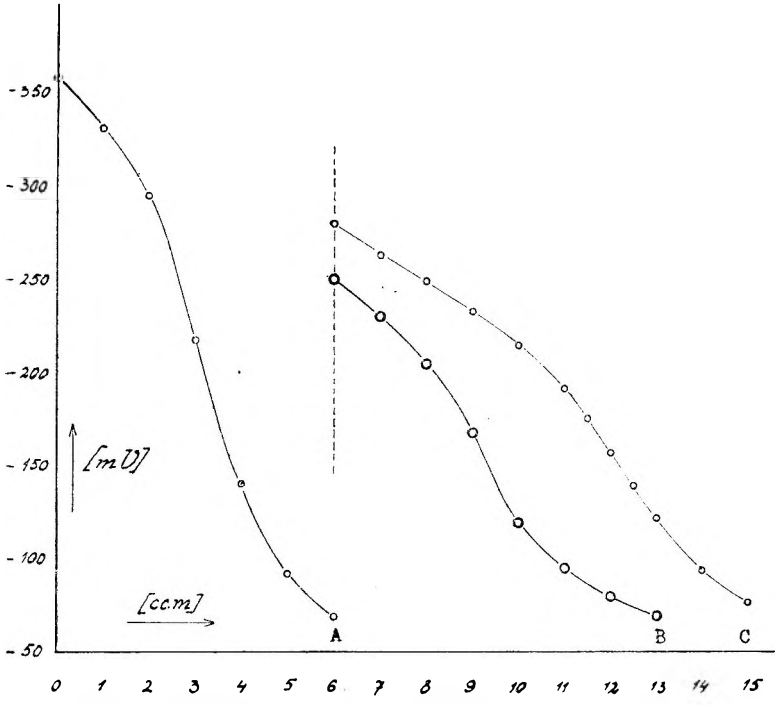

FIG. 4. A. 0·05 g. of strychnine in alcohol. B. Tincture of nux vomica. C. 0·05 g. of strychnine in tincture of nux vomica.

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THE EFFECT OF DRUGS ON THE MOTILITY OF ISOLATED STRIPS OF HUMAN STOMACH MUSCLE

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THE STRUCTURE and nerve supply of the human stomach, the movements of its muscle under a variety of conditions in health and disease, and the effect of various drug substances on gastric activity have been the subject of much research. Carlson[1] and Danielopolu[2] have written extensively on the subject and McSwiney[3] reviews the literature widely. The majority of investigators have made use of a method which records the changes in pressure within balloons placed in the oesophagus, antrum, fundus, pylorus or duodenum of animals or man, or have attempted to assess the alterations in gastric motility by observation and photography with the aid of X-rays. The preparations were all in the fasting state; in some cases contractions of the stomach muscle were stimulated by the previous administration of insulin. Animals might or might not be under the influence of a variety of anæsthetics, or be subjected to ablation of varying portions of the central nervous system. Sundry nerves have been cut and stimulated or allowed to degenerate. From all this work much information has accrued but there has also arisen controversy as to the precise action of a number of drugs of therapeutic importance. One source of error may have arisen from misinterpretation of viscerographic records where the balloon has recorded the activity of the pyloric sphincter when it was supposed to lie in the body of the stomach, or where interpretation of roentgenological observations has been unwittingly influenced by subjective factors during visualisation on the screen.

Apart from the reports of Smith[4] and of Tezner and Turolt[5] very little work has been done with isolated strips of muscle from the human stomach wall, though innumerable experiments have been done with isolated pieces of intestine from animals. It would appear desirable to record the effects of various drugs on isolated portions of gastric muscle before proceeding to investigate and interpret the more complex pictures seen in intact animals and in man. With the recent spread of the practice of partial gastrectomy in cases of peptic ulceration it is now easy to obtain fresh samples of human stomach for investigation.

METHOD

Freshly prepared ice-cold Tyrode solution (sodium chloride 0·8 per cent., potassium chloride 0·021 per cent., calcium chloride (anhydrous) 0·02 per cent., magnesium chloride (anhydrous) 0·001 per cent., dextrose 0·1 per cent., sodium acid phosphate (anhydrous) 0·005 per cent., sodium bicarbonate 0·1 per cent.) was brought into the theatre during operation. As soon as possible after removal of the specimen from the patient, a healthy area of stomach was selected as far as

possible from the diseased area and a portion of stomach wall some 5 cm. square cut off. The mucosa was separated from the muscle layers and the latter conveyed to the laboratory at once in fresh cold Tyrode solution. There a suitable piece of muscle approximately 2 cm. by 1/3 cm. was cut in the long axis of the muscle fibres which is clearly marked, and mounted in the usual isolated organ bath in oxygenated Tyrode solution at 37·5°C. attached to a frontal writing lever. Preparations so mounted showed spontaneous activity of varying degree for 8 to 10 hours; portions preserved at 4°C. could be revived after 24 hours. Drugs were added in solution to make various final concentrations in the 75-ml. bath used. Between tests the preparation was washed twice with Tyrode solution and allowed 15 minutes or more to recover.

Material

The specimens used in this work were from 8 cases of chronic duodenal ulcer operated upon for intractable pain. The muscle fibres used for test were taken from the anterior or posterior wall of the stomach as far from the pyloric region as possible, i.e., the reactions to be described are those of the longitudinal muscle of the body of the human stomach. The stomachs were free from disease in themselves as there was no gastric ulcer or inflammation and no pyloric stenosis or gastric atony or stasis.

The Normal Contraction

Anderson[6] using a viscerographic method describes a phase of relative quiescence characterised by small flat topped waves of about $2\frac{1}{2}$ minutes duration, a phase of active contractions and a phase of tonus waves seen in the course of spontaneous gastric activity and a tetanic phase seen in special circumstances. In the present work all these types of contraction were noted. The tetanic phase only occurs under the influence of an abnormal stimulus such as the addition of a parasympathomimetic drug (see Fig. 1A). The phase of quiescence is the usual finding after the preparation is first mounted and also occurs spontaneously for long and variable periods. It is characterised in the muscle strip by the occurrence of small irregular contractions of some 10 to 20 sec. duration with a return to the base line between each contraction (see Fig. 1A). A modified form of this activity may be found to occur during the period of relaxation between the powerful contractions of the phase of active or hunger contractions (see Fig. 4B) though this is not always the case (see Fig. 2B). The phase of tonus contractions is often found and is characterised by flat-topped contractions of about 2 minutes duration, with variable degrees of relaxation in the course of the wave plateau but no return to the base line (see Fig. 4A). The active or hunger contraction is the most characteristic activity and occurs in spontaneous bursts which reach a maximum degree of contraction after some 10 contractions, continue for a variable period from 5 minutes to 2 hours or more, and cease abruptly or more commonly by declining in vigour to assume the characteristics of the tonus wave or the phase of relative quiescence. Thus

it is seen that the essential characteristics of all the types of wave contraction described by clinical investigators as occurring in the intact stomach of man are to be found in the muscle strip with its absence of circulation, its absence of connection to the central nervous system and its relatively constant environment in a bath of nutrient fluid. It is not suggested that muscular contraction in the normal intact stomach is independent of vascular and nervous influence, but that all the elements for the various types of contraction recorded are available in the muscle and are capable of being carried on by the muscle acting independently. The initiation and regulation of the particular type of contraction found at any one time may well be under the influence of a wide variety of extraneous circumstances.

The Action of Drugs

(a) *Parasympathomimetic compounds.*—Strips of isolated human stomach muscle do not respond to parasympathomimetic drugs in the same low concentrations as do isolated segments of gut from rabbits, guinea-pigs, etc. Acetylcholine in a concentration of 10^{-8} produces only a slight increase in motor activity, but a concentration of 10^{-7} gives rise to a much greater response. If the muscle is in a resting state (phase of relative quiescence) it contracts violently and may pass into a tetanic

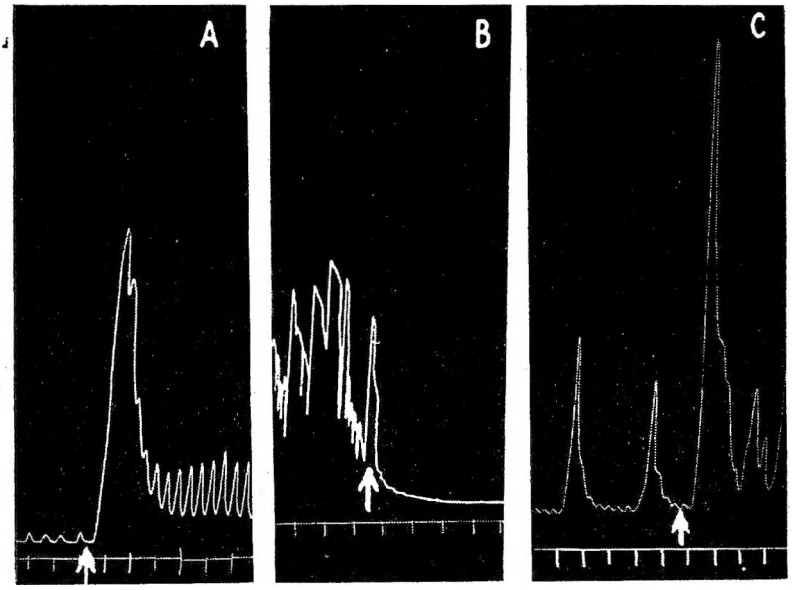

FIG. 1.—Record of movements from a strip of longitudinal muscle from the anterior wall of the human stomach, mounted at 37·5°C. in oxygenated Tyrode solution. Time in 30 sec. 1A shows the phase of relative quiescence changed into a marked motor response and a tetanic spasm by acetylcholine 10^{-7}. 1B shows the phase of active contractions inhibited by adrenaline 10^{-7}. 1C shows phase of active contractions (less violent than in 1B) stimulated by histamine (base) 10^{-6}.

phase (see Fig. 1A). If the muscle is already contracting actively it will pass into a tetanic phase. Carbaminoylcholine in a concentration of 10^{-5}, or eserine 10^{-5} bring about a gradual increase in rhythmic motor activity which may pass into the tetanic phase. The effect of the latter compounds, as in other preparations, is less violent and longer lasting than that of acetylcholine.

(b) *Sympathomimetic compounds.*—Adrenaline in all concentrations relaxes the fibres of the gastric muscle. A concentration of 5×10^{-9} was the greatest dilution which gave definite evidence of activity. If the muscle was in a state of quiescence it relaxed its normal degree of tone under the influence of adrenaline; if it were contracting actively or even in partial tetany all movement ceased and the tone declined. Larger doses up to a concentration of 10^{-7} caused a further degree of relaxation. This

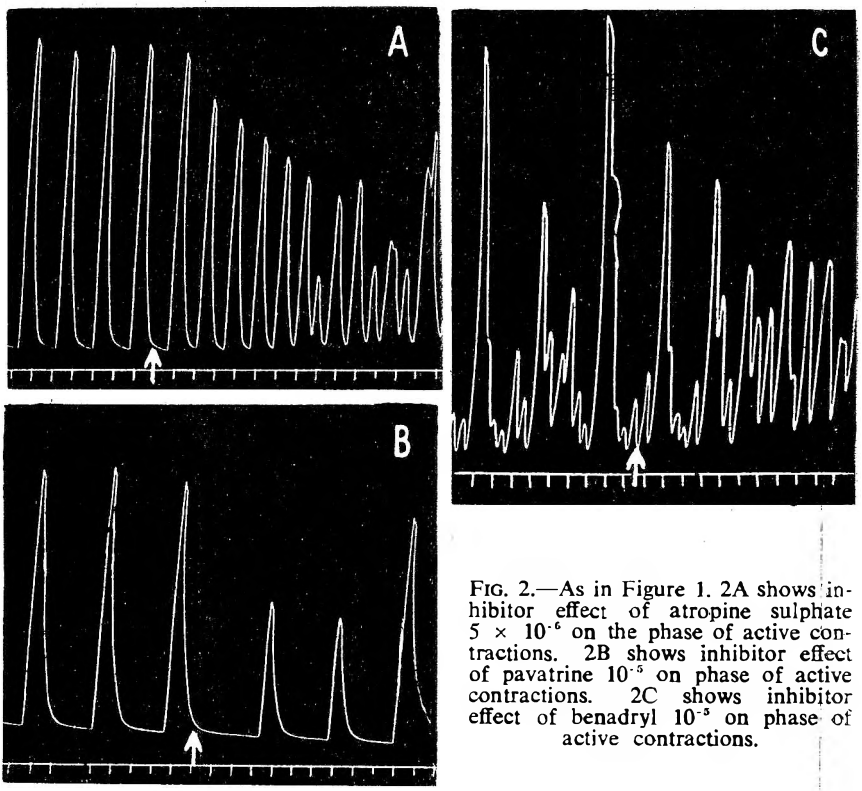

FIG. 2.—As in Figure 1. 2A shows inhibitor effect of atropine sulphate 5×10^{-6} on the phase of active contractions. 2B shows inhibitor effect of pavatrine 10^{-5} on phase of active contractions. 2C shows inhibitor effect of benadryl 10^{-5} on phase of active contractions.

action is shown in Figure 1B. Amphetamine and ephedrine in a concentration of 10^{-5} also inhibit gastric motility.

(c) *Histamine and Barium.*—Histamine in concentrations of 10^{-6} to 10^{-5} of histamine base has a motor effect on the muscle strip. The lesser dose causes a transient increase in contractions, but the larger dose may cause violent contractions and a phase of tetany. Figure 1C illustrates

the effect of histamine 10^{-6}. Barium chloride 10^{-5} also causes increased motor activity of an irregular type and may give rise to tetany.

(d) *Spasmolytic compounds.*—The action of atropine sulphate on this preparation was examined in detail because of the controversy as to whether this drug produces a motor response in certain small doses, as Danielopolu[2] and Anderson and Morris[7] suggest, or is invariably inhibitor in action as Henderson and Sweeten[8] maintain. In the majority of specimens atropine sulphate produced an inhibitor response in all concentrations of the drug which showed any action. If the muscle was in a phase of active contraction the contractions were diminished in extent though they might become rather more frequent. The inhibitory action of atropine in a concentration of 5×10^{-6} on a muscle strip in the phase of active contraction is shown in Figure 2A. Lesser concentrations than 10^{-7} had no effect, and usually 10^{-6} was needed to produce any great degree of inhibition. The activity termed the phase of tonus waves was likewise inhibited by atropine and the action of acetylcholine prevented or abolished. If the muscle was in a phase of relative quiescence the small movements diminished or disappeared.

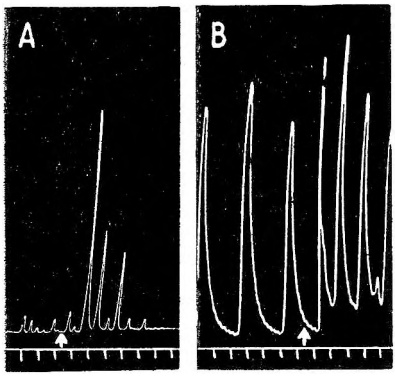

FIG. 3.—As in Figure 1. 3A shows interruption of a long period of relative quiescence by a short burst of active contractions after adding atropine sulphate 10^{-7}. 3B shows the increase in tone and rate of contraction caused by adding atropine sulphate 10^{-7} during phase of active contractions.

In 3 specimens out of the 8 examined atropine sulphate in a concentration of 10^{-7} produced a motor response. This consisted of either a speeding up of the contractions seen in a phase of active contraction (see Figure 3B) with a slight rise in tone, or the production of a short burst of active contractions in the middle of a prolonged period of relative quiescence (see Figure 3A). Further addition of atropine caused inhibition of the stomach muscle, but the effect could be repeated after a period of absence of drug of $\frac{1}{2}$ to 1 hour.

Pavatrine (diethylaminoethyl fluorene carboxylate), which has been shown by Lehmann and Knoefel[9] to have less than 1/20 of the potency of atropine in reducing the hypermotility caused in the stomach of the anæsthetised dog by previous injection of insulin, was active as an inhibitor of contractions in the muscle strip. The concentration required to produce an effect similar to that of atropine 5×10^{-6} was about 10^{-5} (see Fig. 2B). In the small number of tests made on isolated human gastric muscle it would therefore appear that pavatrine approximates more nearly to the activity of atropine than the work of Lehmann and Knoefel[9] on anæsthetised insulin-injected dogs would suggest.

(e) *Anti-histamine Compounds.*—Recently Graham[10] compared the spasmolytic potency of benadryl, neoantergan (2786RP) and antistine. In view of the property displayed by these compounds of inhibiting contractions of gut muscle caused by barium and acetylcholine as well as histamine it is not surprising that a concentration of 10^{-5} neoantergan or of benadryl reduces the extent of the active contraction and the tonus wave of gastric muscle (see Fig. 2C). Benadryl is less powerful than neoantergan in abolishing spasm induced by the addition of histamine but is the more active of the two in inhibiting spontaneous contractions of the gastric muscle.

(f) *Morphine.*—The action of morphine has been the subject of much discussion. Myers[11] working with a viscerographic record of decerebrate cats reported mostly an inhibition of gastric muscle activity with occasional responses by increased movement after injection of morphine. Anderson and Morris[7] working on human subjects reported a diminution of gastric movement followed by a temporary increase and a final state of relative quiescence in fasting patients showing a phase of active contraction.

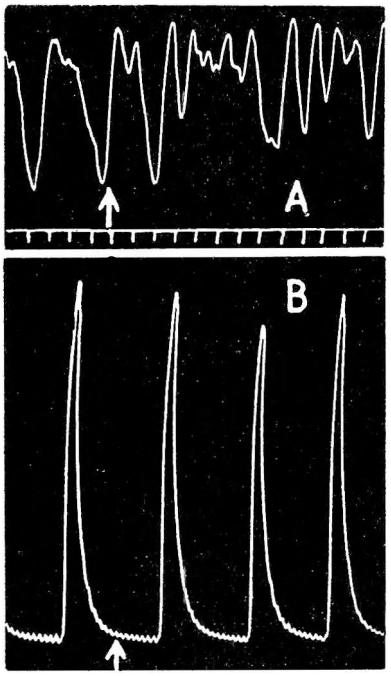

FIG. 4.—As in Figure1, 4A shows increase of tone during phase of tonus wave changes after adding morphine tartrate 10^{-6}. 4B shows the absence of effect after adding morphine 10^{-5} during the phase of active contractions.

With isolated strips in the present work varying effects were obtained. The quiescent muscle and the actively contracting muscle were not affected by morphine tartrate in concentration form 10^{-7} to 10^{-5} (see Fig. 4B). The muscle in tonus waves showed an increase in tone with concentration from 10^{-6} to 10^{-4}. No tetanic spasm was produced nor did the amplitude of the individual wave contractions increase (see Fig. 4A).

DISCUSSION

The statement that the fasting stomach is never entirely at rest is supported by the observation of activity of some sort in isolated strips of human gastric muscle. Hollow smooth muscle viscera with a variable content are seldom at rest. The stimulant action of parasympathomimetic compounds found in this work supports the findings of Smith[4] and Barron[12], and that of histamine agrees with the findings of Schenk[13] while disagreeing with Anderson and Morris[7]. The inhibition of isolated

gastric muscle by adrenaline is in agreement with Smith[4], Tezner and Turolt[5], Dickson and Wilson[14], Barron[12] and Anderson and Morris[7]. It would appear that adrenaline and acetylcholine and their synthetic analogues act in a similar manner upon the isolated gastric muscle as upon the intact stomach in man and in animals. Since histamine is used clinically to promote a flow of gastric juice its action on stomach movements is of some importance. According to Schenk[13] it increases gastric motility (roentgenological studies); according to Anderson and Morris[7] (viscerographic studies) it inhibits gastric activity. Histamine has a motor effect upon a wide variety of isolated preparations of smooth muscle, but not on all. Nevertheless it is unusual for the action to vary in any one species between *in vitro* and *in vivo* preparations of the same type of muscle, especially in the absence of anæsthesia. The dose used by Anderson and Morris[7] was 1·0 mg. per patient, which was less than that used by Schenk[13], who gave 6 to 8 mg. per patient. The latter dose may approximate more closely to that used in the present work (10^{-6} concentration). Both X-ray and viscerographic recording in intact human beings have certain weaknesses, not least of which is the possible introduction of autonomic activity in the patient following upon psychic disturbance as a result of hypodermic injection.

The action of atropine and other spasmolytic compounds is mainly that of inhibition of gastric motility as Bastedo[15] and Henderson and Sweeten[8] claim, but the finding of Danielopolu[2] and Anderson and Morris[7] that small doses of atropine may increase gastric activity is also supported. The nature of this activity is obscure. The differing effects of morphine found with the isolated muscle may help to explain the confusing effects reported by Tolley and Abbot[16], Myers[11] and Anderson and Morris[7].

The general conclusion reached was that the actions of the drugs in common use described were essentially the same in intact patients and in isolated portions of stomach muscle and that the differences observed could probably be explained by complexities in interpretation of recordings from the intact human being, and complexities due to modification of the action of the drugs by influences from the nervous system of the patient (psychogenic and otherwise), influences from the varying content of the viscera, and influences from varying doses and routes of administration of the drugs in clinical use. The beneficial effect of belladonna in the treatment of peptic ulceration may well be explained by the sedative effect of adequate doses of atropine on gastric motility and the blocking effect of this drug on the part played by vagal activity in gastric secretion.

Summary

1. Strips of longitudinal muscle were obtained from the anterior and posterior wall of the human stomach removed at gastrectomy for duodenal ulceration. The preparations were mounted in an isolated organ bath and movements recorded.

2. Spontaneous movement included periods of relative quiescence, periods of slow wave changes in tonus, and periods of active contraction. Periods of tetanic spasm could be induced by drugs.

3. Acetylcholine, carbaminoylcholine, eserine, barium and histamine stimulated the muscle, and adrenaline, ephedrine and amphetamine inhibited it.

4. Atropine, pavatrine, benadryl and neoantergan (2786 RP) inhibited spontaneous and drug induced contractions. Small doses of atropine (concentration of 10^{-7}) occasionally had a motor effect on the muscle strip.

5. Morphine had no effect on the quiescent or actively contracting muscle, but increased the tone of the slow wave of tonus change. Irregularity of spontaneous activity and response to drugs was a marked feature of the preparation.

This work was done during the tenure of an I.C.I. Fellowship in Pharmacology. It is desired to thank Prof. C. F. W. Illingworth and Mr. R. A. Jamieson, of the Peptic Ulcer clinic at the Western Infirmary, Glasgow, for the specimens of human stomach.

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THE EVALUATION OF TRAGACANTH BY MEANS OF THE APPARENT VISCOSITY DETERMINED IN A STANDARD U-TUBE VISCOMETER

PART II.—WHOLE GUM

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From the Laboratory of Damancy & Co., Ltd.

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IN A PREVIOUS COMMUNICATION[1] a method was suggested for the routine comparison of the apparent viscosities of powdered tragacanth by the use of a U-tube viscometer.

It was found that in order to use this type of instrument the sample of mucilage must be relatively homogeneous, a condition that was arrived at by the combined application of heat treatment and mechanical homogenisation. Using the U-tube viscometer it was not found possible to attain homogeneity by heat treatment alone without reducing the viscosity of the sample to figures that bore but little relation to the original viscosity; and as the object was to exhibit the gum at a point as near as possible to the maximum viscosity, both heat and mechanical treatment were found to be necessary. The problems involved in the evaluation of the whole gum are essentially those encountered in the powder, but modified to some extent by the intractable nature of the flake.

EXPERIMENTAL

An examination of a number of commercial samples of whole gum showed great diversity in size, thickness, texture and colour; while attempts to prepare mucilages from flake, without preliminary treatment and within those limits which are known to be without deleterious action on the viscosity, resulted in complete failure. That mucilages can be prepared from whole gum by treatment with boiling water or by immersion in a water-bath over a sufficiently long period is, of course, recognised, but as previous experiments show, such a method of ensuring homogeneity does not yield comparable results when determinations are carried out in a U-tube viscometer. It has long been recognised that the quality of tragacanth is adversely affected when the whole gum is reduced to powder by the customary commercial methods, and this has usually been thought to be due to mechanical destruction or to the heat created during grinding; but whatever the cause, it is advisable that both these attributes to a reduction of viscosity be maintained at the lowest level.

The method as finally adopted consists of "kibbling" in a mortar a suitable quantity of a representative sample of the flake until it passes a No. 30 sieve, after which the sample is thoroughly mixed. Attempts at preparing mucilages from this treated material, although an advance over the whole gum, resulted in products which, even after standing

for several days, showed large aggregations of undispersed gum. The problem of effecting "solution" of the gum was finally solved by the use of a mechanical stirrer. A simple Pyrex all-glass stirrer operated by compressed air was found to be very satisfactory. The addition of alcohol prior to the addition of water serves to keep the particles of gum sufficiently separated until the stirrer can be introduced into the flask. In the absence of alcohol, aggregates of gum are formed which are not usually dispersed by subsequent stirring. Fairly good dispersion is usually effected in less than one hour's stirring.

It soon became obvious that the employment of the same technique as that used for the powder, namely, allowing to stand at room temperature for 48 hours, would not result in full hydration of the gum. In addition, most samples contained a proportion of woody debris in amounts that would probably block the jet of the homogeniser. The mucilage, therefore, after standing for about 24 hours, was "cleaned" by passing it twice through a No. 100 sieve by means of reduced pressure. It was usually necessary to assist the mucilage through the sieve by means of a very small stencil brush, finally removing any mucilage adherent to the under surface of the sieve by means of a spatula. For this purpose it is desirable to use a piece of apparatus which can easily be dismantled and a suitable combination may be made from a Phœnix filter funnel, diameter $3\frac{1}{2}$ inches, and a Sifting Investigator outfit carrying B.S.S. sieves of $3\frac{7}{8}$ inches diameter. With a view to accelerating the rate of hydration and studying the effect of maintaining mucilages at different temperatures, a series of samples were stored in an oven operating at controlled temperatures. Table I shows the effect of such treatment on four different gums.

TABLE I

THE EFFECT ON THE VISCOSITY (TIMES OF FLOW IN SEC.) OF 0·5 PER CENT. MUCILAGES, PREPARED FROM WHOLE GUM, OF MAINTAINING THEM AT VARIOUS TEMPERATURES

Period	48 hours	24 hours	48 hours	48 hours	48 hours	48 hours
Temperature °C.	Room	40°	40°	50°	60°	70°
1	243, 283 258	272, 260 342	351, 355, 374 422, 367, 361	447, 451, 450 478, 439, 430	421, 408, 412 418, 423, 439	189, 246, 171 237, 210
2	2500	2734	2520, 3022	3300, 2820	1805, 1735	925
3	3840	3720	5015, 4800	3670, 4080	2440, 2930	813
4	1735	2160	2280, 2290	2100, 2040	1680, 1765	814

From these experiments it emerges that, in general, using a U-tube viscometer, maximum hydration is not attained by allowing the mucilage to stand at room temperature for 48 hours. The viscosity is seen to rise until a maximum is reached around 40°C., above which a falling off occurs, while a temperature of 70°C. has a very marked effect in reducing the viscosity. It may reasonably be assumed, therefore, that in employing an oven temperature of 40°C. for 48 hours, the hydration

is thereby accelerated to an approximate maximum and at the same time such temperature is without undue significance in its destructive effect on the viscosity.

As the strengths of the mucilages made from whole gum were the same as those previously used in experiments on the powder, namely, 0·5 per cent., it was at once apparent in the samples so far examined that the viscosities obtained when using whole gum were far higher than those encountered in the case of the powdered drug. Using a No. 3 U-tube viscometer (K = 0·306) it is unusual, at a strength of 0·5 per cent., to find times of flow for the powdered gum in excess of 1000 sec., yet in the case of whole gum times of the order of 2000 to 5000 sec. are quite common.

It would thus appear that the whole gum is a far superior article, as judged by apparent viscosity, than the generally available commercial powder. The knowledge that the gum yields a product of inferior quality when ground leads naturally to the assumption of different standards for the two forms; and this fact renders the use of the U-tube viscometer more rational than otherwise would be the case. In Table II are recorded a few results showing the figures for times of flow of mucilage of whole gum, when partly ground and when fully ground, and although the complete history of the gums was not fully known, the figures nevertheless suggest that a reduction of viscosity may be expected on grinding.

TABLE II

THE EFFECT OF GRINDING ON THE VISCOSITY (TIMES OF FLOW IN SEC.) OF 0·5 PER CENT. HOMOGENEOUS MUCILAGES PREPARED FROM WHOLE GUM, PARTLY GROUND AND FULLY GROUND GUM

Sample	Whole Gum	No. 80 Powder	No. 140 Powder
1	1657	1302	840
2	300	205	121
3	167	172	98
4	2830	—	1830
5	2760	—	1962
6	286	—	183

Strengths of mucilage that are applicable to the powder are, when applied to the whole gum, found to yield times of flow that are altogether too time-consuming for routine comparisons, and it is necessary to reduce the strength of the flake to yield figures that are comparable to those obtained when using the powder. A reduction in weight from 1·0 g. to 0·75 g. results in times of flow for the flake which are of the order of those obtained for the powder. The effect of reducing the strength of the mucilage is seen to be roughly proportional over the range of concentrations examined (Table III).

Method.—Kibble a representative sample of whole gum in a mortar until it passes a No. 30 sieve. To 0·75 g., accurately weighed, contained in a 300-ml. conical flask, add 5 ml. of alcohol (95 per cent.) followed

by 200 ml. of water, swirling the contents of the flask during the addition. Immerse the flask up to the neck in a water-bath maintained at from 40° to 50°C., insert a suitable mechanical stirrer and stir at a speed sufficient to maintain movement of the particles for about one hour. Remove from the bath, stopper the flask and place for a period

TABLE III

THE EFFECT OF VARIATION IN THE PERCENTAGE STRENGTHS OF GUM ON THE VISCOSITY (TIMES OF FLOW IN SEC.)

Concentration	0·25 per cent.	0·33 per cent.	0·375 per cent.	0·50 per cent.	Ratios
1	163	455	694	3000	1 : 2·8 : 4·3 : 18·4
2	165	460	838	3700	1 : 2·8 : 5·1 : 22·6
3	129	296	525	2300	1 : 2·3 : 4·1 : 17·8

of 24 hours in an oven maintained at 40°C. Attach a reflux condenser and immerse the flask up to the neck in a bath of boiling water for 5 minutes, swirling the contents of the flask vigorously for 5 seconds at the end of the first, second, third and fourth minutes. Remove from the source of heat and allow to cool to about 40°C. with occasional shaking and replace in the oven for 3 or 4 hours. Remove from the oven and by means of reduced pressure pass the contents of the flask as completely as possible through a No. 100 sieve twice. Replace in the oven until about 48 hours have elapsed from the time of commencement of the assay, after which pass the mucilage 3 times through a Q.P. homogeniser using a uniform and even pumping speed of about one complete stroke per sec. Determine the apparent viscosity (time of flow in seconds) at 20°C. in a No. 3 B.S. U-tube viscometer. The results of the application of this method to a number of samples of flake tragacanth are recorded in Table IV.

TABLE IV

THE RESULTS OF THE EVALUATION OF WHOLE GUM SAMPLES BY DETERMINATION OF THE APPARENT VISCOSITY (TIMES OF FLOW IN SEC.) IN A NO. 3 B.S. U-TUBE VISCOMETER

Sample	A	B	C	D	E	F	G	H	I
	637	709	45·0	126	377	572	740	145	657
	661	645	44·0	132	370	600	805	143	674
	665	690	46·0	129	382	579	827	142	702
	623	660	45·2	126	373	566	785	142	730
	643	675	45·5	131	350	576	720	144	712
	657	730	45·1	120	390	586	790	141	700
Mean	648	685	45·1	127	374	580	778	143	696
Standard deviation per cent.	± 2·5	± 4·6	± 1·47	± 3·4	± 3·7	± 2·1	± 5·2	± 1·1	± 3·8

Results of viscosity determinations on whole gum are seen to be considerably more variable than those on the powdered drug, and this is thought to be caused by a more variable rate of hydration resulting from the use of a kibbled product, which presents a surface to the hydrating medium that is only a fraction of that presented by a fairly uniform fine powder; in addition to the methodic errors the operative errors are also greater.

SUMMARY

1. A method is described for the routine comparison of the apparent viscosity of whole gum.

2. When determined in a U-tube viscometer, mucilages prepared from whole gum reach maximum hydration, and therefore maximum viscosity, after storage for 48 hours at a temperature of 40°C. With shorter periods at lower temperatures hydration is not complete and the viscosity is below the maximum, while with higher temperatures over the same period, although hydration is complete, the viscosity is reduced by virtue of the destructive effect of the higher temperatures.

3. At the same concentration, mucilages of whole gum yield higher viscosities than when commercially powdered, thereby indicating a loss of quality mainly due to grinding.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***l-nor*-Ecgonine, Complete Extraction of the Bases and Acid Esters from Coca Leaves.** A. W. K. de Jong. (*Rec. Trav. chim. Pays-Bas*, 1948, **67**, 153.) A considerable quantity of a number of bases has been extracted for quantitative determinations, from coca leaves at 55°C. with a mixture of benzene, methyl alcohol and N/1 ammonium hydroxide, but some of these are not ecgonine alkaloids. Since the ecgonine alkaloids are soluble in ether, the benzene and methyl alcohol extraction was replaced by ether extraction in a Soxhlet apparatus, but this method too had its disdavantages. When it was proved that coca leaves contain *l-nor*-ecgonine, which could be converted by methylation into *l*-ecgonine, and thus was of value for preparing cocaine, a return to the benzene and methyl alcohol extraction at ordinary temperature, to prevent decomposition of the methyl ester, was indicated. A smaller quantity of the bases was extracted at ordinary temperatures than at 55°C. and it was necessary to add water to the menstruum, care being taken not to add more than was required to dissolve the salts of the bases. For the extraction now proposed, coca leaves, 20 g., are mixed with finely powdered calcium oxide, which rapidly absorbs all the water contained in the leaf tissue, and dried for 24 hours. They are then shaken with 100 ml. of anhydrous benzene, 5 ml. of absolute methyl alcohol, and a volume of ammonia solution, containing exactly 1·938 g. of water. After another 24 hours, a quantity of finely powdered calcium oxide, corresponding to the amount of water used in the ammonia solution, is added, and after thoroughly mixing, the extractor is closed and the mixture allowed to stand. The calcium oxide absorbs the water and the calcium hydroxide formed sets free the ammonia and the bases, while the acid esters of *l-nor*-ecgonine are converted into their calcium salts; decomposition of the alkaloids is thus prevented. Percolation is started after 24 hours at a slow rate (about 5 drops per minute), and when the solvent mixture has been used, a quantity of a mixture of anhydrous benzene and absolute methyl alcohol, which boils at 58°C., is added to the leaves. The percolation is stopped when 800 ml. of percolate has been collected, and the benzene-methyl alcohol mixture distilled at 58°C. The remaining benzene is filtered from the calcium salts, which are insoluble in benzene. The residue is shaken with 25 ml. of N/5 hydrochloric acid, and 100 ml. of ether and the quantitative determination of the bases (by titration, using methyl red solution as indicator), of the cocaines (using N/1 sodium carbonate), and finally of *l-nor*-ecgonine is carried out on the hydrochloric acid layer. L. H. P

ANALYTICAL

Apparent Density of Dry Powders, a Method for the Determination of. W. B. Ault. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 313.) 40 g. of precipitated calcium carbonate is rubbed through a 30-mesh sieve and transferred, without jolting or shaking, to a 250 ml. glass measuring cylinder. The powder occupies a volume V_0, depending upon its lightness or bulkiness, but it is difficult to obtain reproducible results for this quantity. The cylinder is fitted with a bung, placed in a box and allowed to drop through

1 inch, 50 times at intervals of 2 seconds. The powder then occupies a volume V_d, which is reproducible to within $\pm$ 1 ml. in 150 ml., giving an apparent density (W/V_d) variation of $\pm 0{\cdot}002$ g./ml., using apparatus of specified dimensions. The dropping interval of 2 seconds is the most rapid procedure that can conveniently be carried out. There is no advantage in using a sieve finer than 30-mesh, and there will be a greater tendency for shearing to alter the apparent density. Increasing the height or number of times the cylinder is dropped lessens the difference in V_d for different grades of powder. The test can be modified for use with other powders, such as light magnesium carbonate, diatomaceous earth, chalk and barium carbonate. G. B.

Jaborandi, Assay of. Report No. 5 of the Poisons Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts. (*Analyst*, 1948, **73**, 311.) In the method recommended, powdered jaborandi leaves made alkaline with dilute solution of ammonia, are extracted by percolation with chloroform; this is continued until complete extraction is effected as shown by a test with Mayer's reagent. The alkaloids are extracted from the bulked chloroform solutions using 0·1N sulphuric acid and the resulting acid extract, after making alkaline with ammonia, is finally extracted with successive quantities of chloroform. The chloroform extracts are combined, evaporated, dried, dissolved in a standard excess of 0·05N sulphuric acid and titrated with 0·05N sodium hydroxide. The result obtained expresses the total alkaloid content calculated as pilocarpine. Details of the extraction procedure and a method for separating pilocarpine nitrate from the total alkaloids are given. R. E. S.

Morphine and Apomorphine, Determination of, by a Volumetric-Colorimetric Method. A. Ionesco-Matiu, J. Popa and L. Monciu. (*Ann. pharm. Franc.*, 1948, **6**, 25.) In a 200-ml. conical flask, place a known volume of the morphine solution, 0·5 to 2 ml., and evaporate to dryness on a water-bath; to the residue add 2 ml. of concentrated sulphuric acid, and heat to boiling on a water-bath for 30 minutes; cool, and add with shaking, 100 ml. of distilled water, and neutralise to sodium, using one drop of phenolphthalein solution. Add to the solution 5 drops of a saturated solution of corrosive sublimate and 5 drops of a 10 per cent. solution of sodium acetate; heat to boiling for one minute, when the solution changes from colourless to green, through violet to an intense blue. Cool the solution under a current of water, add 2 ml. of 20 per cent. sulphuric acid and, using a micro-burette, add drop by drop solution of N/10 potassium permanganate, until the solution has changed to a yellowish-brown colour, the permanganate solution maintaining its colour for several seconds; each ml. of N/10 potassium permanganate is equivalent to 0·00495 g. of morphine hydrochloride. The same method may be employed for the determination of apomorphine, commencing with the words " Add to the solution 5 drops . . . . "; each ml. of N/10 potassium permanganate is equivalent to 0·00294 g. of apomorphine hydrochloride. The determination of either morphine or apomorphine may be made even in the presence of other alkaloids. S. L. W.

Plant Extracts, Identification of. G. di Bacco. (*Boll. chim-farm.*, 1948, **87**, 124.) Extracts of medicinal plants can be identified and their strength and purity established by chromatographic tests. Aluminium oxide is used as the medium, in a glass tube 15 mm. in diameter and 180 mm. long, drawn off to a point so that the liquid can flow out at the rate of

30 drops a minute. The lower end of the tube is plugged with cotton wool and about 10 g. of aluminium oxide suspension in light petroleum is poured in; the column of oxide should occupy 90 mm. When the light petroleum has flowed out, leaving a layer 8 or 10 mm. above the oxide, the solution or suspension of the extract to be examined is poured on, allowed to pass through, and the chromatogram developed by washing with two lots of light petroleum. For liquid extracts of rhubarb, cascara and frangula, 1 g. of the extract is shaken from time to time during 30 minutes with light petroleum 10 ml., benzene 10 ml., ether 5 ml. and the clear supernatant liquid is poured on the oxide. The liquid extract of genuine rhubarb of the Italian Pharmacopœia gave a 2 mm. yellow band, an 8 mm. red band, a 10 mm. pink fringe; a liquid extract of genuine rhubarb soluble in syrup gave a 1 mm. yellow band, a 6 mm. red band, a 12 mm. pink fringe; a liquid extract of European rhapontic rhubarb gave a 0·5 mm. yellow band, a 12 mm. pink fringe; a mixture of equal parts of genuine and rhapontic liquid extracts gave a 0·5 mm. yellow band, a 3 mm. red band, a 12 mm. pink fringe. Evidently the red band is due to anthraquinone derivatives, and shows the rhapontic rhubarb to be inferior to the Chinese and the soluble extract inferior to the official. The liquid extract of cascara of the Italian Pharmacopœia gave a 0·5 mm. yellow band, a 1 mm. red band, a 3 mm. bright red band, a 16 mm. pink fringe (the column viewed from above is coloured yellow). The aromatic, bitterless extract of cascara of the Italian Pharmacopœia gave a similar chromatogram with a more accentuated yellow band; liquid extract of frangula gave a 2 mm. bright red band and a 4 mm. pale pink fringe. To verify an extract, the chromatogram should be compared with one obtained from an extract of proved authenticity and the activity may be judged to be proportional to the length of the coloured bands. H. D.

FIXED OILS, FATS AND WAXES

Whale Oil, Component Acids and Glycerides of. T. P. Hilditch and L. Maddison. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 253.) The component acids of Antarctic whale oil have been previously separated by means of their lithium and lead salts, with subsequent analysis by ester fractionation. They have now been re-examined by the more recent process of crystallisation from solvents at low temperatures. This method is easier and quicker and gives results agreeing with the earlier. The results are given below.

COMPONENT ACIDS OF ANTARCTIC WHALE OIL

	By lithium and lead salt separations	By low-temperature crystallisation	
		(*a*)	(*b*)
	per cent. (wt.)	per cent. (wt.)	per cent. (wt.
Lauric	0·2	Trace	0·3
Myristic	9·3	9·2	9·3
Palmitic	15·6	15·6	15·6
Stearic	2·8	1·9	2·3
Arachidic	0·3	0·6	0·2
Unsaturated C_{14}	2·5	2·5	2·6
,, C_{16}	14·4	13·9	13·8
,, C_{18}	35·2	37·2	36·9
,, C_{20}	13·6	12·0	12·2
,, C_{22}	5·9	7·1	6·8
,, C_{24}	0·2	—	—

The component glycerides have also been segregated by crystallisation from acetone at $-60°C$. upwards. The following figures are similar to previous results, though differing in some respects: about 16 per cent. of disaturated and 2·5 per cent. of trisaturated glycerides, about 30 per cent. of tri-unsaturated glycerides, and about 50 per cent. of glycerides containing one saturated acid, one unsaturated C_{18} acid, and one of the other homologous unsaturated acids. About 45 per cent. of the oil contains acids of the C_{20} and C_{22} series, and oleic groups are present in over 90 per cent. of the oil. H. F.

INORGANIC

Sulphur Absorbed by Clays, Chemical Activity of. A. Malquori. (*Ann. Chim. appl., Roma*, 1948, **38**, 146.) The chemical activity of the sulphur absorbed by clays when heated with them was tested by boiling a weight of the clay containing 0·5 g. of sulphur with 0·5 g. of calcium hydroxide and 100 ml. of water and boiling for exactly 1 minute, cooling rapidly away from air and determining the sulphur in 10 ml. by oxidation and conversion to barium sulphate. If these sulphurised clays are kept in a moist atmosphere the sulphur rapidly becomes insoluble in calcium hydroxide; the loss in solubility varies for different clays from 8 to 45 per cent. in 14 days. The author connects this behaviour with the hygroscopic water. H. D.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Anti-pernicious Anæmia Factors from Liver, Purification of. E. Lester Smith. (*Nature*, 1948, **161**, 638.) This is a report of the preparation from ox liver of two pigments, both highly active in pernicious anæmia. The crude extract was purified by the methods employed by Emery and Parker (*Biochem. J.*, 1946, **40**, iv) and then by repeated chromatography; subsequently, proteolysed liver extract was used as the starting material and gave better yields of a product with a higher activity. Four tons of material yielded barely 1 g. of the red material from 6 separate lots of liver. The minimum effective single dose for the best 4 preparations from non-proteolysed liver was assessed at about 0·6 mg., proteolysed liver yielding 1 preparation (L.E.445) effective at 0·3 mg. Ten batches of red material all proved clinically active (in 26 cases), and it was found that activity and colour are inseparable. Further chromatographic purification of one of these preparations, following the action of mixed bacteria, gave a product with 8 times the colour intensity of L.E.445, which, if activity remains proportional to colour, should be effective at 0·04 mg. The products are amorphous solids, with the colour of cobalt salts, but showing only general absorption of visible and ultra-violet light, and with molecular weights, as indicated by the diffusion method, of about 3,000 for the pigments from both proteolysed and non-proteolysed liver. On exposure to daylight there is a gradual change in colour from red to orange, accompanied by a marked change in chromatographic behaviour. The products are exceedingly soluble in water, soluble in nearly anhydrous alcohol, acetone, and glacial acetic acid, but insoluble in ether, chloroform and non-polar solvents. The author concludes that the two pigments are differing forms of the classical liver fraction first postulated by Minot and Murphy, and are not an incomplete substitute, such as folic acid or thymine. True pernicious anæmia and its associated neurological disturbances do not require a multiplicity of factors but respond to a single

factor. With an effective dose equivalent to some 20 mg. (and possibly only 2·5 mg.) daily, this is one of the most potent of known physiologically active substances. S. L. W.

Œstrogens, Preparation from Urine by Application of High Temperatures. Felix Sulman. (*Nature*, 1948, **161**, 605.) The usual methods of extracting œstrogens from the urines of pregnant women, pregnant mares, or stallions, with solvents which are not miscible with water, have certain inherent disadvantages. Large extraction vessels and large volumes of urine are required and this involves using a large distillation apparatus and consequently high fuel consumption for evaporation of the solvent; in addition the œstrogens are usually contaminated with organic material. In overcoming these difficulties it was found that the urine could be evaporated till a sticky, gum-like residue remained; this was heated to a temperature not exceeding 245°C. for 5 minutes so as to carbonise the bulk of the organic matter. The œstrogens, such as œstradiol, œstrone, œstriol, hippulin, equilin and equilinin were not destroyed by this procedure. The carbonised mass was extracted with organic solvents and the extract, containing the œstrogens in rather high concentration, was evaporated. The dry residue obtained possessed a high degree of purity, since most organic impurities had been carbonised, and was further purified by the usual methods. L. H. P.

Stilbœstrol Monoglucuronide, Isolation from Human Urine. K. S. Dodgson and R. Tecwyn Williams. (*Nature*, 1948, **161**, 604.) It has been claimed by Wilder Smith (*Nature*, 1947, **160**, 787) that 50 per cent. of stilbœstrol administered to human beings is excreted as a monoglucuronide. The authors were able to isolate the benzylamine salt of stilbœstrol monoglucuronide in a pure crystalline state from the urine of two women, immediately after parturition. These patients had received a total of 100 mg. of stilbœstrol each in 24 hours. The benzylamine salt had m.pt. and mixed m.pt. 223°C. and was lævo-rotatory with $[\alpha]_D^{20°C} = -55°$ ($c = 0{\cdot}2$ in 50 per cent. aqueous acetone). It appeared identical with a sample previously prepared from pure stilbœstrol monoglucuronide isolated from rabbit urine. The yield of the benzylamine salt corresponded to 35 per cent. of the stilbœstrol which had been administered. In addition to the monoglucuronide, small amounts of free stilbœstrol were also detected in these urines. L. H. P.

***o*-Thymotinic Acid, Preparation and Inhibitory Properties of Derivatives.** J. P. Street, C. E. Georgi and P. J. Jannke. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 180.) o-Thymotinic acid (1-methyl-2-carboxy-3-hydroxy-4-*iso*propylbenzene) is structurally related to thymol and salicylic acid. Prepared by treating a solution of thymol in boiling xylene with metallic sodium and dry carbon dioxide at atmospheric pressure, it was obtained in a yield of 71.1 per cent, as colourless, needle-like crystals, m.pt. 126°C., soluble in organic solvents. The preparation of the mono- and di-sodium, silver, magnesium, calcium, barium, mercuric, zinc, cupric, lead, ferrous, ferric, aluminium and bismuth salts, and the mono- and di-hexamine complexes is described. The determination of their phenol coefficients, using *Staphylococcus aureus* at 37·5°C., showed the silver and mercuric salts to have the greatest activity. Fungistatic activity was investigated using the cup-plate technique and five organisms. The mono- and di-hexamine complexes showed the greatest activity against *Epidermophyton floccosum*, *Microsporum canis* and *Tricophyton purpureum;* the magnesium salt also had a considerable effect on the growth of these organisms. The mercuric

salt was the most effective against *Tricophyton mentagrophytes* and also had a considerable effect on *E. floccosum* and *T. purpureum. Candida albicans* was the most resistant of the organisms studied, only the bismuth and magnesium salts having any inhibitory effect. The silver, zinc, ferrous, ferric and lead salts had no fungistatic activity against any of the organisms.

G. R. K.

Trichothecin: an Antifungal Metabolic Product of *Trichothecium roseum* Link. G. F. Freeman and R. I. Morrison. (*Nature*, 1948, **162**, 30.) The substance responsible for the antifungal activity in cultures of *Trichothecium roseum* has been isolated in crystalline form in yields of 20 to 30 mg./l. The name "trichothecin" is suggested for the active substance; after extraction with ether or chloroform from the culture filtrate it was purified chromatographically. From light petroleum it crystallised as colourless needles m.pt. 118°, $[\alpha]_D^{18°C.}$ +44°. On the basis of microanalytical results the formula $C_{19}H_{18}O_4$ is suggested. The compound is neutral, only slightly soluble in water, contains one ketonic and one ethylenic group and the results suggest three $CH.CH_3$ groups. The antifungal activity of this compound is shown against Fungi Imperfecti, Zygomycetes and Ascomycetes. Aqueous solutions of trichothecin were stable at *p*H 1 to *p*H 10 for at least 48 hours at 20°C. At *p*H 12 the antifungal activity was rapidly destroyed even at room temperature. R. E. S.

BIOCHEMICAL ANALYSIS

Cup-Plate Method in Microbiological Assay, with special reference to Riboflavine and Aneurine. A. L. Bacharach and W. F. J. Cuthbertson. (*Analyst*, 1948, **73**, 334.) An assay procedure is developed which follows the principles of the cup-plate assay of penicillin. The medium is made deficient in the single substance to be assayed and the organism chosen is one that will not grow in its absence. Solutions containing the missing substance are put into the cup and after incubation, a "zone of exhibition" is shown, the diameter of which may show a relation to the concentrations of the added nutrient. Conditions are described for producing sharply defined zones of growth together with the results of investigations into the relations between inoculum density, concentration of test solution and diameter of growth zone. Details of the procedure for the determination of riboflavine and aneurine are given. The relative insensitivity and potentialities of the method are discussed. R. E. S.

Histamine, an Improved Colorimetric Method for the Estimation of. S. M. Rosenthal and H. Tabor. (*J. Pharmacol.*, 1948, **92**, 425.) Attempts to utilise the diazo reaction given by imidazole compounds for the estimation of histamine have shown it to be unsatisfactory because of lack of specificity, instability of colours and because of numerous substances in biological extracts which inhibit or interfere. To overcome these difficulties and extend the sensitivity of the test, the authors compared a large number of aromatic amines and finally selected the diazonium salt of 4-nitroaniline as the most satisfactory. The coloured azo compounds formed by 4-nitrodiazobenzene in alkaline solution are extracted with an organic solvent, which concentrates and stabilises the colour. At a suitable *p*H, using certain solvents, the azo compounds of most interfering substances either remain in the aqueous phase or pass into the solvent, methyl *iso*butyl ketone, with a yellow or amber

colour, while that of the histamine goes into the solvent with a rose colour. Ammonia reacts like histamine to give a rose colour in the solvent, but on shaking the solvent with a barbitone buffer of *p*H 7·7 the rose colour obtained with histamine or acetylhistamine is intensified, while that of ammonia and other interfering substances is abolished or changed to a pale yellow. A method for overcoming the effects of inhibitory substances, including those in liver extracts and urine, is described; preliminary results on various tissues and with histamine indicate a satisfactory degree of specificity for the method, which has a sensitivity of approximately 0·5 mg. S. L. W.

Penicillin, Determination of, by Alkaline Hydrolysis. Stella J. Patterson and W. B. Emery. (*Analyst*, 1948, **73**, 207.) The alkaline hydrolysis method has been modified for routine assays on a large number of samples of solid penicillin having a high potency. Several indicators, singly and in combination, were tried, to avoid the use of a *p*H meter; cresol red, which gives a sharp colour change at the required *p*H even in the presence of the yellow pigment of commercial penicillin, was finally chosen. For the assay, 0·1 g. to 0·2 g. of penicillin, accurately weighed, was dissolved in 50 ml. of distilled water, previously boiled for 15 minutes to remove carbon dioxide, and cooled in a flask fitted with a soda-lime tube. 1·0 ml. of 0·1 per cent. neutral solution of cresol red in alcohol (70 per cent.) was added and N/10 sodium hydroxide run in slowly from a micro-burette, delivering drops of from 0·02 to 0·03 ml., until a red colour was obtained; a further 10 ml. of N/10 sodium hydroxide was added, the flask was stoppered with a rubber bung and the mixture was left for 3 hours at room temperature. 10 ml. of N/10 hydrochloric acid was then added, and the excess of acid was immediately back-titrated with N/10 sodium hydroxide, till the indicator changed to the original red colour. The difference between this reading, and a blank, carried out in exactly the same manner but omitting the penicillin, multiplied by 59,340 gives the total number of I.U. in the sample, and the I.U./mg. can then be calculated. The factor 59,340 applies only to salts of penicillin G; quantities of penicillin K, or of other penicillins, which are present in commercial samples, will affect the accuracy of the results. This method has been used to investigate the stability of penicillin. There are several limitations to the method ; it cannot be used to assay the official ointment (500 I.U./g.) or lozenge (500 I.U.) and is applicable only for powders containing more than 900 I.U./mg. Also samples which showed no biological activity still indicated considerable potency when assayed chemically, and it is, therefore, necessary to confirm the results by biological methods from time to time. For penicillin in oil and beeswax the penicillin is separated by extraction which dry anæsthetic ether. The authors also describe an alternative procedure, using α-naphthophthalein as indicator. L. H. P.

Penicillin G in Small Broth Samples, Estimation of. J. A. Thorn and M. J. Johnson. (*Anal. Chem.*, 1948, **20**, 614.) The method described is based on the fact that, on a column of Super Filtrol (an acid-treated bentonite), penicillin G is more strongly adsorbed under given conditions than any other known penicillin and may be eluted as a separate fraction. The method is particularly applicable to fermentation broths and is not affected by the number of types of penicillins occurring in the sample to be analysed. The method is not intended for use on purified samples, for which the more accurate physical and chemical methods are available. A broth liquid adjusted to *p*H 4·6 with 50 per cent., phosphoric acid is used, diluted if necessary with 0·05M potassium monobasic phosphate so as to contain between 50 and 200 units of penicillin

per ml. The column is washed through with a phosphate buffer solution adjusted to *p*H 6·1, the elution of the penicillin occurring in the following sequence: first, X and dihydro F; second, F; last, G. Penicillin K appears to be largely if not entirely inactivated upon adsorption. The average recovery of adsorbed penicillin G varied from 75 to 90 per cent., depending on the adsorbent. A factor is thus needed for a particular adsorbent; this is obtained by adsorbing known amounts of penicillin G. The application of aqueous chromatography to resolution of mixtures of the other known penicillins is described as well as the effect of other acids as eluents. R. E. S.

Pregnanediol, Rapid Method for Estimation of. J. Rabinovitch. (*Nature*, 1948, **161**, 605.) Pregnanediol can be detected rapidly by a method using zinc dust to protect the hormone during acid hydrolysis, and to prevent discoloration which would affect the final colour reaction. 1·5 g. of zinc dust is added to 100 ml. of urine and the mixture is heated to boiling-point, when 10 ml. of concentrated hydrochloric acid is added and the mixture boiled for 5 minutes. The flask is immersed in cold water and the zinc allowed to settle. The supernatant liquid is poured on to a sand column, and the residue of zinc is washed successively with 25 ml. of N/1 hydrochloric acid, 25 ml. of N/10 hydrochloric acid and three quantities each of 25 ml. of water; the washings are poured on to the sand column, and the column is dried by sucking hot air through it. The residual zinc is shaken with three quantities, each of 20 ml. of hot alcohol (95 per cent.), and the hot extract is decanted on to the sand column. The alcoholic extract is passed rapidly through the sand and the filtrate is evaporated to dryness. The residue is dissolved in a mixture of 5 ml. of alcohol (95 per cent.) and 20 ml. of N/10 sodium hydroxide, and allowed to stand in the cold for 1 hour. A precipitate, which contains the pregnanediol fraction, is formed, and is separated by filtration through the sintered glass, washed with water and dried. It is extracted with 10 ml. of hot alcohol, the solution may be used for the Gutterman colour reaction. In this the solution is evaporated and the residue dissolved in 5 ml. of concentrated sulphuric acid, a deep yellow or orange colour quickly develops if more than 0·5 mg. of pregnanediol was present in the original 100 ml. of urine. A negative result is shown by a colourless or pale yellow solution. The method may also be used for quantitative estimations by using larger quantities of urine and gravimetric methods. The sand-zinc method gives results of practically the same order as the quantitative method of Astwood and Jones, and is rapid and easy to carry out. L. H. P

Tryptophane, Methionine, Cystine and Tyrosine, A Modified Method for the Microbiological Assay of. E. C. Barton-Wright and N. S. Curtis. (*Analyst*, 1948, **73**, 330.) In this modified method, peptone is treated with hydrogen peroxide to destroy these 4 amino-acids and the resulting product is substituted in the basal medium for the usual series of individual amino acids. Details of the treatment of the peptone with hydrogen peroxide are given and the individual assay media are described. The organism recommended for the tryptophane assay is *Lactobacillus arabinosus* 17/5, the incubation temperature is 30°C. and the range of tryptophane to establish a standard curve is 2 to 10 μg. For the assay of L-methionine, L-cystine and L-tyrosine the organism used was *Leuconostoc mesenteroides* P.60. The range of L-methionine was 15 to 40 μg., that of cystine 5 to 35 μg., and that of tyrosine 10 to 50 μg., to establish a standard curve. The assay of DL-methionine was accomplished using *Lactobacillus fermenti* 36. Protocols of typical standard curves are given. R. E. S.

CHEMOTHERAPY

Curariform Activity of Certain Chondrodendrine Derivatives. D. F. Marsh, C. K. Sleeth and E. B. Tucker. (*J. Pharmacol.*, 1948, **93,** 109.) The authors compared the activity of *d*-N-methyl-chondrodendrine iodide and *d*-*o*-methyl-N-methyl-chondrodendrine iodide with *d*-tubocurarine chloride pentahydrate and *d*-*o*-methyltubocurarine iodide trihydrate in rats, rabbits, cats and man. All these compounds produce skeletal muscular paralysis and differ only in quantitative activity. *d*-N-Methyl-chondrodendrine is about one-half as active as the isomeric *d*-tubocurarine in rats and rabbits but only about one-fourth to one-eighth as active in cats and man. Although the *d*-*o*-methyl-N-methyl-chondrodendrine is about equipotent with *d*-tubocurarine, it is only one-sixth to one-eighth as active as its diasteroisomer, *d*-*o*-methyltubocurarine. S. L. W.

Curariform Activity of *iso*Chondrodendrine Derivatives. D. F. Marsh and M. H. Pelletier. (*J. Pharmacol.*, 1948, **92,** 454.) A comparison with *d*-tubocurarine chloride pentahydrate and *d*-*o*-methyltubocurarine iodide trihydrate in rats, rabbits and cats, showed *d*-N-methyl-*iso*chondrodendrine to be about a twentieth, and *d*-*o*-methyl-N-*iso*chondrodendrine about a fourth as paralysing as *d*-tubocurarine, which in turn is only about one-tenth as active as *d*-*o*-methyltubocurarine. Like the tubocurarine compounds, these *iso*-chondrodendrine derivatives have relatively little effect in intact animals other than lissive action on skeletal muscles. The authors obtained the *d*-*iso*chondrodendrine from *Pareira brava* by a modification of the method of King (*J. chem. Soc.*, 1940, 737) and prepared the derivatives as the iodide salts by the method of Dutcher (*J. Amer. chem. Soc.*, 1946, **68,** 419). S. L. W.

Curarising Properties of R.P.3697. R. Wien. (*Arch. int. Pharmacodyn.*, 1948, **77,** 96.) This compound, the triethyliodide of tri(diethyl-amino-ethoxy):1:2:3 benzene, was studied as a possible substitute for *d*-tubocurarine. Its curarising properties were assayed in comparison with *d*-tubocurarine by the rabbit head-drop method, the frog rectus abdominis preparation, the cat sciatic gastrocnemius preparation, and the rat, rabbit or kitten phrenic nerve-diaphragm preparations. The results of the assays showed that by the rabbit head-drop method it was one-third as active as *d*-tubocurarine, on the frog rectus abdominis preparation it was only one-twentieth as active, and on the rat phrenic nerve-diaphragm only one-eightieth as active; on the rabbit and kitten phrenic nerve-diaphragm, however, it was one-fifth as active. The curarisation effects were easily reversed by neostigmine or eserine. Compared with similar doses of *d*-tubocurarine there was no effect on blood pressure and less effect on respiration in rabbits anæsthetised with ether and thiopentone, in chloralosed cats and in decerebrate preparations. Unlike some other synthetic curarising compounds it compared very favourably with *d*-tubocurarine for its absence of anticholinesterase properties.

S. L. W.

Thio-antimonials, Organic, in Schistosomiasis. L. W. Clemence and M. T. Leffler. (*J. Amer. chem. Soc.*, 1948, **70,** 2,439). Oil-soluble substances of the general formula $(RS)_3Sb$ have been prepared, where R may be *n*-octyl, *n*-decyl, *n*-undecyl, *n*-dodecyl, *n*-tetradecyl, *n*-hexadecyl, *n*-octadecyl, β-phenylethyl, β-(1-naphthylethyl), β-(*p*-di-*iso*butylphenoxy-ethoxy)-ethyl, β-*cyclo*hexylethyl, ω-*cyclo*hexylamyl, ω-(β-tetralyl)-butyl, ω-(β-decalyl)-butyl, or β-(2-pyridyl)-ethyl. These substances are prepared by the

reaction of mercaptan and antimony trichloride in chloroform, and show some promise in preliminary experiments on schistosomiasis. During the investigation the following substances, not previously described, were synthesised and characterised: ω-(β-tetralyl)-butyl and ω-(β-decalyl)-butyl alcohols; β-*cyclo*hexylethyl, ω-*cyclo*nexylamyl, ω-(β-tetralyl)-butyl and ω-(β-decalyl)-butyl *iso*thiouronium bromides and mercaptans. G. B.

PHARMACY

DISPENSING

Folic Acid in Liquid Prescriptions. S. Scheindlin. (*Amer. J. Pharm.*, 1948, **120**, 103.) Folic acid is unstable to oxidation, reduction, acid, alkali, dry heat, acylation esterification, methylation, benzylation, nitrous acid, bromine, hypobromite, hydroxylamine, zinc dust and acetic acid (stable to acetic acid alone) and 1 per cent. hydrogen peroxide, and it is unstable to light but not destroyed by autoclaving in the dark. Folic acid is only very slightly soluble in water, but its sodium salt is water-soluble. An elixir of folic acid containing 5 mg. of folic acid in 4 ml. of a solution containing 10 per cent. of alcohol, artificial red colour, flavouring agents and preservatives. The folic acid was present as the sodium salt and the *p*H was 8·2. A series of mixtures with a wide range of commonly used medicaments was then prepared and stored in clear glass bottles exposed to electric light (but not to direct sunlight) for the first week, and then transferred to a box where they were protected from light. The mixtures were examined for precipitation, colour change, liberation of gas, or change of *p*H after 1 day, 1 week, and 1 month. Varying concentrations of alcohol, glycerin and propylene glycol produced no change in any of the solutions after one month. At and below *p*H 4·1 immediate precipitation of folic acid occurred; at *p*H 5·1 the mixture remained clear for over 24 hours, but at the end of 1 week some precipitation had taken place. No change was observed at any other *p*H. Mixtures of folic acid elixir with the following drugs showed a precipitate which could not be easily suspended or which contained a potent medicament, and the author recommends that such mixtures should not be prescribed:—phenobarbitone, chloral hydrate, tinctures of hyoscyamus, stramonium, nux vomica and digitalis, and quinine dihydrochloride; in addition, sulphadiazine was found to destroy folic acid activity very rapidly. S. L. W.

GALENICAL PHARMACY

Suppository Bases, Examination of Physical Characters of. P. Malangeau. (*Ann. pharm. Franc.*, 1948, **6**, 50.) The essential requirements of a suppository are: (1) that it should melt at a sufficiently low temperature to become liquid in the rectum (without being water-soluble), and (2) that it should offer sufficient mechanical resistance to enable its easy introduction. A simple method of determining the melting-point is as follows: when the melted mass is poured into the mould, a thin, polished metal rod is placed upright in the centre of the suppository cavity and maintained in that position until the suppository has set, so that when the mould is unscrewed the suppository is fixed on the end of the rod. The rod with the suppository attached is then placed in a water-bath containing 2 l. of water, the suppository being placed

at the same level as the bulb of a thermometer and immersed under not less than 3 cm. of water. The temperature of the water is then gradually raised, at the rate of 1° every 2 or 3 minutes, to 30°C., the water being stirred mechanically. The melting-point is taken when the mass of the suppository slides off the metal rod. For the determination of mechanical resistance, the author describes a simple apparatus, by means of which a solid cylinder of the suppository is subjected to varying degrees of vertical pressure at different temperatures. Experiments conducted with this apparatus, using cocoa butter, cocoa butter with the addition of propyleneglycol stearate, hydrogenised oil with stearates of propyleneglycol and triethyleneglycol, and hydrogenised oil with propyleneglycol stearate, showed that, whereas at ordinary temperatures the mechanical resistance of these four bases is fairly comparable, at higher temperatures cocoa butter loses its mechanical resistance much more quickly and is less likely to lend itself to the manufacture of suppositories containing a high percentage of liquid ingredients. S. L. W.

Tablet Disintegration Testing. V. M. Filleborn. (*Amer. J. Pharm.*, 1948, **120,** 233.) Tablets are immersed for a definite time in an artificial saliva bath, and enclosed in a plastic tablet container which is placed in a glass vessel containing artificial gastric juice, agitated by a pump and maintained at 37°C. Fresh artificial gastric juice is admitted by a drip-feed, and the excess allowed to flow out of the vessel. Particles of sterilised sponge may be added to simulate the presence of food. Disintegration is regarded as complete when the tablet is broken into pieces small enough to pass through the 1/16th inch holes of the plastic tablet container. Tests in which the disintegration of radio-opaque tablets, which have been swallowed whole, is observed in human subjects, show that the disintegration times obtained by the "artificial stomach" method are approximately the same as those in the human stomach. When phenobarbitone, ephedrine hydrochloride, mepacrine hydrochloride, sulphathiazole, sulphapyridine, sulphanilamide and sulphadiazine tablets are submitted to the disintegration tests of the Swiss Pharmacopœia and of the 7th Addendum to the British Pharmacopœia, 1932, the observed disintegration times are generally smaller than for the "artificial stomach" method and there appears to be no relationship between the results obtained by the three methods. G. B.

PHARMACOLOGY AND THERAPEUTICS

Amellin Ineffective in Diabetes. H. Whittaker. (*Brit. med. J.*, 1948, **1,** 546.) A mixture of amellin (an extract of *Scoparia dulcis*), calcium gluconate and lactose was given to 2 patients with diabetes in doses of 5 gr. (0·32g), by mouth, thrice daily for 3 months. One patient also received insulin and the progress of his disease was unaltered by the administration of amellin. The patient receiving amellin without insulin became progressively worse and heavy glycosuria and hyperglycæmia were constantly present. After 3 months' treatment with amellin, traces of ketone bodies were found, and the blood-sugar was 348 mg./100 ml. After doses of 16 units of protamine-zinc-insulin daily, the urine became sugar-free and blood sugar 4 hours after breakfast was 206 mg./100 ml. G. R. B.

Amidone (Methadon), Clinical Evaluation of. Elizabeth B. Trioxil. (*J. Amer. med. Ass.*, 1948, **137,** 920.) Amidone (physeptone) was administered by mouth, as capsules, tablets, or elixir, or by hypodermic or intravenous

injection. Onset of action occurred in 2 minutes after intravenous injection, 15 to 20 minutes after hypodermic injection or administration as elixir, and 30 minutes after administration as capsules or tablets; the average duration of action by all routes was 3 to 4 hours, but sometimes the effect lasted for 8 to 12 hours. Usually the hypodermic route was used, but the elixir was found to be equally efficacious and more suitable for prolonged use. When tested on a group of 400 patients showing all degrees of pain from a variety of clinical conditions, amidone gave complete and adequate relief to 81 per cent. in doses varying from 2·5 to 20 mg. Side-effects occurred in 13 per cent. of the patients and included nausea and vomiting, sedation (generally a slight drowsiness) and dizziness. When compared with morphine and pethidine on a group of 90 patients, the analgesic effect of 10 mg. of amidone was found to be equivalent to that of 15 mg. of morphine or 150 mg. of pethidine. No evidence of addiction was encountered in three patients who were treated with the drug for one year. Morphine, pethidine, " pantopon " and dihydromorphinone addicts experienced no withdrawal symptoms when the narcotic was replaced with amidone, or after the end of treatment. No contraindications were met with, but the routine use of elixir in patients with dysmenorrhœa was not encouraged because of the high incidence of nausea and vomiting. For the relief of obstetric pain, amidone is inferior to pethidine. G. R. K.

Arsenicals, an Improved Method for Assay of Toxicity. W. L. M. Perry (*Nature*, 1948, **161**, 975.) In a quantal response assay, each animal can contribute only a positive or a negative reading (in this case, death or survival), and in the event of death there is no indication whether the dose given was the exact minimum individual lethal dose, or whether it was considerably in excess. Thus, the method is wasteful of information, and only when a continuous variate such as survival time cannot be used is recourse to quantal response methods necessary. In the case of the arsenicals there seems to be no such difficulty. Using the survival time as the continuous variate it has been found possible to perform an assay with increased speed, accuracy, and economy in animals; a definite numerical estimate of the toxicity of the drug for that particular animal is obtained, and provided a linear dose-response relationship can be employed it is to be expected that more information will be gained per animal used. A series of experiments with neoarsphenamine, so designed that the methods of quantal responses and measurement of survival times could be compared, was carried out. The dose range in the latter case was chosen to ensure that all the animals treated should die, and that the longest period of survival should not exeed 10 hours. A linear relationship between the dose of drug and mean survival time was established by using logarithmic transformations. Statistical analysis of the results shows the graded response method to be accurate and unbiassed. The limits of error for the graded response assay were shown to be about half as wide as those for the quantal response assay, and the accuracy of the estimation of potency about 4 times as great.

S. L. W.

Atropine Poisoning, Acute. R. B. Welbourn and J. D. Buxton. (*Lancet*, 1948, **255**, 211.) A report of 9 cases of acute atropine poisoning, arising from a dispensing error, 1/6 grain of atropine sulphate being given by subcutaneous injection, instead of the 1/100 grain prescribed pre-operatively. All the patients were young men with septic conditions requiring minor operations, and the poisoning was not suspected until after operation. Only 4 of the patients showed toxic effects, namely, acute delirium and blurred vision,

and all recovered completely. In these 4 cases difficulty was experienced in anæsthetising with soluble thiopentone and nitrous oxide and oxygen. Of the remaining 5 cases, who showed no toxic effects and in whom no difficulty was experienced in producing anæsthesia, 3 were anæsthetised with trichloroethylene which has a more powerful and lasting depressive action on the central nervous system than nitrous oxide and oxygen or soluble thiopentone. S. L. W.

Aureomycin; Experimental and Clinical Investigations. M. S. Bryer, E. B. Schoenbach, C. A. Chandler, E. A. Bliss and P. H. Long. (*J. Amer. med. Ass.*, 1948, **38**, 117.) Aureomycin is supplied as the hydrochloride of an antibiotic from a strain of *Streptomyces aureofaciens*. It consists of yellow crystals, soluble in water giving a solution of *p*H about 4·5, and slightly less soluble in isotonic sodium chloride solution. Alkaline solutions are unstable. *In vitro*, 0·1 to 5·0 μg./ml. inhibits the growth of various Gram positive and Gram negative bacteria, but *Pseudomonas æruginosa* and strains of *Proteus* are unaffected by 20 μg./ml. Fifty times the concentration is required when 50 per cent. of human serum is present. Mice treated orally with 50 mg./kg. of body weight are protected against β-hæmolytic streptococci (C203), but not against *Klebsiella pneumoniæ* A. and *Diplococcus pneumoniæ* I (S.V.I.). In human patients, coli-aerogenes and *Streptococcus fæcalis* infections of the urinary tract are sterilised by 10 to 60 mg./kg. of body weight per day, orally. Favourable initial responses are obtained in patients with Rocky Mountain spotted fever, and with brucellosis, using 3 mg./kg. per day, intramuscularly. G. B.

***Cyclo*heptenylethylbarbituric Acid, Toxicology and Pharmacology of.** W. A. Halbeisen, C. M. Gruber, Jr., and C. M. Gruber. (*J. Pharmacol.*, 1948, **93**, 101.) The intraperitoneal LD50 of *cyclo*heptenylethylbarbituric acid (medomin) is 284 mg./kg. for mice and 220 mg./kg. for rats. When injected intravenously it is 119 mg./kg. for rabbits and 105 mg./kg. for dogs; both rabbits and dogs appeared to develop a tolerance for the drug. Large doses rapidly given intravenously produce a sudden fall in arterial pressure, the extent being directly proportional to the amount given and the speed of administration. An increased heart rate occurs during the fall and persists for some minutes after blood pressure has returned to the control level. Large doses given rapidly intravenously cause marked slowing of, and may permanently stop, respiration in expiration; the respiratory mechanism fails before the heart. When the fall in blood pressure is not extensive there appears to be dilatation of the vessels of the spleen, intestine, kidney and limb; when it is sudden and extensive, a decrease in the volume of these organs is observed, which the authors believe to be passive in character. There is dilatation of the vessels of the skin. The drug appears to have a less depressant effect on the cardiac vagus nerves than other intermediate-acting barbiturates such as amytal sodium. Like other intermediate-acting barbiturates it is destroyed in the body and is excreted as the parent substance only when excessively large doses are given. S. L. W.

Digitalis Assay; Comparison of Intravenous Pigeon and Intravenous Cat Methods. H. A. Braun and L. M. Lusky. (*J. Pharmacol.*, 1948, **93**, 81.) The procedure used in the intravenous pigeon assay was based on the U.S.P. assay process. Healthy adult pigeons of either sex were employed. After keeping the birds in the laboratory for a week on a commercial pigeon feed, the birds were starved for 18 to 28 hours. On the day of assay they were anæsthetised with ether, weighed, and tied to boards. The alar vein was

exposed and cannulated, the cannula consisting of a blunted 22-gauge hypodermic needle, and the test solution injected from a 10-ml. micro-burette calibrated to 0·1 ml. Very light ether anæsthesia was maintained throughout the assay. The solution to be assayed was diluted so that the estimated fatal dose per kg. was diluted to 15 ml. with 0·9 per cent. sodium chloride solution. When the average death time fell outside 60 to 90 minutes a new dilution was prepared. As the pigeon is slightly more resistant to digitalis than the cat, more tincture per 100 ml. is required; an additional 1 ml./100 ml. of tincture usually suffices. The diluted tincture was injected at the rate of 0·1 ml./100 g. of pigeon at 5-minute intervals until cardiac arrest supervened; the end point is very sharp. At least 6 pigeons were used for every preparation to be assayed. The U.S.P. requirement of a standard error of ±5·7 per cent. was adhered to, and 6 to 8 pigeons were usually sufficient to come within this figure. From a comparison of the results obtained with 30 preparations of digitalis it was found that the potency estimates obtained by this method varied in no case by more than 13·6 per cent. from those obtained by the present U.S.P. method; the average deviation between the two methods was not significantly different from zero. The coefficient of variation of the lethal dose of digitalis for the pigeon was 10·4 per cent., compared with 12·9 per cent. for cats. Preliminary investigations show that not only are pigeons more consistent in a given assay than cats but that various batches of pigeons seem to vary less from each other than various batches of cats.

S. L. W.

Dimercaprol (B.A.L.) Treatment of Gold Dermatitis. N. R. W. Simpson. (*Brit. med. J.*, 1948, **1**, 545.) A 5 per cent. preparation in arachis oil with 10 per cent. of benzyl benzoate was administered by deep intramuscular injection in the treatment of two cases of gold dermatitis. 2 ml. was given 4 times on the first day, 2 ml. thrice daily for 3 days, 2 ml. once daily for 9 days and subsequently, 2 ml. every alternate day. In one case, redness, heat, pain and induration occurred at the site of injection. After treatment was commenced, a lapse of 6 days occurred before improvement in the dermatitis was noted.

G. R. B.

Fluorescein as an Indicator of Antihistamine Activity. S. C. Bukantz and G. J. Daurmin. (*Science*, 1948, **107**, 224.) Fluorescein was used as a tracer substance to investigate the changes in capillary permeability due to antihistamine activity in the skin. The first experiment, determining the fluorescence at skin sites of a dog, showed that the antihistamines NH188 (neohetramine) and benadryl were of approximately equal activity in preventing fluorescence. In a second experiment, fixed concentrations of histamine in varying concentration of the antihistamine drugs NH188 (neohetramine) or benadryl were injected intradermally into each of 5 human subjects and 3 ml. of a 5 per cent. solution of fluorescein was injected intravenously soon afterwards. It was found that there was an inverse relationship between the concentration of the antihistamine drug and intensity of fluorescence; also at dimly fluorescent sites the initial fluorescence took longer to develop and was of shorter duration than at highly fluorescent sites. To determine the effects of histamine and of antihistamines on the rate of absorption of fluorescein injected intradermally, fluorescein (1 in 50,000 of saline solution), fluorescein + histamine (1 in 10,000), and fluorescein + histamine (1 in 10,000) + benadryl (1 in 2,000) were injected into three sites on the forearms of 3 normal and 1 allergic human subject. The fluorescein sites remained visible under ultra-violet light for 30 to 45 minutes in the normal cases, while the fluorescein + histamine sites no longer

fluoresced after 4 to 10 minutes; the fluorescein + histamine + benadryl site fluoresced as long as the fluorescein sites. In the allergic subject the fluorescence of the fluorescein and the fluorescein + histamine sites disappeared within 4 minutes, but that of the fluorescein + histamine + benadryl site remained for 25 minutes. This indicated that normally fluorescein is rapidly absorbed under the action of histamine but that benadryl antagonises this action; in allergic subjects a histamine-like substance released locally causes rapid absorption of fluorescein from the skin and this also is neutralised by the presence of the antihistamine drug. The time for appearance, intensity and duration of fluorescence at histamine-injected sites may thus be quantitively modified by the local presence of antihistamine substances. L. H. P.

Gonadotrophin. Crystalline Human Gonadotrophin and its Biological Action. L. Claesson, B. Hogberg, T. Rosenberg and A. Westman. (*Acid endocrinol.*, 1948, **1**, 1.) A crystalline and electrophoretically homogeneous form of chorionic gonadotrophin was isolated from the urine of pregnant women by a method which is fully described. It possesses a constant biological activity of 6,000 to 8,000 I.U./mg. It shows a marked stimulatory action on the growth and maturation of the follicles and on the formation of corpus luteum in intact mice, rats and rabbits, but fails to do so in hypophysectomised rats; in this latter group the crystalline hormone produces only an extensive development of the ovarian interstitial gland. Administered intravenously, it is well tolerated by patients in daily doses as high at 12,000 I.U. injected on 3 consecutive days, causing increased follicular growth in the human ovary and a forced production of œstrogenic hormones. In amenorrhœa due to pituitary hypofunction, large doses intravenously may induce bleedings from the progestational endometrium. Combined with small doses of serum gonadotrophin from pregnant mares it produces intensive development of the follicles. The granulosa and theca cells show no sign of degeneration. Follicular rupture and corpus luteum formation takes place in contrast to the effects induced by the action of crystalline chorionic gonadotrophin administered alone. S. L. W.

Intestinal Carminatives. Method for Assessing Value. S. Alstead and J. Fleming Patterson. (*Lancet*, 1948, **254**, 437.) A simple method for assessing the value of carminatives for expediting the passage of flatus from the bowel is described. A rubber catheter was passed about 2 inches beyond the anal sphincter. The free end was connected with a glass adapter to a piece of rubber tubing, and the tubing attached to a 500 ml. glass measuring cylinder, inverted in water to act as a gas jar. As bubbles of gas displace the water column, the volume of gas is recorded. The oral administration of a carminative mixture, hot turpentine stupes to the abdomen, radiant heat and the injection of carbachol were found by this test to be ineffective. Pituitary extract was found to be the most valuable; physostigmine and prostigmine were only occasionally effective in increasing the output of flatus. G. R. B.

Myanesin, Relaxant in Children. W. H. Armstrong Davison. (*Brit. med. J.*, 1948, **1**, 544.) A dose of myanesin (α:β-dihydroxy-γ-(2-methylphenoxy)-propane) of the order of 2 ml. per stone (6·36 kg.) of body weight was given to 44 children between the ages of 24 days and 4½ years to obtain relaxation for abdominal surgery. Maintenance anæsthesia was with open ether, nitrous oxide, or ethyl chloride. Relaxation after the dose

of myanesin occurred rapidly and was maintained for 10 to 25 minutes. The injection was made into the intravenous drip, if one was set up, or into the longitudinal sinus at the posterior angle of the anterior fontanelle.

G. R. B.

Neohetramine, A New Antihistamine Drug, Pharmacological Characteristics of. N. B. Dreyer and D. Harwood (*Proc. Soc. exp. Biol., N.Y.*, 1947, 66, 515.) The amount of neohetramine required to abolish the contractions of guinea-pig ileum and uterus, and cat uterus produced by a concentration of 0·03 to 0·3 μg. of histamine base in oxygenated Ringer solution was determined. The ratio of the amount of neohetramine to histamine was then calculated. This was 2·7 : 1 for guinea-pig ileum. 1·4 : 1 for guinea-pig uterus and 0·7 : 1 for cat uterus. In dogs and cats the fall in blood pressure caused by 1 to 2 μg. of histamine was offset by 2·5 mg./kg. of neohetramine. However, the effect of larger doses of histamine was not neutralised. Neohetramine, like pyribenzamine, did not affect the inhibition of rat uterus by histamine, but histamine-induced vasoconstriction in a perfused rabbit ear was counteracted by an equivalent concentration of the drug. Doses of 1 to 5 mg./kg. of neohetramine given to atropinised cats and dogs caused an immediate drop in blood pressure without a change in the heart-rate. The animals recovered in a few minutes. Neohetramine showed little or no effect on sympathetic nerve stimulation. On the parasympathetic nerve system, neohetramine exerted some atropine-like action on the chorda tympani, but even large doses failed to abolish chorda secretion. Neohetramine did not lessen the effect of the vagus on the intestine, and in some cases seemed to potentiate it. The total and free acidities of gastric juice obtained by rhythmic stimulation of the left vagus were unaltered by doses of neohetramine up to 5 mg./kg. A. D. O.

Podophyllin, Effect of, on Transplanted Mouse Tumours. M. Belkin. (*J. Pharmacol.*, 1948, **93**, 18.) Podophyllin dispersed in sesame oil was given subcutaneously in doses of 20 mg./kg. to 12 mice carrying 15-day-old implants of sarcoma 180, a similar group of mice given injections of sesame oil alone serving as controls. Injections were made every 3 or 4 days for 2 weeks on the side opposite the tumour. The controls grew typically, but the tumours in mice receiving podophyllin exhibited a prompt decrease in growth rate; at the end of 2 weeks the average volume of the treated tumours was approximately one-seventh that of the controls. In another experiment similarly conducted the effect of podophyllin was tested on a mammary adenocarcinoma. In this case the terminal volumes of the treated mammary tumours was approximately two-thirds that of the controls. For both kinds of tumours used in these experiments the most prominent and consistent histological finding following podophyllin administration was extensive necrosis. Characteristic nuclear alterations were found in both types of tumour. The podophyllin produced varying degrees of malaise, and diarrhœa. Resistance to repeated administration does not develop, judged by the appearance of the tumours after several injections of the drug. S. L. W.

Procaine Penicillin G. W. E. Herrell, D. R. Nichols and F. R. Heilman. (*Proc. Mayo Clin.*, 1948, **22**, 567.) In the search for methods of prolonging the effective concentration of penicillin in the blood the authors examined the properties of a procaine salt of penicillin G (duracillin). Procaine penicillin G is a crystalline, non-pyrogenic substance prepared by combining one molecule of procaine base with one molecule of penicillin. The resulting compound contains 41·5 per cent. of procaine base and has

a potency of 940 units/mg. It is employed in the form of a suspension in sesame oil, each ml. of the suspension containing 300,000 units of penicillin and 125 mg. of procaine. After preliminary animal experiments to ascertain the non-toxicity of the preparation, intramuscular injections of 1 ml. were given to 10 patients. Determination of the concentration of penicillin in the blood, either by the Fleming slide-cell technique or by the Kolmer serial dilution method, disclosed an effective therapeutic concentration for at least 24 hours after the administration of the injection. The injection appears to be safe and non-toxic; no local irritation, soreness or pain followed. Therapeutic results are the same as would be expected from any other form of penicillin therapy. It is important not to massage the site of injection. S. L. W.

Sulphetrone in Tuberculosis. M. G. Clay and A. C. Clay. (*Lancet*, 1948, **255**, 180.) Of 44 cases of tuberculosis treated with sulphetrone improvement was noted in 22, 5 were unchanged, 6 became worse, and 11 died. Of those improved, 9 improved considerably, 7 moderately, and 6 slightly. Improvement was not dramatic, and at best sulphetrone can only be regarded as an adjuvant and not in any was as a specific for tuberculosis. An attempt was made to keep the blood-sulphetrone level at 7·5 to 10 mg./100 ml. At first sulphetrone was given as long as the patient tolerated it, but later it was given in courses of 14 to 15 weeks, with a rest period of 6 weeks between courses. Parenteral sulphetrone was found to possess no advantages over sulphetrone orally. The hypochromic and nutritional anæmias were corrected by administration of ferrous sulphate 3 to 6 gr. twice daily, and yeast, preferably autolysed or boiled, 2 dr. twice daily. Changes in the alkali reserve were compensated by giving 30 gr. of sodium bicarbonate 3 or 4 times a day. Most patients developed cyanosis, but this was not an indication for stopping treatment. In most patients the treatment caused so little upset that they could continue taking it after they got up. Sulphetrone should be used only if there are facilities for estimating blood-sulphetrone levels and for carrying out blood counts.

S. L. W.

Sulphetrone, Treatment of Experimental Tuberculosis with. G. Brownlee and C. R. Kennedy. (*Brit. J. Pharmacol.*, 1948, **3**, 29.) In an experiment in which two groups of 20 guinea-pigs were infected with a heavy inoculum of a virulent bovine strain of tubercle bacilli, the survival time of the group treated with sulphetrone (0·6 g. daily in the diet) was prolonged, being 77 days compared with 45 days in the untreated group. In a second experiment in which the infection was a heavy inoculum of a human virulent strain, the treated group of 24 animals survived considerably longer than the untreated group of 21 animals. Throughout the entire drug-treated group macroscopic evidence at necropsy showed very much less tuberculosis than in the untreated group. This was confirmed by histological examination, and by the observation that acid-fast organisms were very much less in number than in the untreated group. The most significant histological evidence was the repeated finding of healed tuberculous lesions, often calcified, in the spleen, liver, lungs and lymph nodes. With both the bovine and the human strains the results suggest that sulphetrone exerts a retarding effect on the progressive nature of established experimental tuberculosis in guinea pigs, though it is evident that it is incapable of eliminating the causative organism. S. L. W.

***bis*-Trimethyl Ammonium Compounds, Pharmacology of.** G. E. Glock, G. A. Mogey and J. W. Trevan (*Nature*, 1948, **162**, 113.) The authors confirm the findings of previous workers on the curare-like action of a series

of *bis*-quaternary ammonium polymethylenes. Thus, they find that the C_3 compound curarises but is relatively inactive compared with *d*-tubocurarine chloride on the rat diaphragm, and its action was completely reversed by neostigmine; the C_5 compound had no action on the rat diaphragm. The C_3 compound had marked cholinergic action, together with relaxation of decerebrate activity at a dose of 1 mg./kg.; it produces a response of the rabbit's ileum similar to that of acetylcholine; it has slight activity as an anticholinesterase, but has no action on pseudo-cholinesterase. Its cholinergic activity, and its low curarising activity, render it unsuitable as a clinical substitute for *d*-tubocuraine. The C_5 compound has about the same anti-cholinesterase activity as the C_3. The overlapping of "muscarine," "nicotine" and anticholinesterase activities is a very striking phenomenon which constantly occurs in complex quaternary ammonium compounds. If the chain includes phenyl groups the effect of increasing the distance between the $\overset{+}{N}$ atoms is not to increase the curarising activity but to develop anti-cholinesterase activity. With regard to the species variation, results with the two closely related compounds, *d*-tubocurarine and its dimethyl ether, show that not only does the ratio of the potency vary between different species, but also, especially in the rabbit, the discrepancy may be larger in the intact animal. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

Iodonium Compounds, Antibacterial Activity of. L. Gershenfeld and B. Witlin. (*Amer. J. Pharm.*, 1948, **120**, 158.) In iodonium compounds iodine is present as an integral part of the positive ions. They are strong bases and form stable salts. They are decomposed by heat. Hitherto, investigations of these compounds have been primarily for the preparation and synthesis from the standpoint of valency studies, and they have only recently been studied to determine whether they exert insecticidal or bactericidal effects. The authors conducted antibacterial efficiency tests on the following:—diphenyliodonium chloride, *bis-p*-chlorophenyliodonium sulphate, *bis-p*-bromophenyliodonium iodide, *bis-p*-chlorophenyliodonium iodide, *bis-p*-iodophenyliodonium iodide and diphenyliodonium iodide. These compounds, in powder form, showed bacteriostatic activity when tested by the F.D.A. agar plate technique, but only *bis-p*-chlorophenyliodonium sulphate in saturated aqueous solution showed bactericidal efficiency against *Staphylococcus aureus* at 37°C. within 1 minute. The addition of sodium thiosulphate did not affect the bateriostatic or bactericidal efficiencies. Saturated solutions of the compounds in alcohol (95 per cent.) showed greater bactericidal efficiency than alcohol itself against *Staphylococcus aureus*, and saturated solutions in a solvent consisting of acetone 10 per cent. by volume in alcohol (95 per cent.) were also effective against this organism. A saturated solution of *bis-p*-chlorophenyliodonium sulphate in acetone-alcohol solvent showed bactericidal efficiency in 1 minute against *Eberthella typhosa*, *Serratia marcescens*, *Pseudomonas æruginosa*, *Escherischia coli* and *Proteus vulgaris*, and was capable of killing *Bacillus subtilis* (24-hour culture) and *B. subtilis* spores (4-day old culture) within 4 hours at 37°C.

S. L. W.

Iodonium Compounds, Bacteriostatic Efficiency of. L. Gershenfeld and B. Witlin. (*Amer. J. Pharm.*, 1948, **120**, 170). Bacteriostatic efficiency tests were performed on the following iodonium compounds in aqueous solution:—diphenyliodonium chloride, *bis-p*-chlorophenyliodonium sulphate, *bis-p*-bromophenyliodonium iodide, *bis-p*-chlorophenyliodonium iodide and

diphenyliodonium iodide. The test organisms used were *Staphylococcus aureus, Serratia marcescens, Eberthella typhosa, Pesudomonas æruginosa, Escherischia coli, Proteus vulgaris, Bacillus subtilis, Bacillus mesentericus, Bacillus megatherium* and *Streptococcus hæmolyticus*. The minimum bacteriostatic concentration of the compounds varied from 0·001 mg./ml. for *bis-p*-chlorophenyliodonium iodide (*Staph. aureus*) to 0·8 mg./ml. for *bis-p*-chlorophenyliodonium sulphate (*Proteus vulgaris*). In the over-all picture, considering all test organisms, diphenyliodonium chloride appeared the most generally effective. It was bacteriostatic to all organisms tested, and it showed bacteriostatic efficiency at lower concentrations than any of the other compounds tested in the case of 6 of the 11 organisms for which a reasonable comparison was possible. *bis-p*-Bromophenyliodonium iodide appeared second in general effectiveness, being ineffective only against *Eberth. typhosa* and *E. coli*. It was generally effective at the relatively low concentration of 0·009 mg./ml. From a comparison of the results of diphenyliodonium chloride and iodide with those of *bis-p*-chlorophenyliodonium sulphate and iodide, it would appear that it is the anion which has the effect on the activity. Intraperitoneal injections in mice of diphenyliodonium chloride and *bis-p*-chlorophenyliodonium sulphate caused increased excitability, increased respiration, and paralysis of the hind legs. The lethal dose for both of the compounds was 20 mg./kg. of bodyweight.

S. L. W.

Quaternary Ammonium Disinfectants; a Semi-micro Method for Testing. E. G. Klarmann and E. S. Wright. (*Amer. J. Pharm.*, 1948, **120**, 146.) Two factors interfere with the use of the "phenol coefficient" method in the evaluation of quaternary ammonium compounds, namely, (1) the creation of a condition in the "medication" mixture (of diluted cationic disinfectant plus bacteria), which prevents the transfer of a truly representative bacterial sample to the subculture, and (2) the failure to take due account of, and to suppress, the characteristic and marked bacteriostatic action of the cationic compounds in the transfer tube. The authors have developed a semi-micro method to overcome these factors, using Bacto-Oxgall to suppress bacteriostasis. Depending upon whether *Eberthella typhosa* or *Staphylococcus aureus* is to serve as the test organism, 1 or 5 per cent. respectively of Bacto-Oxgall is used. The composition and preparation for use with *E. typhosa* is as follows:—10 g. of Armour's peptone, 5 g. of Armour's beef extract, 10 g. of Bacto-Oxgall in 1000 ml. of distilled water; boil, and adjust to *p*H 7·4, and autoclave for 30 minutes. Add 5 g. of "Super-Cel" (10 g. with 5 per cent. of Bacto-Oxgall), and filter while hot. Add 5 g. of dextrose per litre, transfer to tubes each containing 20 ml., and autoclave for 30 minutes at 15 lb. pressure. The details of the semi-micro technique are as follows:—pipette 0·05 ml. of a 24-hour F.D.A. broth culture of the test organism on to the bottom of sterile 25 x 150 mm. test-tubes, taking care that the pipette does not touch the walls of the test-tube. Place the tubes in a water-bath at 20°C; add 0·5 ml. of diluted disinfectant, which has also been kept in a water-bath at 20°C, to each tube and mix thoroughly with the culture; 10 minutes later pour 20 ml. of Bacto-Oxgall broth into the tube, using aseptic precautions; incubate all tubes for 48 hours at 37°C. The results obtained by this method suggest that the quaternary ammonium compounds are not entitled to the phenol coefficient figures obtained with the original F.D.A. method; conversely, the latter method does not appear to be directly applicable to the testing of these compounds.

S. L. W.

PHARMACOPŒIAS AND FORMULARIES

THE PHARMACOGNOSY OF THE BRITISH PHARMACOPŒIA 1948

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READING the B.P. 1948, the foreign observer is struck by the up-to-date selection of the contents, so that excellent monographs for the newest synthetics and pure active principles of vegetable and animal drugs, such as penicillin and heparin, are included. On the other hand, certain vegetable drugs, of which the importance and the consumption is still quite considerable, such as cinchona, chamomile, peppermint and linseed, have been dropped. I am of the opinion that a Pharmacopœia should standardise such drugs officially, even though, as in Great Britain, semi-official standards are given by a book such as the Pharmaceutical Codex. Most of the official titles are well chosen from the botanical point of view, but for a few monographs I would suggest the replacement of the designations by the true botanical names, e.g. Aurantii Pericarpium or Flavedo would be more accurate than Aurantii Cortex. Further, the same designation should be used for botanically equivalent drugs, e.g. for the leaves and flowering tops of Belladonna and Hyoscyamus, which should both be called Herba. The definitions of the drugs are generally very clear. In certain cases, however, similar drugs differing somewhat in their properties and in their behaviour during the preparation of extracts and tinctures, are summarised in the same monograph, e.g. on Aloe, where both the hepatic and the vitreous types are admitted, on Benzoin and on Amylum. In the latter case, e.g. rice starch has an inner surface 10 times greater than potato starch, a fact which without doubt will influence the adsorbing effect. Insufficient attention is paid to the standardisation of conditions of harvesting and of conservation of drugs. These two operations affect the qualitative and the quantitative composition of the complex active principles in such a way that the variation will not be detected by the official assay. There are great difficulties in controlling such standardisation, but I believe that the uniformity of the drugs would be improved if directions for harvesting and conservation were included. In the few cases for which special conditions are stated, as for Digitalis and Colchicum, the requirements are on the correct scientific basis.

The standards for minimal content of active principles are in general rather low. This is for instance the case with Carum, Cinnamomum, Colchicum, Filix Mas and especially with Coriandum and Fœniculum, two drugs for which a content 2 to 2½ times higher could easily be prescribed. One of the main duties of a Pharmacopœia is to require high standards for drugs. Only in this way can the suppliers be forced to produce drugs of high quality. For Digitalis Folium no minimal standard of biological activity is prescribed. If Digitalis Præparata and the tincture are required to have a certain potency, it seems to be necessary to ensure at least the same, or better a somewhat higher, potency for the initial crude drug.

The macroscopical and microscopical descriptions are very good and are given in a very detailed manner. In a few instances I would have preferred, however, that more stress had been laid upon the characteristics which are essential for differential diagnosis. For example, there is an excellent detailed

description of the anatomy of the costæ in Caraway fruit but no mention is made of the typical small vittæ to be found on the outer side of the vascular bundle of each costa. Although the B.P. indicates that the parquetry layer is not present in Caraway this layer actually is well developed in this fruit.

The macroscopical and microscopical sizes are worked out very carefully. By putting in "about" or "mostly" in front of the indicated sizes the important fact has been taken into account that the dimensions of organs or cells depend on the conditions of growth and other factors and therefore may vary and exceed the permitted figures. The width of the parquetry layer cells of Umbelliferous fruits is very useful in the differentiation of these drugs, and should be stated in a future revision.

The quantitative microscopical determination of foreign organic matter by the lycopodium method of Wallis is a very useful innovation; in a further edition the characteristic elements for the drug in each case should be indicated.

The methods of assay of active principles work very well; they are accurate and well planned. If I have any criticism to make it is that the British method of extraction and purification of the alkaloid takes more time than e.g. the method of the Swiss Pharmacopœia and this without improving in any high degree the accuracy of the assay. It would be advisable to assay in Ipecacuanha not only the total alkaloids but also the relation of emetine and cephaëline, especially as both the Rio and the Cartagena drugs are admitted, the latter containing a much higher percentage of the rather undesirable cephaëline.

All drugs containing essential oils are assayed, and it is especially valuable that the diminution of content of essential oil produced by grinding is recognised by giving different figures for the oil-content of both the whole and the ground drug.

The treatment of ash values is rather inconsistent. For several drugs (Ergota, Hamamelis, Podophyllum, etc.) no figure is given. In other monographs there are only figures for the total ash, in a further group of drugs there are figures for acid-insoluble ash only, and in a last group the determination of both the total ash and the acid-insoluble ash. Too much importance has been given to the acid-insoluble ash in certain pharmacopœias, especially when it is considered that this value corresponds with the external mineral impurities. My own experience is that the acid-solubility of external mineral impurities is much higher in drugs grown on calcareous soils than in drugs grown on siliceous soils.

In conclusion, I am glad to have the opportunity of saying that Pharmacognosy has been dealt with in the British Pharmacopœia 1948 in a careful and highly critical manner. The small improvements which I have suggested are given for two reasons, first, the somewhat different continental tradition in pharmacognosy and, secondly, the desire and the hope of giving some modest help to our British friends.

BOOK REVIEWS

AMERICAN PHARMACY. Edited by R. A. Lyman. Vol. 1, 2nd edition, 1948. Pp. 552, Figs. 200. Vol. 2, 1947. Pp. 379. Figs. 111. J. B. Lippincott Co., Philadelphia and London.

This work has been produced in the main by about a score of professors of pharmacy, assisted by specialists on medical, veterinary, zoological and commercial aspects. Six advisory editors, a technical editor and an editor-in-chief have undertaken the final task of production. From this it will be seen that the two volumes constitute a serious contribution to pharmacy in general. To a non-American reader it is not so clear why the subject should be designated American pharmacy. Certainly the references to original English work are somewhat scanty and it would seem that the various authors were not able to consult English pharmaceutical literature as freely as that of the U.S.A. As an illustration, the chapter on the extraction of drugs, followed as are most of the chapters by references to original papers, gives about 20 references to historically interesting work up to 1870, but only one reference to English work, i.e. the continuous extraction apparatus of Self and Corfield (1930), now official in the B.P. The many papers on extraction published in the *Quarterly Journal of Pharmacy and Pharmacology* during the last 20 years are not referred to. Volume 1 is divided into three main parts. Part I deals with Fundamental Principles and Processes. Among other subjects its 10 sections, starting with metrology, deal with the following: heat and refrigeration, purification and clarification, solution, colloids, emulsions and suspensions, extraction, bacteriological technique, preservation and packaging. Part 2 describes the various galenical preparations of the U.S.P. and of the N.F. These are classified in accordance with their chief characters, thus the mucilages, creams, glycero-gelatins, glycerites and collodions are brought together. Emulsions form an important section and are treated very thoroughly with much valuable information on the newer emulgents. Part 3 of this volume is devoted to biologicals and describes the vitamins, hormones and endocrine glands, with a short chapter on other biological products, such as the antibiotics, penicillin and streptomycin, with a table of the lesser known substances. Volume 2 is divided into three main parts. (1) Advanced Pharmacy, dealing with such subjects as flavours, colouring agents, deodorants, solvents, parenteral preparations, and tablets. (2) Medical, Surgical and Dental supplies. (3) Animal Health Pharmacy. As is usual in recent American publications the volumes are exceedingly well, even extravagantly, produced. This work expresses a recognition of " the destinies of America as a teacher, administrator and adviser to the professional pharmacist " and thus represents a benign challenge to us all.

H. FINNEMORE.

BOOKS RECEIVED

BACTERIAL AND VIRUS DISEASES by H. J. Parish. Pp. 159 and Index, E. & S. Livingstone, Ltd., Edinburgh, 1948, 7s. 6d.

THE U.F.A.W. HANDBOOK ON THE CARE OF LABORATORY ANIMALS edited by A. N. Worden. Pp. XVI + 368, Balliere, Tindall and Cox, London, 1947, 31s. 6d.

LETTERS TO THE EDITOR

Colour and Fluorescence Reactions for Steroid and Synthetic Hormones.

FOR some time it has been known that warming with concentrated sulphuric acid caused condensation of several natural œstrogens with the production of coloured solutions with varying fluorescence effects. Boscott[1] has recently developed the phosphoric acid reaction of Finkelstein, Hestrin, and Koch[2] for the detection and estimation of steroid and synthetic œstrogens. The need for a reaction to distinguish between tablets containing small amounts of the various steroid and synthetic hormones in general has led us to investigate the possibility of extending Boscott's technique for this purpose, since it is known that the presence of small amounts of tablet disintegrants and lubricants interferes with certain colour reactions for these substances (cf. Cocking[3]). The basic technique described by Boscott was followed after preliminary extractions (when necessary) of the crushed tablets with ether and evaporation of the solvent. It is not certain in cases where extraction had to be used what accompanying tablet constituent was interfering with the fluorescence reaction; starch interfered with the dienœstrol reaction but in other cases merely caused a slight alteration of the fluorescence colour. The results given by a number of steroid hormones not previously examined are also recorded.

STEROID HORMONES.

The crystalline hormone was dissolved in 0·2 ml. of glacial acetic acid, mixed with about 2 ml. of 88 per cent. phosphoric acid and allowed to stand for 1 hour, the colour and fluorescence (under filtered ultra-violet light) being observed at intervals. After 1 hour the solution was diluted with about 3 ml. of glacial acetic acid and the colour and fluorescence again noted. In addition, approximate fluorescence intensities are reported relative to œstrone as standard.

Desoxycorticosterone acetate. 1 mg. gave a violet fluorescence, weaker than œstrone after 1 hour but stronger on dilution (*ca.* 2 x œstrone).

Ethinyl œstradiol. 0·1 mg. gave an intense orange fluorescence. Intensity: 50 to 100 x œstrone. Two tablets (each 0·05 mg.) crushed and extracted with ether gave a similar reaction when applied to the evaporated ether extract.

Ethisterone (ethinyl testosterone). The reaction produced a dichroic (green-violet) solution in acetic and phosphoric acids, turning to deep red in 1 hour. Dilution with acetic acid gave a red solution with an intense peach coloured fluorescence (50 to 100 x œstrone). Tablets (5 mg.) gave this reaction without extraction.

Methyl testosterone. 1 mg. gave a strong yellow fluorescence (*ca.* 10 x œstrone). Tablets gave the same result without extraction using approximately one-fifth of one tablet (5 mg.)

Œstradiol. An immediate light green fluorescence stronger than œstrone and unchanged after 1 hour was produced by 0·1 to 1 mg. Dilution with acetic acid caused partial quenching.

Œstradiol dipropionate. 0·1 to 1 mg. gave no reaction in the cold; heating at 100°C. for 5 minutes initiated a reaction similar to that of œstradiol.

Œstradiol monobenzoate. 0·1 to 1 mg. gave no reaction in the cold but behaved similarly to the dipropionate after heating at 100°C. for 10 minutes. The behaviour of the dipropionate and the monobenzoate indicated that

hydrolysis was occurring at 100°C. and also that an OH group in the 3-position is necessary for the production of fluorescence.

Œstrone. A green fluorescence was produced after 1 minute. After extraction with ether followed by evaporation of the ether extract the residue from tablets of œstrone gave the same reaction.

Progesterone. The reaction produced a weak blue fluorescence; 5 to 10 mg. quantities were required to make the test effective.

Testosterone propionate. This gave no reaction in the cold. On heating at 100°C. for 2 minutes and standing for 1 hour, dilution with acetic acid produced a fairly strong yellow fluorescence. Heating for 5 minutes following a similar procedure gave a red colour with an orange-yellow fluorescence, fairly strong but relatively weaker than œstrone.

Synthetic Hormones.

Dienœstrol. After dissolving 0·1 mg. in 0·2 ml. of glacial acetic acid, adding 1·8 ml. of 85 per cent. phosphoric acid, allowing to stand for 1 hour and then heating for 1 hour at 100°C. followed by dilution with 3 ml. of acetic acid, a purple colour with an intense but rather unstable violet fluorescence was produced, as described by Boscott. This reaction could be applied to dienœstrol extracted from crushed tablets with ether. Hexœstrol and stilbœstrol gave no reaction to this test.

With the exception of hexœstrol and stilbœstrol all the hormones mentioned can be distinguished when in tablet form by means of the colour and fluorescence reactions described, using, when necessary, ether extraction to separate the hormone from interfering tablet material. Owing to the low intensity of some of the fluorescence effects produced, comparison of an unknown fluorescence with fluorescences produced by known hormones when treated similarly should be used to obtain a reliable fluorescence identification.

Finkelstein, Hestrin and Koch postulated that the production of a fluorescence depended on the presence of a conjugated double bond system and on the position of polar groups. The present results have indicated the importance of a free OH group in the 3-position and have shown that a 17-ethinyl group confers intense fluorescence activity.

Preliminary work on the application of the techniques described to the identification and possible estimation of steroid and synthetic hormones in oily solutions for injection has revealed difficulties which necessitate further study.

We desire to thank the Directors of The British Drug Houses, Ltd., for permission to publish these results.

Analytical Laboratory, R. G. Stuart.
The British Drug Houses, Ltd., London, N.1. R. E. Stuckey.

December 20, 1948.

References.

1. Boscott, *Nature*, 1948, **162**, 577.
2. Finkelstein, Hestrin and Koch, *Proc. Soc. exp. Biol., N.Y.*, 1947, **64**, 64.
3. Cocking, *Analyst*, 1943, **68**, 144.

Correction.—No. 1, p. 61, line 2, for 500 read 550.

SCIENTIFIC MEETING

THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND THERAPEUTIC ACTIVITY

By Professor W. H. Linnell, D.Sc., Ph.D., F.R.I.C.

Summary of a paper delivered before the Bristol and South-Western Counties Section of the Royal Institute of Chemistry at the University, Bristol, on January 13, 1948

Arguments whether the physical properties or the chemical properties of any active substance are the more important are largely a waste of time, as both are linked together *via* the molecular structure. The physical properties must be such as will ensure the substance arriving at the seat of action, but once this is attained some " molecular fit " appears to be necessary for the required activity. In fact, some of Pauling's work gives scientific backing to the simile of the lock and key advanced by Fisher.

During comparatively recent times the new conception has been advanced that, in certain attacks on invading organisms, the remedial compound exerts its activity by interference with an essential metabolite. The names of Woods and Fildes are prominent in this respect, the best example being that of the sulphonamides, for which the essential metabolite is *p*-amino-benzoic acid. Progress along this route is to be expected, since it gives a definite direction to research. Difficulty will be encountered from the fact that most cells, whether they belong to an invading organism or the host, are very similar in their essential requirements. Though immediate practical results in the introduction of new substances to medicine will in the near future, in all probability, result from the empirical method, fundamental information will have to take into account the biochemical aspect.

The acridine antiseptics and the synthetic œstrogens provide examples for discussion. During the 1914-18 war the acriflavine type of substance was established as being of great importance in the treatment of wounds, since compounds of this class have the important property of being as active *in vivo* as *in vitro*. In 1935 the two compounds available were both derivatives of 2: 8-diaminoacridine, and the preparation of all the isomeric mono and diamino derivatives of the acridine nucleus and comparison of their potency was undertaken. It was found that the 2 and 5 positions were the most active, but that the 5 position appeared to induce increased toxicity. Whenever a 1 (or 8) amino substitution appeared the compound was entirely inactive, a result which has been explained *via* hydrogen bonding. The 2:7-derivative appeared to possess the best properties of a compound of this type in that, although it exhibited a high toxicity against the invading organisms, it was only very slightly toxic against the host. The work of Manifold and Russell on the use of this compound with brain tissue gives support. Albert has since carried this work farther, and claims that for an acridine derivative to be active its *p*K as a base should be above a certain value, and also that the possibility of tautomerism within the molecule concerning the nitrogen grouping might be of importance. A recent preparation, as yet not published, of 1-dimethylamino-acridine, which cannot give rise to hydrogen bonding, and has a *p*K above the minimum as suggested by Albert, but which is inactive, suggests that the last word has not been said in this respect.

[*Continued on page 136*

NEW REMEDIES

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

Anaxeryl.* The active ingredients of this ointment are dioxyanthranol 0·22 per cent., ichthyol 0·85 per cent., balsam of Peru 1·00 per cent., salicylic acid 0·30 per cent., resorcin 0·20 per cent., and birch tar oil 0·30 per cent. It is indicated in psoriasis, persistent dry eczema, lichen planus and various mycotic infections. Anaxeryl ointment may be applied daily to any part of the body. It is supplied in 40 g. tubes. A. O.

Chloroquine Diphosphate. (*New and Non-official Remedies, J. Amer. med. Ass.*, 1948, **136,** 1049.) Chloroquine diphosphate is 7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline diphosphate, $C_{18}H_{32}O_8N_2ClP_2$; mol. wt. 515·88. It occurs as a white crystalline powder, m.pt. 193°C. to 195°C., or for a second form of chloroquine diphosphate the m.pt. is 215°C. to 218°C., readily soluble in water, almost insoluble in alcohol, benzene, chloroform and in ether; a 1 per cent. aqueous solution has *p*H about 4·5. It has a bitter taste. When dried *in vacuo* over phosphorus pentoxide at room temperature for 48 hours, it loses not more than 2 per cent. of its weight. When a few drops of ammonium molybdate solution are added to 50 mg. dissolved in 3 ml. of water, a white precipitate is produced immediately. On adding 5 ml. of a saturated aqueous solution of picric acid to 20 ml. of a 0·1 per cent. aqueous solution of chloroquine diphosphate, a yellow precipitate is immediately produced, which melts, after washing and drying, between 205°C. and 210°C. (Caution is required for this test !) When 50 ml. of a 0·5 per cent. solution, made alkaline with 1 ml. of strong ammonia solution, is extracted with two quantities, each of 30 ml. of cyclohexane, and evaporated to dryness and allowed to crystallise in a vacuum desiccator over phosphorus pentoxide, the crystals obtained have m.pt. 87°C. to 90°C. *Assay for phosphorus*—50 ml. of a 1·5 per cent. acid solution of bismuth subnitrate is added to 0·2 g., accurately weighed, dissolved in 50 ml. of water, the mixture is digested for 2 hours on a steam-bath, and filtered through a Gooch crucible; the precipitate is washed with dilute nitric acid (2 ml. in 100 ml.), water, alcohol and finally ether, and then dried for 2 hours at 100°C. and weighed. The phosphorus content is not less than 11·8 per cent. and not more than 12·25 per cent. *Assay for chloroquine diphosphate*—0·2 g. accurately weighed, is dissolved in 50 ml. of water, and the solution, made alkaline with 5 ml. of ammonia solution, is extracted with successive quantities of 25, 20, 15, 10 and 10 ml. of ether. The combined ether extracts are filtered and evaporated and the residue, after drying at 100°C. for 30 minutes, is not less than 98 per cent. and not more than the equivalent of 102 per cent. Chloroquine diphosphate is highly active against the erythrocytic forms of *Plasmodium vivax* and *P. falciparum* and is said to have about 3 times the activity of mepacrine against these organisms. It suppresses acute attacks of malaria but is not a prophylactic agent. It is administered by mouth, before or after meals; for suppression of vivax infection 0·5 g. at weekly intervals is recommended; for treatment of acute attacks of vivax or falciparum malaria an initial dose of 1 g. followed by 0·5 g. after 6 to 8 hours and 0·5 g. daily for 2 days is sufficient to terminate the attack. L. H. P.

Depropanex* is a deproteinated pancreatic extract. It is a saline solution of a chemically derived, protein-free, nitrogenous fraction obtained by acid-

alcohol treatment of mammalian pancreas. Depropanex contains no insulin, histamine or acetylcholine, and not more than 2·5 per cent. of solids of which 0·9 per cent. is sodium chloride and 0·5 per cent. is non-protein nitrogen. The *p*H is adjusted to 6·5 to 6·8. Standardisation is carried out by comparing the effect of the extract on the arterial blood pressure of anæsthetised dogs with that of a standard extract. Each batch is adjusted to contain 10 depressor units per ml. A qualitative test is made by observing the heart-blocking effect in mice. The lowering of arterial blood pressure in urethanised rabbits and atropinised dogs is used as a test for the absence of histamine and acetylcholine. Depropanex has been successfully used in intermittent claudication, especially that associated with occlusive arterial disease, in renal and ureteral colic, spastic ureteritis and dysmenorrhœa. In chronic vascular disease 2 to 3 ml. should be given intramuscularly every other day. For ureteral colic or where there is acute contraction of smooth muscles 3 to 5 ml. should be given. An intramuscular dose of 2 to 4 ml. is recommended for primary dysmenorrhœa. It is not advised that depropanex be injected intravenously. The product is supplied in 10 ml. rubber-capped vials. A. D. O.

Mycil* is a fungicide, issued in the form of an ointment and a dusting-powder, the active ingredient of which is *p*-chlorophenyl-α-glycerol ether. It is effective against *Epidermophyton floccosum* and the various species of *Tricophyton*, the usual causative organisms of athlete's foot. The ointment is used for treatment of the infection, and the dusting-powder is sprinkled in the socks or shoes as a prophylactic measure. S. L. W.

Neurinase* is a combination of the active principles of fresh valerian with soluble barbitone. It is claimed that volatile oil containing bornyl *iso*valerianate, obtained from the fresh rhizome, acts synergetically with the barbiturate. Neurinase is indicated as a hypnotic in insomnia of nervous origin and as a sedative in psycho-neurotic disorders and migraine. It is issued as a solution, containing in a teaspoonful about 2 gr. of soluble barbitone, and in tablets, containing, in each, about 3·3 gr. of soluble barbitone. S. L. W.

Nitrogen Mustard Hydrochloride,* is di-(2-chloroethyl)methylamine hydrochloride, the nitrogen mustard derivative known in America as bis(β-chloroethyl)amine hydrochloride, or "Bis." It is indicated in cases of Hodgkin's disease which have become resistant to radiation therapy, producing a remission of symptoms, and rendering the case amenable to further X-ray treatment. It does not appear to be more effective therapeutically than radiation therapy in the treatment of lymphosarcoma or lymphatic and myelogenous leukæmia. The results obtained with nitrogen mustard in the treatment of polycythæmia rubra are comparable with those obtained with radio-active phosphorus. It is administered intravenously in a dose of 0·1 mg./kg. of bodyweight for a total of 3 to 6 days; the maximum single dose should not exceed 8 mg. and an interval of 6 to 8 weeks should be allowed between courses of injections. Solutions for injection must be freshly prepared, 10 ml. of a 0·9 per cent. sterile solution of sodium chloride being added to 10 mg. of the salt. Nausea and vomiting and a tendency to hæmorrhage may occur. Extravasation should be avoided. Nitrogen mustard hydrochloride is issued in boxes of 10 vials each containing 10 mg. S. L. W.

Promizole* is a proprietary brand of 2: 4′ diamino-5-thiazolylphenyl sulphone, and is used in the oral treatment of leprosy. No claim is made as to the ultimate value in leprosy of promizole given orally, but the therapeutic results so far

obtained are considered sufficiently encouraging to warrant further clinical study. Doses of 1·5 g., increasing to 6 g., have been given daily for periods of a year or more; the drug is well tolerated. Initial clinical reports indicate that it may also be of value in tuberculosis. Tablets of 0·5 g. are supplied in bottles of 100 and 1,000. S. L. W.

Prothricin* is an antibiotic nasal decongestant which contains 2·00 per cent. of tyrothricin and 1·5 per cent. of propadrine hydrochloride. The solution is buffered to *p*H 5·5 to 6·5 and contains 0·002 per cent. of phenylmercuric acetate as a preservative. The shrinking effect of propadrine on the nasal mucosa lasts for 2 hours and causes little or no irritation or side reactions. Prolonged use does not cause the ill-effects of ephedrine on the nasal mucosa. The extensive use of tyrothricin has not given rise to drug sensitivity nor has has it caused tissue damage. In this respect it is superior to the sulphonamides. The concentration of tyrothricin in prothricin is effective in the presence of body fluids and tissue exudates against the Gram-positive organisms commonly infecting the respiratory tract and it is moderately effective against the Gram-negative meningococci and gonococci. It is recommended for acute catarrhal rhinitis, rhinosinusitis and ethmoiditis, and the incidence of otitis media and other complications may be reduced by its use. Chronic infections respond less readily. Applied by means of a dropper or spray, prothricin should be used every 15 to 30 minutes or as necessary. Unless there is constant medical supervision it is contra-indicated in heart or thyroid disease, high blood pressure and diabetes; otherwise prothricin seldom causes side effects. The preparation is supplied in dropper-bottles containing 1 fl. oz. A. D. O.

Scobenol* is a stable emulsion containing 25 per cent. of benzyl benzoate for the treatment of scabies. After bathing and drying, the emulsion is applied over the whole body from the neck downwards with a flat paint brush, and is allowed to dry on. Two such treatments, either on successive days or within a period of 8 days, are sufficient. Scobenol is issued in bottles containing 4 fl. oz., which is sufficient for the complete treatment of an adult. S. L. W.

T.E.A.B.* is a proprietary form of tetraethylammonium bromide supplied as a 10 per cent. solution for intramuscular or intravenous injection. The tetraethylammonium ion produces a fall in blood pressure, depression of gastro-intestinal motility, pupillary changes, cessation of sweating, dry mouth, and postural hypotension. It should not be administered to patients with low blood pressure or with vasomotor instability, and only with caution to patients with severe hypertension and poor renal function. It is indicated in the treatment of peripheral vascular disease and functional vascular disorders such as Raynaud's syndrome, in thrombo-angiitis obliterans and thrombophlebitis, for the relief of pain in causalgia and in neuralgia following herpes zoster, for the relief of hypertension, and for the alleviation of the pain of peptic ulcer, abdominal cramps and diarrhœa; by distension of the bladder it also relieves pain in certain types of vesical dysfunction. The recommended dosage for intravenous use is 0·2 to 0·5 g. in 10 per cent. solution; intramuscularly the dose should not exceed 20 mg./kg. of body-weight or 15 ml. of the 10 per cent. solution, but usually 5 to 10 ml. is sufficient. Intravenous injection produces an immediate response, but the effect is less prolonged than with the intramuscular injection. It is issued in boxes of 12 and 25 ampoules, each ampoule containing 1 ml. or 5 ml. of 10 per cent. solution. S. L. W.

NEW REMEDIES

Tivlolac* is a solution of colloidal calcium and vitamin D for injection, subcutaneously or intramuscularly, in allergic states such as urticaria, allergic rhinorrhœa, migraine and asthma, or in vasomotor disorders such as chilblains or angioneurotic œdema. It may also be employed prior to tonsillectomy and dental extraction to reduce capillary hæmorrhage, and it may be given to hasten bony union where this is delayed owing to known calcium deficiency. The average dose is 1 ml. daily for 2 days, followed by a similar dose at 4 to 6-day intervals. Tivlolac contains 0·05 per cent. of colloidal calcium and 5000 I.U. of vitamin D in 1 ml., and is issued in boxes of 6 and 12 ampoules of 1 ml., and in rubber-capped bottles containing 15 and 30 ml. S. L. W.

Vibelan* tablets contain aneurine hydrochloride 0·5 mg., riboflavine 0·75 mg. and nicotinamide 7·5 mg. in a yeast extract base; it is claimed that the daily administration of 4 tablets supplies the normal adult requirement of these 3 vitamins, the yeast extract providing small unstandardised amounts of other members of the vitamin B group. The use of the tablets is indicated in all vitamin B deficiency states, in seborrhœic dermatoses and some forms of acne, and in patients receiving glucose-saline infusions. Since inactivation of œstrogens by the liver is impaired by vitamin B deficiency, the use of the tablets is also suggested in the treatment of functional uterine hæmorrhage and other hyperœstrogenic states in either sex, including benign prostatic hypertrophy. Vibelan is issued in bottles of 50, 250, and 1,000 tablets. S. L. W.

THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND THERAPEUTIC ACTIVITY (*continued from page* 132).

With regard to the synthetic œstrogens, it was suggested that the high activity of the stilbœstrol which resulted from the work of Robinson, Dodds *et al* was due to molecular simulation of œstradiol. To probe this contention 3-monohydroxy, 4-monohydroxy and 3:4-dihydroxy-$\alpha\beta$-diethylstilbene were prepared and tested for œstrogenic activity. The 4-hydroxy compound was very active, an activity which was highly potentiated by the introduction of a second 4-hydroxy group; the 3-hydroxy derivative showed little activity, and the 3:4 dihydroxy showed a lower activity than the 4-monohydroxy compound. These results proved that molecular simulation could not be the whole story, and with any derivative of diethylstilbene a *para*-hydroxy group was of paramount importance. Further exploration of the molecular skeleton, $\alpha\beta$-diethylstilbene, has resulted in obtaining an activity similar to that of deoxycorticosterone in 4-hydroxy-4-ωhydroxyaceto-$\alpha\beta$diethylstilbene and recently cardiatonic activity in a 4-butenolide.

It will be observed that the examples chosen illustrate the empirical method of attack, but that once an activity has been obtained further work of a systematic character may be prosecuted within the group. Many thousands of different researches, having for their aim the production of something of use in medicine, have produced the comparatively few important synthetic compounds in use to-day, but, although many of the researches lead to a negative result, they none the less contribute to the knowledge of the relationship between chemical structure and therapeutic activity.

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