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1 *Lancet*, 1952, i, 742
2 *Lancet*, 1953, i, 1154.
3 *Lancet*, 1953, i, 1024.
4 *Canad. Med. Ass. J.*, 1953 68, 464

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REVIEW ARTICLE

ION EXCHANGE RESINS IN ORGANIC ANALYSIS

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The possible applications of ion exchange resins to organic and pharmaceutical analysis are now being actively explored by a number of workers. Further developments, particularly in the study of the physical chemistry of exchanges with organic ions should lead to notable simplifications of some of the older analytical processes.

The development of synthetic ion exchange resins dates from the paper of Adams and Holmes[1] describing the properties of comparatively unstable, weak cation and anion exchangers, prepared from phenol-formaldehyde and phenylenediamine polymers respectively. These authors were able to forecast some of the future applications of the resins in chemistry. During the last decade, much effort has been spent on the design and manufacture of stable, insoluble exchange resins having only one functional group each. Accounts of the preparations and properties of these new resins are given in books by Nachod[2] and Kunin and Myers[3], and reviews dealing with their applications have been written by Schubert[4] and Kressman[5].

A number of unifunctional resins are now available commercially which provide the analyst with a new group of reagents with consistent properties. Their particular advantage in analysis is that they can be set up in columns through which flows the solution with which they react. Considerable evidence exists to show that column reactions between resin and solution can be made quantitative.

The modern resins consist of a stable hydrocarbon matrix formed by co-polymerising unsaturated materials such as styrene and divinylbenzene. The reactive, functional groups may be introduced either before or after polymerisation. The weak cation exchangers can be prepared by the former method, for example, methacrylic acid and divinylbenzene are co-polymerised to give a resin containing carboxyl groups throughout its mass[6]. The strong cation exchangers are made by the latter method, spherical beads of the styrene, divinylbenzene co-polymer are sulphonated[7]. After sulphonation the resin beads retain their insolubility but they are able to swell up in water.

If the sulphonated resin is washed with water until the washings are neutral and is then put into a sodium chloride solution, the latter rapidly becomes acid owing to an interchange of cations between the solutions and the resin. The sulphonate ions are fixed onto the resin matrix but the hydrogen ions associated with them are free to move out into the solution, if an equivalent number of sodium ions diffuse into the resin to replace them. Eventually an equilibrium is set up:—

$$\underset{\substack{\text{resin,}\\ \text{completely}\\ \text{ionised}}}{(RSO_3^- + H^+)} + \underset{\text{solution}}{(Na^+ + Cl^-)} \rightleftharpoons \underset{\substack{\text{sodium form}\\ \text{of resin}}}{(RSO_3^- + Na^+)} + \underset{\text{solution}}{(H^+ + Cl^-)}$$

The increase of acidity of the sodium chloride solution gives a simple method for determining the extent of exchange[8]. The exchange occurs throughout the resin particle and not simply at the resin/solution boundary.

Ion exchange equilibria of this type can be interpreted qualitatively by the law of mass action. More exact interpretation involves consideration of the non-ideal behaviour of the ions, particularly in the resin phase where they are concentrated in a "solution" of the order 5N. Also, the volume changes of the resin on going from one ionic form to another involve energy changes due to the elastic forces of the resin matrix, which can effect the equilibria, e.g., these forces will tend to oppose conversion to a form which has a large specific volume. It is probably in consequence of this swelling effect that the strong exchangers show a marked preference for divalent ions.

The reversible ion exchange between a solution and a resin can be driven to completion in one direction by using a column technique. An excess of one of the reactants, the resin (usually a 20:1 or greater excess) is put into the column and the solution is allowed to flow through it, in this way one of the reaction products is continuously removed from the resin. For example, if a potassium chloride solution flows over an excess of strong anion exchanger in the hydroxyl form the effluent from the column consists of a potassium hydroxide solution. Under suitable conditions the exchange of chloride for hydroxide ions in the solution is quantitative.

The four types of unifunctional ion exchange resins of principal analytical interest are shown in Table I.

TABLE I

RESIN TYPES

Type	Functional group	Manufacturers		
		Permutit (G.B.)	Rohm and Haas (U.S.A.)	Dow Chemical Co. (U.S.A.)
A. Strong cation exchanger ..	Sulphonic acid	Zeo Karb 225	Amberlite IR-120	Dowex 50
B. Weak cation exchanger ..	Carboxylic acid	Zeo Karb 226	Amberlite IRC-50	
C. Strong anion exchanger ..	Quaternary ammonium	De Acidite FF	Amberlite IRA-400	Dowex 1 and 2
D. Weak anion exchanger ..	Primary amine	De Acidite E	Amberlite IR-45	

Some of these resins can be obtained in the convenient form of small spherical beads. In addition to their water insolubility, they are stable in organic solvents such as ethanol and acetone and they retain an appreciable exchange capacity in these solvents though rates of exchange are usually slower than in water.

Resins of type A and B are activated by conversion to the hydrogen form and this is achieved by passing an excess of 2N hydrochloric acid over the exchanger (resin) contained in a column. The resin is then washed with water until the washings are neutral. Types C and D are

similarly activated by conversion to the hydroxyl form with 2N sodium hydroxide solution.

In addition to the nature of the functional group of the exchanger, three other properties determine its characteristics. These are (1) the capacity, i.e., the concentration of exchangeable group in the resin, usually expressed in milli-equivalents/g. of dry resin, (2) the amount of cross-linking in the resin matrix and (3) the particle size of the resin. The degree of cross-linking is determined by the proportion of divinylbenzene used in the co-polymerisation and this can have an appreciable effect on the chemical behaviour of the exchanger. For example, Partridge, Brimley and Pepper[9] report that variations in the degree of cross-linking of a sulphonated polystyrene resin give rise to important differences in its behaviour towards organic bases, and Saunders and Srivastava[10] state that a decrease in the degree of cross-linking of a carboxylic acid resin increases its equilibrium capacity for a large organic cation such as quinine. Variation of resin particle size has a very marked effect on rates of exchange, particularly if large ions are involved. Srivastava[11] found that an 80/120 B.S.S. sieved fraction of a carboxylic acid resin absorbed more quinine in 5 minutes from solution in ethanol (50 per cent.) than did a 20/40 B.S.S. fraction in 1 hour. The equilibrium capacities for quinine were however found to be the same in both cases.

In choosing or designing a resin for a particular application all these factors have to be taken into account. A low degree of cross-linking of the resin will facilitate exchange of large ions, but it will also cause large volume changes of the resin on conversion from one form to another. Similarly, the use of a strong exchanger will give rapid rates of exchange, but it may cause hydrolysis of labile organic materials since the strong exchangers are effective acid-base catalysts. Also it is often difficult to secure quantitative elution from the strong exchangers with a reasonably small volume of liquid. The weak cation exchangers have the disadvantage for column work, that they undergo a large increase in volume on conversion in water from the relatively unionised hydrogen form to the almost completely ionised salt form. This swelling is mainly an electrostatic effect arising from the mutual repulsions of ionised carboxyl groups held close to one another by the resin matrix. It can be very much reduced by using a solvent of low dielectric constant such as ethanol.

Physico-Chemical Studies with Exchange Resins

Most of the work so far published in this field has been concerned with the exchange of inorganic ions. Detailed studies of exchange equilibria have revealed the important part which volume changes and resin cross-linking play in determining positions of equilibrium[12,13]. The kinetics of exchange of inorganic ions have been examined by a number of workers[14,15,16], a recent paper being that of Reichenberg[17].

Saunders and Srivastava[10] have studied both equilibrium and kinetics of the absorption of quinine by a carboxylic acid resin from aqueous ethanol solutions. A period of several days was required for equilibrium

to be reached. The rate of absorption, being governed by the rate of diffusion of quinine through the resin particles, was greatly increased by reducing the particle size of the resin. They also examined in detail the absorption of a number of organic bases on to the resin[18]. Both rates of absorption and equilibrium capacities were found to be governed by the dissociation constants and molecular sizes of the absorbates. Kressman and Kitchener[19] have studied ion exchange equilibria with large organic cations and a phenolsulphonate cation exchanger. They found that the affinities of some of these ions for the resin increased with increasing ionic size. Kressman[20] has also studied separations of materials on the resins based on differences of ionic size.

Hale, Packham and Pepper[21] have observed the exchange of quaternary ammonium ions on sulphonated polystyrene resins and have reported an irreversible change in the swelling properties of the resin after such exchanges.

As has been pointed out by Tompkins[22], there is a paucity of background data concerning the constants of exchange reactions which makes difficulties in the development of new procedures in which the exchangers are used. There is no doubt that the full value of these resins in organic analysis will not be realised until more physico-chemical data concerning their interactions with organic compounds have been accumulated.

Resin columns. In analytical work the quantity of resin used should be as small an amount as will give a quantitative exchange. A tube containing 5 to 10 g. of resin in a column 20 cm. high is very suitable. There should be at least a 20:1 excess of resin. The resin should be slurried into a column already filled with solvent, in order to avoid formation of air bubbles. It is useful to have a capillary siphon at the outlet of the column to avoid accidental drying out. Solutions used should be roughly of 1 per cent. concentration. To achieve quantitative exchanges operating conditions such as flow rates, resin/solution ratio and volumes of eluting and washing liquids must be carefully evaluated, after elution and washing the column should be drained of liquid and washed again.

Applications of Ion Exchange Resins to Organic Analysis

These can be classified according to the function which the resin performs with respect to the solution.

(i) Complete removal of small ions from a solution.
(ii) Replacement of either the cations or the anions in the solution by hydrogen or hydroxyl ions respectively.
(iii) Removal of a material to be estimated from the solution onto the resin, followed by washing on the column and quantitative displacement from it.

Group I

Deionisation of water. This is an application of interest to all analysts. The process of removing dissolved salts from water by allowing it to flow successively over an anion and then a cation exchange resin was

first described by Adams and Holmes[1], using weak exchangers. The quality of the water obtained has been very much improved since then by using a column containing a mixture of strong anion and strong cation exchanger. This removes all small ions, including silicate and carbonate, from the water, giving a product containing only 1 part of solids per 25 million with a specific conductivity of 10^{-7} mhos./cm. at 20° C., comparable with the best conductivity water obtained by distillation in quartz apparatus.

Originally, the mixed resin column could not be easily regenerated and had to be discarded when the resins were spent, i.e., when the product no longer had an acceptable specific conductivity. However, a method has been developed[23], suitable for large-scale operation by which the two exchangers can be separated on the column, regenerated separately with acid and alkali, washed and then mixed together again, so that the column can be used for an almost continuous supply of deionised water. The deionised water so obtained easily fulfils the specification for distilled water of the British Pharmacopœia, the main contamination to be expected, organic matter, is well within the prescribed limits.

If the product is stored under suitable conditions it provides a supply of carbonate-free water, avoiding the necessity for boiling large volumes of ordinary distilled water.

Deionisation of colloids. Colloidal ions are only slowly taken up by ion exchange resins. Janus, Kenchington and Ward[24] have used this effect to produce gelatin sols of very low salt content. A 2 per cent. gelatin sol was passed through a column containing mixed strong anion and strong cation exchangers, at 40° C. so as to lower the viscosity of the sol. The exchangers removed all small ions from the liquid leaving a sol of specific resistivity, greater than 50,000 ohm/cm. and having an ash content, based on the weight of dry gelatin in the sol, of 0·003 per cent. The *p*H of the effluent was that of the isoelectric point of the gelatin.

Thompson[25] mentions the use of a strong cation exchanger for removing amino-acids from protein sols and Partridge[26] has discussed the use of ion exchange resins as molecular sieves for separating small and large ions.

The reviewer has found that it is possible to develop a quantitative assay of glycine in a gelatin sol, using a mixed resin column. The flow rate of the solution of glycine plus gelatin can be adjusted so that a negligible amount of gelatin is absorbed while the glycine is completely removed from the solution. The concentration of glycine in the sol is measured by the difference of the titres with caustic soda in the presence of formaldehyde of the solution before and after passage through the column.

Group II

Estimation of total ionic concentrations in water or colloid sols. In this type of estimation, the water or sol is allowed to flow over a strong exchanger to remove either the small cations or the small anions. The

effluent is then titrated with alkali or acid. Egner, Erikssen and Emanuelson[27] have used this method of determining the salt content of rain-water. Polis and Reinhold[28] have employed it to determine the total concentration of cations in serum; the serum is allowed to flow over a cation exchanger in the hydrogen form to give an acidic effluent, containing the anions of the serum, chloride, bicarbonate, phosphate, proteinate in the form of their acids. The effluent is aerated with carbon dioxide-free air to remove any free carbon dioxide present and is then titrated with alkali. The titre gives an estimate of the number of equivalents of total cation present in the original serum.

Preparation of carbonate-free alkali. It has always been a difficult problem to prepare solutions of sodium and potassium hydroxide completely free from carbonate. The standard method of preparation consists of making a saturated solution of the alkali in water and allowing it to stand for some time, the carbonate which is sparingly soluble is allowed to settle out. A much simpler procedure has been devised by Davies and Nancollas[29]. This is based on the preferential uptake of carbonate ions by a strong anion exchanger. The alkali solution is made up without any particular precautions and is allowed to flow through a column of strong anion exchanger, the effluent is almost free from carbonate. This technique has been developed further by Grunbaum, Schöniger and Kirk[30]. They required a very dilute (0·001N) carbonate-free caustic potash solution with a factor which would remain constant over a considerable period. They were able to achieve this by attaching a column containing a strong anion exchanger to the filling tube of an automatic burette. When the alkali solution was required a standard 0·001N solution of potassium chloride was run through the column and the effluent consisting of 0·001N potassium hydroxide was used for the titration. Quantitative conversion of chloride to hydroxide was effected by the column and since the standard solution was stored as inert potassium chloride, the hydroxide only being prepared immediately before titration, a solution was obtained whose factor remained constant over a period of several months.

Estimation of base in alkaloidal salts. This assay has been devised by Jindra and Pohorsky[31]. The alkaloidal salt solution is run through a column of preferably strong anion exchanger, though Jindra's earlier work was carried out with weak exchangers. Under suitable conditions a quantitative exchange of hydroxyl ions for the salt anions occurs and the liquid coming out of the columns consists of a solution of the alkaloid. This can be estimated by direct titration with standard acid. Fleming and the reviewer have found this method to be very suitable for the estimation of ephedrine hydrochloride and nicotine sulphate. In the latter case the exchanger not only removes the sulphate anions, but also takes out coloured components from impure solutions giving a colourless eluate which can be estimated polarimetrically. Jindra and Rentz[32] have further extended the method for the assay of local anæsthetics. One disadvantage of this method when the final estimation is carried out by acid titration, is that any inorganic salts present in the original material

will be converted to their hydroxides and so will add to the titre. This difficulty can perhaps be overcome by ashing or otherwise oxidising the sample and if appreciable amounts of inorganic matter are present, they can be dissolved in water and run through the column, the titre for this liquid being subtracted from that obtained for the original salt. This method could also be applied to the estimation of acid in salts of organic acids, by using a strong cation exchanger. The column effluent would then consist of a solution of the organic acid which could be estimated by titration.

Group III

This type of application of ion exchange resins provides an analytical tool by means of which substances in complex mixtures such as biological fluids can be removed and purified for estimation. In some cases it may give better results than older processes, such as extractions with immiscible solvents.

Separation of plant and animal tissue extractives. Partridge[33] has outlined a systematic procedure by which the water-soluble extractives of tissues can be separated into groups, using ion exchange columns. Individual members of groups were then separated by fractional displacement from the resins on a scale large enough to permit characterisation of new components. He describes a model experiment in which 16 amino-acids were isolated in a crystalline condition from the hydrolysis product of a protein. The hydrolysate was first treated to remove unchanged protein, either by dialysis or by precipitation with ethanol, coloured material was then removed by treatment with charcoal which also removed the aromatic amino-acids. The resulting solution was passed through a cation exchange resin which took out the positively charged amino-acids. A sample of the acid effluent was titrated with alkali to give an estimate of total anions present, the remainder was passed through a weak anion exchanger which removed the negatively charged amino-acids from the solution giving a final product which contained sugars and other neutral substances. Weakly cationic amino-acids such as histidine and lysine could be displaced from the cation exchanger by ammonia while arginine was displaced with caustic soda. Negatively charged amino-acids were removed from the anion exchanger with dilute hydrochloric acid. This technique has been applied successfully to the preparation of isotope-labelled amino-acids from proteins biosynthesised by organisms fed with labelled compounds.

Analysis of nucleic acid hydrolysates. Cohn[34] has used an exchange resin column to separate the amphoteric degradation products of nucleic acid hydrolysis. These mono ribonucleotides contain both phosphate and amino-groups and Cohn worked out the *p*H at which an optimum separation of components on an anion exchanger could be expected. From this he deduced that the mixture should be run through the exchanger (finely powdered strong anion exchanger) at *p*H 6, at which all the components carry a net negative charge. By lowering the *p*H of the eluting liquid in a stepwise fashion, the nucleotides were removed one after

another. Ultra-violet spectrophotometry and paper chromatography were used to analyse each fraction. By this method he was able to achieve a quantitative analysis of the acid hydrolysates and to identify previously unknown isomers of adenylic and geranylic acids.

Estimation of streptomycin in fermentation broth. Doery, Mason and Weiss[35] have developed an ion exchange method for the quantitative assay of streptomycin in broth. The broth was diluted with phosphate buffer to *p*H 9 and then clarified by centrifugation. A 5-ml. sample was then run through a column 4 mm. in diameter and 12 mm. high, of a weak cation exchanger which had been equilibrated with sodium bicarbonate. The resin retained the streptomycin cations quantitatively. It was washed with water and the streptomycin was displaced from it with 25 ml. of 0·2N hydrochloric acid. The effluent was then analysed by the standard maltol assay.

Estimation of sugars. The separation and assay of mixtures of sugars developed by Khym and Zill[36] is an example of the use of the resins with compounds which are normally non-ionic. The sugars were treated with borate solutions, with which they react to give complex negatively charged ions. The column used was charged with 200–400 mesh, strong anion exchanger, which was converted to the borate form with potassium tetraborate solution and then washed with water. The test solution, consisting of a mixture of sugars in dilute potassium borate solution, was put onto the column and elution was carried out with boric acid–borate buffer solutions. The eluate fractions were analysed for the various sugars by standard colorimetric methods and by paper chromatography. Sharp separations of fructose, galactose and glucose; mannose and fructose; ribose, arabinose and xylose; sucrose, fructose and glucose; sucrose and maltose were obtained in these borate columns.

Chambers, Zill and Noggle[37] have used similar borate columns to separate mixtures of glycosides, though in this case the separation appeared to be due to variations in the aglycone part of the glycoside molecules rather than to differences in the borate complex anion formed.

Analysis of aldehydes and ketones. Samuelson and his co-workers have also developed a specialised technique for using ion exchange resins with non-ionic organic compounds. Gabrielson and Samuelson[38] report that both aldehydes and ketones were taken out of solution by a strong anion exchanger column converted to the bisulphite form by pre-treating it with an excess of sodium bisulphite solution. By elution with water at 75° C., the ketone was removed from the column, but no aldehyde was detectable in the eluate. The aldehyde could be eluted quantitatively with an excess of sodium chloride solution.

Samuelson and Sjostrom[39] have extended this method to the estimation of sugar solutions. They found that sugars were quantitatively removed from aqueous ethanol solutions by the bisulphite form of a strong anion exchanger. They could then be fractionally eluted from the column.

Alkaloidal assay. For assaying alkaloids, particularly in coloured or otherwise impure solutions, the weak cation exchangers should be useful. To obtain fairly rapid results the exchanger should be in the form of a

fine powder and the alkaloid solution should be slightly alkaline. After absorption onto the exchanger, it is washed with solvent to remove non-basic impurities such as tannins, and then displaced with ammonia in the case of weak bases such as quinine or with hydrochloric acid if the base is stronger, for example, ephedrine. The purified alkaloid solution can then be assayed by titration, by evaporation to dryness or by polarimetry. Huyck[40] has shown the practicability of this method for ephedrine while Saunders and Srivastava have shown that quinine can be quantitatively removed from aqueous ethanolic solution by a weak cation exchanger and can be quantitatively displaced from the resin by ammonia solution.

Bjorling and Berggren[41] have used an inorganic silicate exchanger in the analysis of preparations containing tropa alkaloids. The exchanger was activated with a mixture of potassium chloride and acetic acid solutions, the solution of alkaloidal salt was flowed through the column when the alkaloid was transferred to the exchanger. It was then eluted with 0·2N hydrochloric acid and estimated spectrophotometrically.

Riboflavine determination. Fujiwara and Schimizu[42] have used a cation exchanger in the estimation of riboflavine in biological extracts, elution being carried out with pyridine–acetic acid solutions.

Estimation of methonium ions in serum. Child, working at the School of Pharmacy, has found that methonium ions can be quantitatively removed from aqueous solution or from serum by a weak cation exchange resin. In the latter case, the resin can be washed free of proteins and the methonium ion can then be displaced from the resin with hydrochloric acid. Results based on biological tests indicate that the displacement is quantitative.

Miscellaneous developments. Several groups of workers have prepared resins containing groupings other than the normal acidic or basic groups. Cassidy[43] has prepared so-called electron exchange resins containing reversible oxidation-reduction groups, by polymerising vinyl hydroquinone. Gregor, Taifer, Citarel and Becker[44] have prepared resins containing chelating groups, e.g., from *m*-phenylene diglycine, which give better possibilities of separating metal cations than the conventional resins.

Manecke[45] has suggested the use of an applied electric field across a horizontal resin exchanger column, in order to improve the sharpness of separation of ions on the column.

Application of Ion Exchange Resins in Pharmaceutical Analysis

Now that these resins are available commercially as standardised materials, comparable with other analytical reagents, it is worth considering whether they can be applied to simplify some of the standard pharmaceutical assays.

The most direct field of application would be in the estimation of salts. Alkaloidal and other organic base salts could be determined by the Jindra method and this is very much more simple and convenient than,

say, the steam distillation method recognised for estimating ephedrine in its salts[46]. Similarly, treatment of salts of organic acids with a strong cation exchanger followed either by direct titration or by extraction with organic solvent and subsequent titration would be more straightforward than the two-stage titration and extraction method used in some cases[47].

In the determination of small quantities of material in complex mixtures such as biological fluids, there is little doubt that ion exchange methods can offer a simpler and in some cases more reliable method of separation for assay than the older methods, such as solvent extraction.

Further work is required not only on direct empirical studies of optimum conditions for various assays, but also on the physico-chemical principles governing the uptake of organic materials by the resins and their subsequent displacement from them.

The resin manufacturers could assist further development of the applications of their products to organic analysis if they would supply them in the form of fine powders of mean particle diameter about 70 μ. Exchanges and absorptions with organic ions are often slow and they can be speeded considerably by reducing the particle size of the resin.

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RESEARCH PAPERS

THE EFFECTS OF ANAPHYLACTIC AND PEPTONE SHOCK ON THE COAGULABILITY OF RABBIT AND GUINEA-PIG BLOOD

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In the dog, in both anaphylactic and peptone shock, there is an increase in the clotting time of the blood. This was first shown for peptone shock by Schmidt-Mülheim[1], and for anaphylactic shock by Biedl and Kraus[2]: and has been amply confirmed by other workers. Howell[3] first suggested that the anticoagulant might be heparin, and some later work by Quick[4] on peptone shock lent support to Howell's view. Waters, Markowitz and Jacques[5]—following the demonstration by Chargaff and Olson[6] that protamine combined with heparin—found that the protamine titre of the blood of an anaphylactic dog was very high, and hence inferred the presence of abnormal amounts of heparin in it. The presence of heparin in such blood was finally established by its isolation in crystalline form by Jaques and Waters[7], who also showed that its source was the liver.

In the guinea-pig and rabbit in anaphylactic or peptone shock the situation is not so clear. Not only have no attempts been made to demonstrate the liberation of heparin in these animals, but there is even dispute as to whether the clotting time of guinea-pig blood is in fact increased in anaphylactic shock. Thus, while Friedberger[8] and Dale[9] reported a prolongation of clotting time, Eagle, Johnson and Ravdin[10] found no change. Dragstedt[11], in a review of anaphylaxis, stated that there is usually no change, although sometimes the blood may become incoagulable. In the rabbit in anaphylactic shock, Auer[12], Scott[13], and Eagle, Johnson and Ravdin[10] noted a delay in blood coagulation. The last-named workers suggested that this was due to an increase in antithrombic activity. They did not, however, suggest that this increase was to be accounted for by heparin—nor, indeed, was there at that time any satisfactory test by which they could have determined the point.

The present work on rabbits and guinea-pigs was undertaken to clarify this confused situation and to determine if heparin is liberated by anaphylactic and peptone shock in these animals, as it is known to be liberated by the same conditions in the dog.

Methods and Results

(a) Anaphylactic shock in rabbits

Rabbits were sensitised by injecting 2 ml. of horse serum on 3 occasions at intervals of 4 days. The first injection was given intravenously, and the second and third intraperitoneally. Anaphylactic shock was induced

4 weeks from the beginning of sensitisation, by the intravenous injection of 6 ml. of the antigen.

Initial experiments indicated that the clotting power of the blood was greatly reduced. It remained to determine if heparin was concerned in this change. For this purpose blood samples from shocked animals were treated with protamine sulphate, following control experiments which indicated that protamine had itself no action on the clotting time of normal blood.

For the control experiments sets of 9 small test tubes were set up in a water bath at 37° C. To the first 5 were added, in 0·9 per cent. saline solution, 0·025, 0·05, 0·10, 0·20 and 0·40 mg. of protamine sulphate respectively. To the sixth and seventh were added 10 units of heparin, the seventh tube having in addition 0·1 mg. of protamine sulphate, which was the amount found to neutralise 10 units of heparin *in vitro*. The eighth and ninth tubes were controls. The volume in each tube was made up to 0·4 ml. with normal saline solution.

In each of 4 experiments approximately 12 ml. of blood was collected by heart puncture from normal rabbits. 1 ml. was added immediately, and at random, to each of the tubes, and the clotting times determined. The results are shown in Table I. These indicate that protamine sulphate has no action on the formation of the normal clot.

TABLE I

CLOTTING TIMES OF NORMAL RABBIT BLOOD AFTER TREATMENT WITH HEPARIN AND PROTAMINE SULPHATE

Normal rabbit No.	Protamine sulphate added to each tube					Heparin 10 units	Heparin 10 units + protamine 0·1 mg.	Control	Control
	0·025 mg.	0·05 mg.	0·10 mg.	0·20 mg.	0·40 mg.				
	minutes							minutes	
1	3½	4½	4	5½	6	>60	5	4	5
2	2	2	2	2½	2	>60	2	2	2
3	3½	3½	4	4½	5½	>60	5	3	5
4	2	2	2	2	3	>60	3	2	2

Similar observations were then made with blood withdrawn from sensitised rabbits 10 minutes after administration of the shocking dose of antigen. The first 5 tubes were set up as in the experiments just described, but the sixth, seventh and eighth tubes were all controls. The

TABLE II

CLOTTING TIMES OF THE BLOOD OF ANAPHYLACTIC RABBITS

Shocked rabbit No.	Protamine sulphate added to each tube					Control	Control	Control	Remarks
	0·025 mg.	0·05 mg.	0·10 mg.	0·20 mg.	0·40 mg.				
			minutes				minutes		
1	—	>60	>60	>60	>60	>60	>60	>60	Slight clot formation
2	31	26	30	27	30	30	25	22	Formation of semi-solid clots only
3	—	30	35	30	36	38	31	29	
4	25	26	21	21	27	29	20	24	
5	28	26	60	37	60	27	31	40	
6	>60	>60	>60	>60	>60	>60	>60	>60	Slight clot formation

blood from shocked animals, unlike that from normal rabbits, did not produce hard and solid clots, and the end-points were consequently more difficult to determine. The times reported, therefore, refer in the main, to the formation of semi-solid clots. The results of these observations are given in Table II. It seems reasonable to infer from them that the lowered coagulability of the blood of rabbits in anaphylactic shock is not due to heparin, since the presence of protamine sulphate, which inactivates heparin, does not affect the clotting time.

(b) Anaphylactic and peptone shock in guinea-pigs

Anaphylactic shock was produced by the intravenous injection of horse serum or 1 per cent. egg white solution, following a sensitising period of at least 14 days. Peptone shock was produced by a single intravenous injection of 1·25 g./kg. of peptone-oxoid, which had been treated with permutit in order to remove most of the free histamine.

Clotting times were obtained in the following way. The heart was exposed at death, which usually occurred within 3 to 5 minutes of the injection, and a glass melting point tube, 150 mm. × 1·0 mm., was inserted into the left ventricle. The tube quickly filled with blood, since the heart continued to beat for a short time after respiration had ceased. A small piece of the tube was then gently broken off one end, every 30 seconds, until thin fibrin threads appeared. The blood was then considered to have clotted. We have found previously that this method, though without the high degree of accuracy of some techniques, has given good results when determining clotting times following the administration of heparin. The results of this series of experiments are given in Table III, the first row of this gives the mean clotting time for a group of 36 normal animals, the others the mean clotting times in various experimental circumstances.

TABLE III

CLOTTING TIMES OF GUINEA-PIG BLOOD IN CONDITIONS OF ANAPHYLACTIC AND PEPTONE SHOCK

Group	Type of shock	Time of death after injection	Time of blood samples	Number of animals	Mean clotting times in minutes ± S.D.
1	Controls	—	—	36	3·7 ± 1·5
2	Anaphylactic	<5 minutes	At death	12	3·4 ± 0·8
3	Peptone	<5 minutes	At death	12	3·2 ± 0·9
4	Anaphylactic	Protected by antihistaminic	15 minutes after injection	12	4·0 ± 1·8
5	Peptone	Protected by antihistaminic	15 minutes after injection	12	3·6 ± 1·0
6	Peptone	10 to 30 minutes	At death	12	3·8 ± 0·4

As previously noted, most animals die from anaphylactic shock within 5 minutes of the injection. There was, therefore, a distinct possibility that even if heparin had been liberated during the initial stages of the shock it would not have been able to exert its influence on coagulability

before the animal died. Groups 2 and 3 in Table III are animals in which this state of affairs obtained. It is evident that the clotting times in both groups do not differ from the control values derived from the normal animals of Group 1.

To overcome the difficulty just mentioned, groups of animals were protected from the fatal effects of the shock by an antihistaminic, and the clotting times determined 15 minutes after administration of the antigen or peptone. Columns 4 and 5 of Table III give the results in these animals. Once more the clotting times do not differ from the control values. Similar results are obtained, in the absence of an antihistaminic, in those animals whose death from peptone shock is delayed, and occurs 10 to 30 minutes after the shocking dose, and in which, therefore, any heparin that might be liberated would certainly have had time to exert an anticoagulant action by the time the blood was withdrawn at death. Here again, however (Group 6, Table III), the clotting times are normal. In guinea-pigs, therefore, it is clear that heparin is not liberated in anaphylactic or peptone shock and, furthermore, that the clotting times in shock are no different from those in normal animals.

Discussion

It is now well established that a common factor, and indeed the salient feature, in anaphylactic and peptone shock in animals is the liberation of histamine. It is clear from previous work that the lowered coagulability of the blood in anaphylactic shock in the dog is due to the liberation of heparin from the liver. It is equally clear from the present work that the blood of shocked rabbits has also a lowered coagulability but that this is not due to heparin. Finally, in guinea-pigs there is no alteration of the blood coagulability in shock. It remains to account for these differences in the blood changes in shock, in face of the common factor of histamine liberation.

In the dog in anaphylactic shock the main site of histamine liberation, as of heparin liberation, is the liver; and this organ is extremely rich in mast cells. Riley and West[14] have recently shown a striking correlation between the mast cell and heparin content of a tissue, and the amount of histamine the tissue contains. Riley[15] had previously shown that when lethal doses of the histamine liberator stilbamidine were administered to rats some of it was temporarily trapped in the mast cells, which then disrupted. From these two sets of observations Riley and West concluded that heparin and histamine coexist in the mast cells. While there is as yet no direct evidence that this is so, their hypothesis accords well with what happens in anaphylactic shock in the dog—especially when one considers the extremely rich distribution of mast cells in the liver of this animal, and the fact that in shock it is from the liver that both the histamine and the heparin are derived.

The absence of heparin liberation in the rabbit is not entirely inconsistent with Riley and West's view. The anaphylactic reaction in this animal is mainly confined to the blood, and the main source of histamine appears to be the platelets[16], which are devoid of heparin. Since tissue

mast cells may accordingly not be involved in the shock reactions, the absence of heparin in these conditions would be explained. This, however, does not account for the lowered blood coagulability which is in fact observed in shocked rabbits, and which must clearly be due to some other factor.

The absence of prolonged clotting times in anaphylactic and peptone shock in the guinea-pig is difficult to explain. There is no doubt that death from shock is caused by liberation of histamine from the lungs, which, in this animal, are comparatively rich sources of it[17]. Histological examination has confirmed the presence of mast cells in the guinea-pig lung. If, therefore, histamine and heparin coexist in these mast cells one would expect their disruption to liberate heparin in an amount sufficient to prolong the clotting time of the blood. It may well be, of course, that mast cells show species differences, and that those of the guinea-pig contain little or no histamine.

Alternatively, the histamine liberated by shock in the guinea-pig may come mainly, or even exclusively, from the reacting structures themselves—the bronchiolar muscle cells—and thus be intrinsic histamine in the sense defined by Dale[18].

There is some evidence that the liberation of only small amounts of histamine within the lungs may cause death in the guinea-pig[19,20,21], and that the source of this histamine may be the mast cell. In that event, if the disruption of only small numbers of mast cells is concerned, the amount of heparin liberated may be insufficient to alter the clotting time of the blood.

Summary

1. While it has been established that anaphylactic shock in the dog causes a lowered coagulability of the blood, and that this is due to the liberation of heparin, what happens to rabbit and guinea-pig blood in similar circumstances is not clear.

2. Experiments are described on anaphylactic shock in the rabbit, and on anaphylactic and peptone shock in the guinea-pig, which seek to clarify this confused situation.

3. The results show (*a*) that though in anaphylactic shock the clotting time of rabbit blood is prolonged, this is not due to the liberation of heparin, and (*b*) that in neither anaphylactic nor in peptone shock is the clotting time of guinea-pig blood prolonged.

4. The results are discussed in relation to the hypothesis that heparin and histamine coexist in mast cells.

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THE GRAPHICAL EVALUATION OF RESULTS OF SIMPLE AND MULTIPLE SLOPE-RATIO ASSAYS

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Graphical methods for the analysis of results of parallel-line quantitative assays have been presented by several writers, for example[1,2,3,4]. The statistical analyses of results of slope-ratio assays, based on a linear relationship between the response and the dose itself, are generally more tedious than for parallel-line assays, particularly when more than one test preparation is investigated at a time. Hence a method for rapid computation of such assays by means of nomograms may prove especially useful for routine work.

A simplified procedure for computation of slope-ratio assays of any number of preparations[5] has been used to develop the nomograms to be described here. In each of the examples to be presented there are a common zero dose and two non-zero doses of each test preparation, with doses evenly spaced; this is the most economic arrangement of doses which will allow tests of validity, and these examples have been chosen as being of the most useful type for routine assays. The nomograms used to estimate the relative potencies are independent of the number of replications used, but for finding the fiducial limits fresh charts are needed for different numbers of replications. A brief description of the construction of charts for assays with more dose-levels, or without a common zero dose, will also be given.

The range has been used to estimate the error variance, with a consequent further saving in computation, and the only calculations necessary involve some addition and subtraction, and a few divisions which may conveniently be done on a slide rule.

Throughout, the notation will be that used in the earlier paper[5] but all symbols will be explained here for completeness.

Examples

Simple assay (one test preparation)

Table I shows a computing sheet for a 5-point assay of one test preparation with four replications. The common zero dose and two non-zero doses of each preparation are evenly spaced, and the upper level of each preparation is taken as one unit.

The total and range for each treatment are entered on the sheet, the ranges are totalled to give the value r, and the treatment totals are used as shown in the table to give the values Q and H which are themselves totalled to give $S(Q)$ and $S(H)$. The values of Q_T/Q_S and Q_S/r are calculated and noted (a slide rule gives sufficient accuracy).

The tests of validity described by Finney[6] as "blanks" and "intersections" are made by examining the values of H. The numerical values of

TABLE I

COMPUTING SHEET FOR SIMPLE SLOPE-RATIO ASSAY

Preparation Dose (units)	Control 0	Standard ½	 1	Test ½	 1	Total
Response ..	1·3	3·6	6·1	3·2	4·7	—
	1·7	3·6	6·3	3·2	4·9	—
	1·5	3·7	6·0	3·1	4·5	—
	1·4	4·1	6·3	2·9	5·0	—
	T_0	S_1	S_2	T_1	T_2	—
Total ..	5·9	15·0	24·7	12·4	19·1	77·1 = G
Range ..	0·4	0·5	0·3	0·3	0·5	2·0 = r
Validity tests						
$H = 2S_1 - S_2$, etc.		5·3		5·7		11·0 = $S(H)$
		$S(H) - 2T_0 = -0·8$				$t_1r = 3·10$ (5 % level)
		$Hs - HT = -0·4$				$t_2r = 2·62$ (5 % level)
Estimation of relative potency and fiducial limits						
$Q = 5(S_1 + 2S_2) - 3G$, etc.		90·7		21·7		
Q_T/Q_S		—		0·239		
Q_S/r		45·35		—		
R (relative potency) ..		—		0·71		
R_L, R_U (5 per cent. fiducial limits)		—		0·66, 0·76		
Calculated values:						
Relative potency				0·707		
5 per cent. fiducial limits				0·658, 0·755		

$S(H) - 2T_0$ and $H_S - H_T$ should not exceed t_1r and t_2r respectively, where t_1 and t_2 for the 5 per cent. and 1 per cent. levels of significance are shown in Table II.

If the validity tests are satisfied, the chart in Figure 1 is then used to estimate the relative potency and its fiducial limits. The points corresponding to Q_T/Q_S are found on the curves A_1A_1', A_2A_2' and joined. The intersection of this line with the vertical axis BB′ gives the value of R, the

TABLE II

VALUES OF t_1 AND t_2 FOR SIGNIFICANCE TESTS IN A SIMPLE 5-POINT SLOPE-RATIO ASSAY WITH n REPLICATIONS

n	t_1		t_2	
	5 per cent.	1 per cent.	5 per cent.	1 per cent.
2	2·41	3·78	2·04	3·20
3	1·71	2·43	1·44	2·05
4	1·55	2·14	1·31	1·81
5	1·50	2·05	1·27	1·73
6	1·49	2·02	1·26	1·70

relative potency. The point corresponding to Q_S/r is then located on the horizontal axis CC′ and joined, in turn, to each of the two Q_T/Q_S points; the points of intersection of these lines, produced if necessary, with the vertical axis BB′ give the 5 per cent. fiducial limits (R_L and R_U).

A scale for R may be put on either of the curves A_1A_1', A_2A_2', in which case the first operation may be avoided, but as the vertical scale for R is identical with that for R_L and R_U, and since both Q_T/Q_S points must be located, the first method avoids some detailed scaling.

In the example, $Q_T/Q_S = 0{\cdot}239$ and, using the chart in Figure 1, on joining the points found on A_1A_1' and A_2A_2', a value of 0·71 for R is found on BB′. The point $Q_S/r = 45{\cdot}35$ on CC′ is joined to the points $Q_T/Q_S = 0{\cdot}239$ on A_1A_1' and A_2A_2', and the intersections of these lines with BB′ give values of R_L and R_U equal to 0·66 and 0·76 respectively. These results compare favourably with the calculated values ($R = 0{\cdot}707$; $R_L = 0{\cdot}658$; $R_U = 0{\cdot}755$).

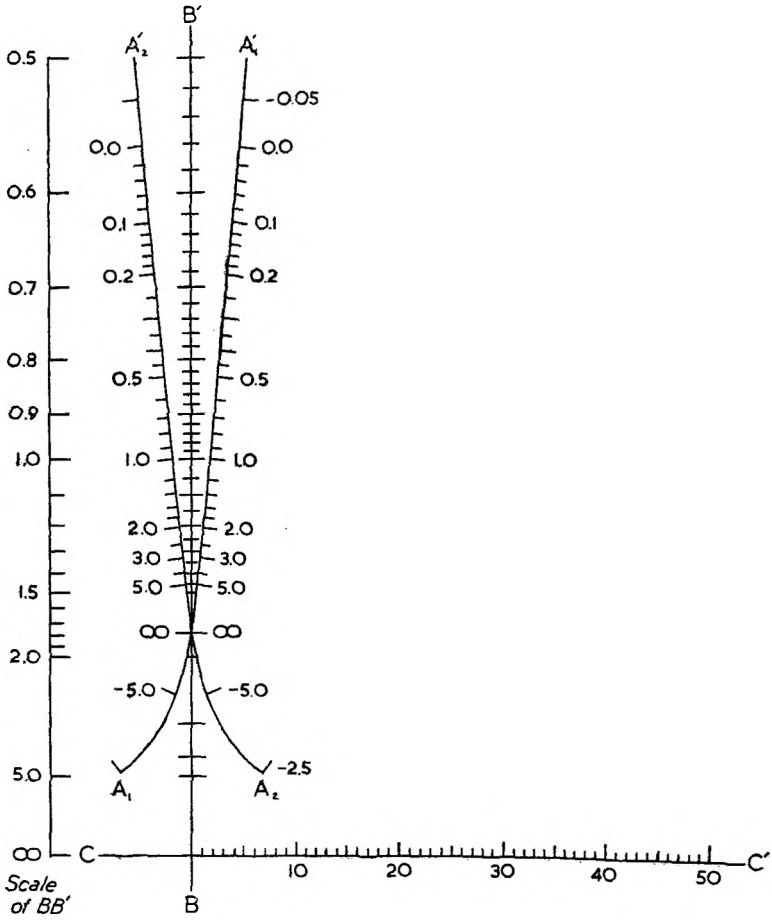

FIG. 1. Nomogram for simple 5-point assay with 4 replications.

(i) Join Q_T/Q_S on A_1A_1' to Q_T/Q_S on A_2A_2' and read R on BB′.

(ii) Join $|Q_S|/r$ on CC′ to Q_T/Q_S on A_1A_1' and A_2A_2' in turn and read R_L, R_U on CC′.

Multiple assay (several test preparations)

The example given here is an assay of three test preparations using a $\{1 + 4(2)\}$-point design, with a common zero dose and two levels of each of the four preparations; there are two replications of each treatment.

TABLE III

COMPUTING SHEET FOR A MULTIPLE SLOPE-RATIO ASSAY

Preparation Dose (units)	Control 0	Standard ½	 1	Test (1) ½	 1	Test (2) ½	 1	Test (3) ½	 1	Total
Response ..	1·10 1·40	4·06 4·23	7·20 6·90	4·05 3·80	6·94 6·40	3·25 3·18	5·10 5·20	3·60 3·60	5·75 5·79	— —
Total ..	T_0 2·50	S_1 8·29	S_2 14·10	T_{11} 7·85	T_{12} 13·34	T_{21} 6·43	T_{22} 10·30	T_{31} 7·20	T_{32} 11·45	$81·46 = G$
Range ..	0·30	0·17	0·30	0·25	0·54	0·07	0·10	0·00	0·05	$1·78 = r$
Validity tests										
$H = 2S_1 - S_2$, etc. ..		2·48		2·36		2·56		2·95		$10·35 = S(H)$
		$S(H) - 4T_0 = 0·35$ Range of $H = 0·59$			$t_1 r = 3·36$ (5 per cent. level) $t_3 r = 2·10$ (5 per cent. level)					
Estimation of relative potencies and fiducial limits										
$Q = 9(S_1 + 2S_2) - 3G$, etc.		84·03		66·39		− 1·11		26·52		$175·83 = S(Q)$
$S(Q)/Q$		2·09		2·65		− 158·4		6·63		—
bs		5·75		—		—		—		—
bs/r		3·23		—		—		—		—
R (relative potency) ..		—		0·93		0·67		0·78		—
RL, RU (5 per cent. fiducial limits ..		—		0·87, 0·99		0·62, 0·73		0·72, 0·84		
Calculated values:—										
Relative potency				0·932		0·672		0·779		
5 per cent. fiducial limits				0·871, 0·993		0·617, 0·728		0·721, 0·836		

Treatment totals and ranges are entered and combined as shown in Table III. The range of the values of H is noted.

For validity, $S(H) - 4T_0$ should not numerically exceed $t_1 r$, and the range of H should not exceed $t_3 r$, where t_1 and t_3 for different significance levels are shown in Table IV. These tests correspond respectively to the tests for blanks and intersections described by Clarke[5].

TABLE IV

VALUES OF t_1 AND t_3 FOR SIGNIFICANCE TESTS IN A MULTIPLE $\{1 + 4(2)\}$-POINT SLOPE-RATIO ASSAY WITH n REPLICATIONS

n	t_1		t_3	
	5 per cent.	1 per cent.	5 per cent.	1 per cent.
2	1·89	2·72	1·18	1·51
3	1·43	1·96	0·93	1·17
4	1·33	1·79	0·87	1·09
5	1·30	1·74	0·86	1·07
6	1·30	1·73	0·86	1·07

If the assay appears to be valid, the relative potencies are found from Figure 2 as follows. The points corresponding to $S(Q)/Q_S$ and $S(Q)/Q_T$ are found on the lines OO′ and P_1P_1' respectively and joined; the intersection of this line with P_2P_2' gives the value of R, the relative potency.

Thus in the example, $S(Q)/Q_S = 2·09$, and for the first test preparation $S(Q)/Q_T = 2·65$. A line joining the points corresponding to these values gives an estimate of the relative potency as 0·93 (the value obtained by calculation is 0·932).

To find the fiducial limits of the relative potencies, it is first necessary

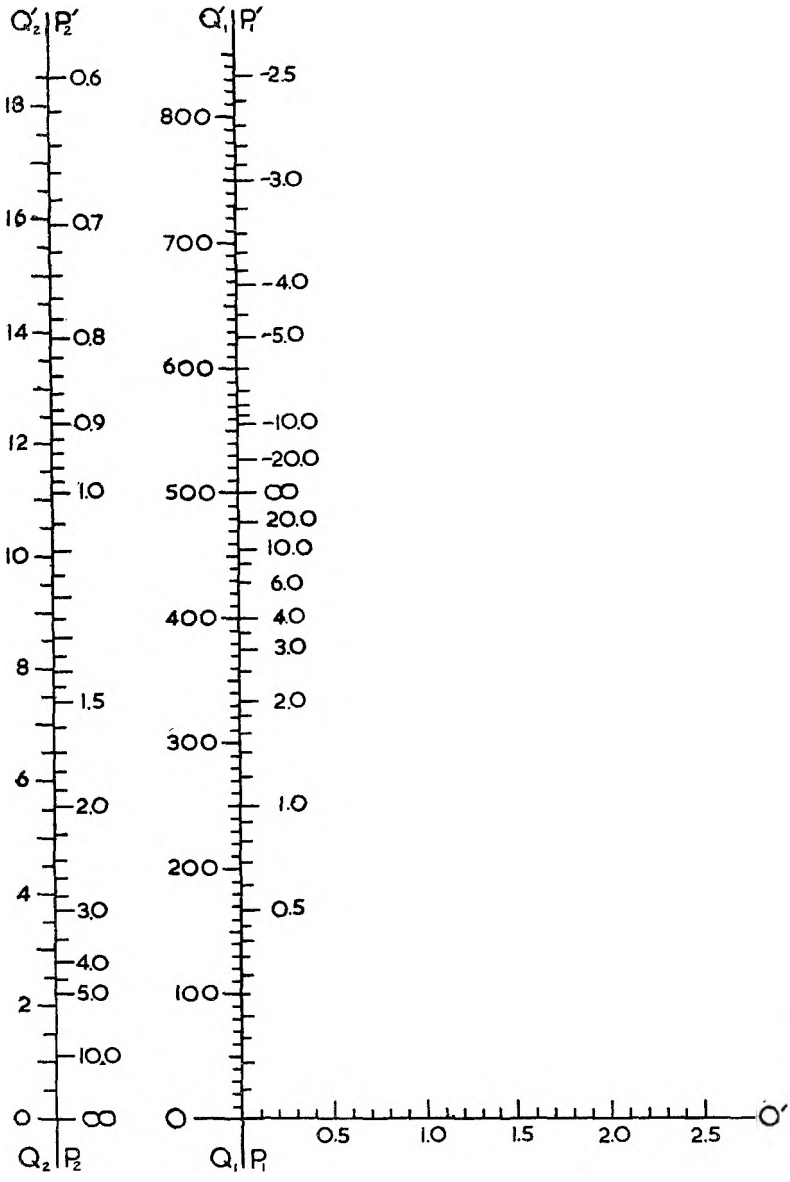

FIG. 2. Nomogram for multiple {1 + 4(2)}-point assay with 2 replications—I.
(i) Join $S(Q)/Q_S$ on OO′ to $S(Q)/Q_T$ on P_1P_1' and read R on P_2P_2'.
(ii) Join $S(Q)/Q_S$ on OO′ to $|S(Q)|$ on Q_1Q_1' and read $|b_S|$ on Q_2Q_2'.

to estimate $|b_S|$, the numerical value of the slope of the dose-response line for the standard preparation. This is also obtained from Figure 2, by joining the points corresponding to $S(Q)/Q_S$ and $|S(Q)|$, located on OO′ and Q_1Q_1' respectively, and reading off the value of $|b_S|$ given by the

intersection of this line with Q_2Q_2'. In this example $|b_s|$ is found to be about 5·75 (calculated value 5·775).

The chart in Figure 3 is then used to find the 5 per cent. fiducial limits of the relative potencies. The values of $|b_s|/r$ is calculated as 5·75/1·78, i.e., 3·23, and the corresponding point on the horizontal axis XX′ is joined, in turn, to the points on the curves Y_1Y_1' and Y_2Y_2' corresponding to the relative potency of any one test preparation. The intersections of these two lines with the vertical axis ZZ′ give the upper and lower fiducial limits. For the first test preparation in this example the 5 per cent. fiducial limits are found to be 0·87 and 1·00 (calculated values 0·871 and 0·993). The corresponding values for the other two test preparations may be rapidly read off in the same way.

Construction of the Charts

The nomogram for the simple slope-ratio assay is derived from the relations

$$R = \{1 + J\,Q_T/Q_S\} \,/\, \{J + Q_T/Q_S\}$$

and $R_L, R_U = R \pm Kd_n^{-1}\, t\, (r/Q_S)\, (J - R)\, [\gamma(R-1)^2 + 2R]^{\frac{1}{2}}$,

where $\gamma = (5k^2 + 5k + 2)/2\,(k^2 + k + 1)$,

$$J = \gamma/(\gamma - 1),$$

$$K = \frac{1}{(J+1)}\sqrt{\frac{nk\,(k+1)\,(2k+1)}{6}},$$

k is the number of non-zero dose-levels of each preparation, n is the number of replications of each treatment and d_n, the factor used in estimating the standard error from the range, is given in Table V.

TABLE V

Values of d and ν*

i	d_i	ν_i
2	1·128	7·27
3	1·693	10·95
4	2·059	14·49
5	2·326	17·86
6	2·534	21·08
7	2·704	24·11
8	2·847	27·02
9	2·970	29·82

* The above values, constants of the range distribution, have been obtained from tables given by Cox[7] based on data published by Pearson[8].

A suitable nomogram for solving these equations is obtained by first drawing rectangular coordinate axes, marking off on the x-axis a scale for $|Q_S|/r$ such that $x = \mu|Q_S|/r$, and on the y-axis marking off a scale for R_L and R_U such that $R_L,\ R_U = \delta/y$, where δ and μ are any convenient constants. The curves for R are then given by the equations

$$x = \pm\,\mu K\, d_n^{-1}\, t\, (J - R)\, [\gamma(R-1)^2 + 2R]^{\frac{1}{2}}/R,\ y = \delta/R,$$

where the value of t for the appropriate significance level is obtained from standard tables, entering the tables with $(2k + 1)(n - 1)$ degrees of freedom. As explained in the example, it is convenient to mark off the

curves only with scales for Q_T/Q_S given by $y = \delta(J + Q_T/Q_S)/(1 + JQ_T/Q_S)$, and to use the y-axis to read off values of R.

For a simple slope-ratio assay without a zero dose, the procedure is just the same, except that γ is now equal to $(5k + 1)/2\ (k - 1)$, Q is given by, for example, $Q_S = 4(S_1 + 2S_2 + \ldots + kS_k) - (k + 1)\,G$, K is replaced by L, where

$$L = \frac{1}{(J + 1)}\sqrt{\frac{2n(k + 1)(2k + 1)}{3k}},$$

and the t-table is entered with $2k$ (n $-$ 1) degrees of freedom.

When more than one test preparation is investigated at a time, the corresponding expressions for the relative potency and the fiducial limits are $R = \{\gamma - 1 + Q_T/S(Q)\}/\{\gamma - 1 + Q_S/S(Q)\}$

and $R_L, R_U = R \pm \frac{t}{(vk + 1)\,d_n}\left(\frac{r}{b_S}\right)\sqrt{\frac{6k\,[\gamma(R - 1)^2 + 2\,R]}{n\,(k + 1)\,(2k + 1)}}$,

where $\gamma = \frac{vk\,(k - 1) + (3k + 1)\,(k + 2)}{v\,k\,(k - 1) + 2(2k + 1)}$, v is the number of preparations including the standard, t is found from tables with $(vk + 1)\,(n - 1)$ degrees of freedom, and

$b_S = 6[Q_S + (\gamma - 1)\,S(Q)]/n\,(k + 1)\,(2k + 1)\,(vk + 1)$.

These relations lead to three nomograms of which two can be drawn on the same chart. To find the value of R and of $|b_S|$, rectangular coordinate axes are drawn as in Figure 2. The x-axis is marked off to give values of $S(Q)/Q_S$ using the linear scale $x = \eta\,(\gamma - 1)\,S(Q)/Q_S$, where η is a convenient scaling constant. The y-axis is marked off with two scales: one, linear, for $S(Q)$ given by

$$y = 6\theta\,(\gamma - 1)|S(Q)|/n(k + 1)\,(2k + 1)\,(vk + 1)$$

and the other, non-linear, for $S(Q)/Q_T$ given by

$$y = \phi[(\gamma - 1)S(Q)/Q_T]/[1 + (\gamma - 1)S(Q)/Q_T],$$

θ and ϕ being further constants chosen for convenience. Another vertical axis is drawn at $x = -\eta$ and marked off with a linear scale for b_S given by $y = \theta|b_S|$, and an inverse scale for R given by $y = \phi/R$.

The nomogram for finding the fiducial limits of each value of R is exemplified in Figure 3. The scale for $|b_S|/r$ on the x-axis is given by $x = \mu|b_S|/r$, and that for R_L, R_U on the y-axis by $R_L, R_U = \delta/y$. The equations of the curves for R are

$$x = \pm\frac{\mu\,t}{(vk + 1)d_n}\sqrt{\frac{6k[\gamma(R - 1)^2 + 2R]}{n(k + 1)\,(2k + 1)R^2}},\ y = \frac{\delta}{R}$$

so that the vertical scale for R is the same as that for the fiducial limits.

For an assay without a common zero dose, the charts are similar, but γ is then equal to $[v\,(k - 1) + 3(k + 1)]/v(k - 1)$, Q is given by, for example, $Q_S = 2v\,(S_1 + 2S_2 + \ldots + k\,S_k) - (k + 1)\,G$, the scale for $S(Q)$ is $y = 3\theta|S(Q)|(\gamma - 1)/n\,v\,(k + 1)\,(2k + 1)$, the equation of the curve for R has vk instead of $(vk + 1)$ in the denominator of the expression for x, and the t-table is entered with $vk(n - 1)$ degrees of freedom.

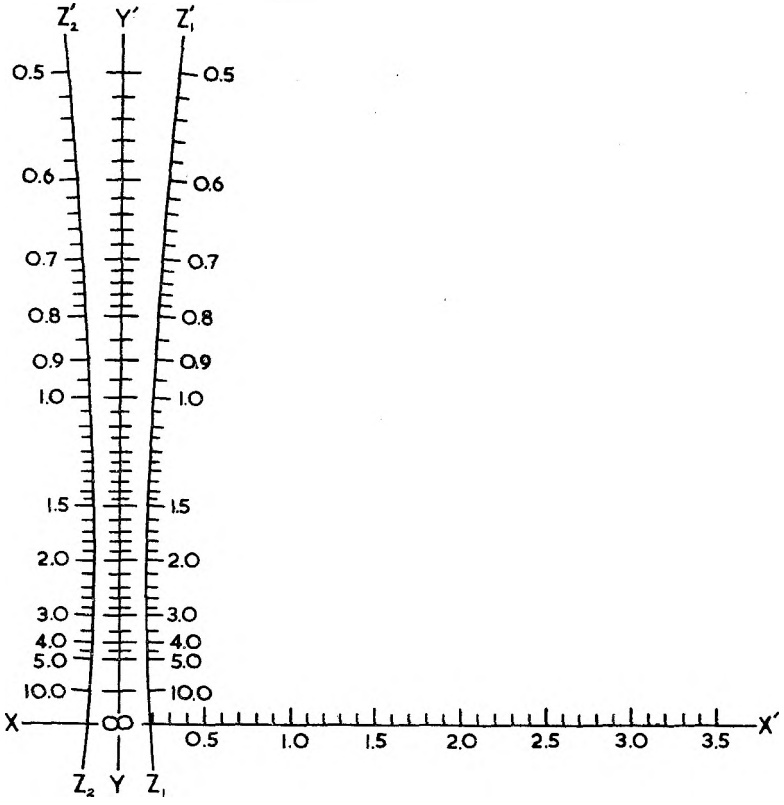

FIG. 3. Nomogram for multiple {1 + 4(2)}-point assay with 2 replications—II. Join $|b_S|/r$ on XX′ to R on Z_1Z_1' and Z_2Z_2' in turn and read R_L, R_U on YY′.

VALIDITY TESTS

The first step in the general application of validity tests as used in the above examples is the calculation of values of H where

$$H_S = 2(k-1)S_1 + [2(k-1) - 3]S_2 + [2(k-1) - 6]S_3 + \ldots - (k-1)S_k,$$

and so on.

The test for "blanks" involves calculating the quantity $S(H) - vk(k-1)T_0/2$, and comparing it with $t_1 r$, where

$$t_1 = \frac{tk}{2(vk+1)d_n}\sqrt{\frac{3vn(k^2-1)}{\gamma-1}},$$

d_n is given in Table V and t is found from standard tables with $(vk+1)(n-1)$ degrees of freedom. No test for "blanks" is made if there is no common zero dose.

In the case of a simple assay with a common zero dose the test for "intersections" is made by comparing the difference between the values of H for the two preparations with $t_2 r$, where $t_2 = t\sqrt{nk(k-1)/(2k+1)}/d_n$ and t has $(2k+1)(n-1)$ degrees of freedom; when there is no common zero dose $t_2 = t\sqrt{n(k-1)(2k+1)/k2}d_n$ and t has $2k(n-1)$ degrees of freedom.

For a multiple assay, the corresponding test for "intersections" may be made using a range test described by Cox[7]. When there is a common zero dose the range of the values of H should not be greater than $t_3 r$ where $t_3 = d_v F_{v_1,v_2} \sqrt{nk(k-1)(2k+1)/2}/(vk+1)d_n$. F is found from variance ratio tables with v_1 and v_2 degrees of freedom, where $v_1 = v_v$ and $v_2 = (vk+1)v_n$, using the values of v given in Table V. When there is no common zero dose, $t_3 = d_v F_{v_1,v_2} \sqrt{n(k-1)(2k+1)/2k}/v\, d_n$, $v_1 = v_v$ and $v_2 = vkv_n$.

If k is greater than 2, further tests of validity may be made by examining the deviations from linearity for the responses to non-zero doses; such tests are easily effected by using the usual orthogonal coefficients for the treatment totals (see, for example[9,10]). Thus for $k = 3$, the quantities $C_S = S_1 - 2S_2 + S_3$, etc., are computed for each preparation. The deviation from linearity for any one preparation will be significant if the corresponding value of C is greater than $t_4 r$, where $t_4 = t\sqrt{6n}/(vk+1)d_n$, and t is found for $(3v+1)(n-1)$ or $3v(n-1)$ degrees of freedom, depending on whether there is or is not a common zero dose. Alternatively an overall test of curvature may be made by examining whether the total of the values of C exceeds $\sqrt{k}\, t_4 r$. It is unlikely that in routine assays k will exceed 3, and consequently further tests for $k > 3$ will not be given here.

Discussion

Charts such as those presented here considerably reduce the time required for evaluation of the estimates of relative potencies and their fiducial limits in slope-ratio assays, and should prove particularly useful for multiple assays, which are common in microbiological work. Once the experimental design has been decided on, the charts are drawn up and may be used repeatedly.

The use of range in place of standard deviation cuts down the necessary computing, but does not give the best estimate of error except when $n = 2$, and may introduce a bias in the estimates of fiducial limits if the responses are not normally distributed. When n is small, however, these limitations are unlikely to be of practical importance. The procedure described in this paper for estimating the fiducial limits also applies only when the observations are not arranged in blocks. A further qualification of the method is that the fiducial limits are obtained from the approximate variance of R, applicable only when the assay is of good precision (see, for example[9,10]); this condition should, however, be aimed at before an assay method is adopted for routine use and, in any case, holds for most microbiological assays. The estimates of relative potency obtained from the chart are unaffected by these considerations.

Summary

Charts are presented and described for the rapid evaluation of results of simple and multiple slope-ratio assays with and without a common zero dose.

We wish to acknowledge the assistance of Miss A. Jordan in the early stages of this work, and of Miss B. K. Allchorne and Miss K. Rhymes in the computation.

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A COMPARISON OF PHYSICAL AND CHEMICAL METHODS WITH BIOLOGICAL ASSAY OF VITAMIN A*

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IN 1946, Morton and Stubbs proposed their correction procedure for the spectrophotometric determination of vitamin A[1]. Since then it has been adopted as an official method by the U.S. Pharmacopeia[2] and the Association of Official Agricultural Chemists[3]. Both bodies specify that the correction be applied only after saponification. The British Pharmacopœia[4] has also adopted this method but requires saponification only for cod-liver oils, the correction being applied directly to whole oil solutions of halibut-liver oil and concentrates. Since 1951, the Food and Drug Laboratories have used a method essentially the same as that of the U.S.P. XIV[5].

Although used extensively, the method also has been subjected to criticism. Gridgeman[6] discussed the theory of the three-point correction very fully and concluded that direct evidence of its validity is needed. Cama, Collins and Morton[7], in a very comprehensive paper, have stated that the correction procedure gave erroneously low values. They supported this statement by the results of detailed analysis of a cod-liver oil and two high potency oils. For good quality, low potency oils, they recommended the use of uncorrected *E* values, measured on unsaponifiable extracts, with a correction made for vitamin A_2 only. These authors believed that the application of the correction to solutions of whole oils of this type gave low results, unless a suitable adjustment for the presence of *neo*vitamin A was also made. In the analysis of high potency oils they recommended the use of solutions of the whole oil, with correction of the results by the application of two or more formulæ. They stated that results obtained in this way also required adjustment for the presence of *neo*vitamin A. The correction procedure when applied to unsaponifiable extracts gave erroneously low results, apparently because the irrelevant absorption was not linear. They suggested that the non-linear irrelevant absorption was caused by isomerism during saponification. The criterion used by these authors was chemical purity as determined by a comprehensive analysis of 3 oils. In reaching these conclusions they assumed that *neo*vitamin A was equal in biological potency to all-*trans* vitamin A and that vitamin A_2 had no biological activity. Both Cama *et al.*[7] and Bagnall and Stock[8] have pointed to the need for further data regarding these points. Swann[9] discussed the determination of vitamin A in cod-liver oil and stated that corrected *E* values, as determined on the unsaponifiable matter, underestimated this type of oil. This conclusion

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was reached as a result of comparisons with other methods, including a chromatographic procedure.

Melnick *et al.*[10] also reached the conclusion that the geometric correction, applied to unsaponifiable fractions of high potency oils, gave low results. They based their conclusions on a comparison with rat growth assays and attributed the low results obtained by the U.S.P. method to the presence of *neo*vitamin A and vitamin A_2. Unpublished results of a similar comparison conducted by an informal committee of the U.S.P.[11] indication of over-correction to the extent reported by Melnick *et al.* conservative estimate of biological potency. There was, however, no indication of overcorrection to the extent reported by Melnick *et al.*

Analysts concerned with market samples frequently have to take into account the presence of oxidised vitamin A as well as *neo*vitamin A and vitamin A_2. In some samples slight oxidation will be indicated by a decrease in the absorption at 325 mμ accompanied by a relative increase in the absorption at 310 mμ. More extensive oxidation will result in a shift of the maximum absorption from 325 mμ so that the application of the correction is no longer valid. This problem has been discussed by Bagnall and Stock[8], who pointed out that while the practice of multiplying gross E values by a factor of 1600 may be useful when dealing with fresh oils, it could lead to very erroneous results with oxidised samples. Since these samples are generally below label claim, the problem is important in practice if an exact potency must be assigned.

Another aspect of the correction procedure that has been criticised, particularly by manufacturers, is that it is unsuitable for routine analysis because of the length of time required to complete an assay. The elimination of either saponification or correction would do much to simplify the procedure. Swann[9] has reported that for cod-liver oils there is fair agreement between E (gross) $\times$ 1600 and E (corr.) $\times$ 1900 as determined on the whole oil. He intimates, however, that results obtained in this manner are high. Bagnall and Stock[12] have reported that for halibut-liver oils the agreement is good between E (gross) $\times$ 1600 and the geometric correction. As noted above, this is not suitable for samples which have become oxidised.

Finally, there appears to be one other point deserving mention at this time. The U.S. Pharmacopeia has described a single method for all pharmacopœial substances. The British Pharmacopœia, on the other hand, gives considerable freedom, particularly with regard to the saponification of concentrates. Cama *et al.*[7] have proposed a scheme by which, if assumptions made are correct, a very precise and accurate estimate of the true vitamin A content may be obtained. Such methods as the latter, however, are not readily adapted to routine analysis where hundreds of samples are being assayed. For most effective control, uniformity of procedure, while possibly sacrificing the ultimate in accuracy, has much to recommend it as far as governmental control or industrial transactions are concerned.

Since the correction procedure has been widely adopted for routine control, and since there have been few published comparisons involving

this method, it seemed of prime importance to determine the relative merits of the Morton–Stubbs method as an indicator of biologically active vitamin A under conditions of routine analysis. Any discussion of chemical methods for the determination of vitamin A is based on certain assumptions of biological activity. One of these assumptions involves the biological potency of *neo*vitamin A which has not, as yet, been definitely established. It seemed important, therefore, to obtain further data on this point. It was also of interest to determine the effect of oxidised vitamin A on the various assay procedures. This paper presents the results of studies on these problems.

Methods

The biological potencies were estimated by the vaginal smear method developed in this laboratory by Pugsley, Wills and Crandall[13], and by the U.S.P. XIV growth assay. 3 dosage levels for both the standard and sample were used in all assays and the results of each assay were

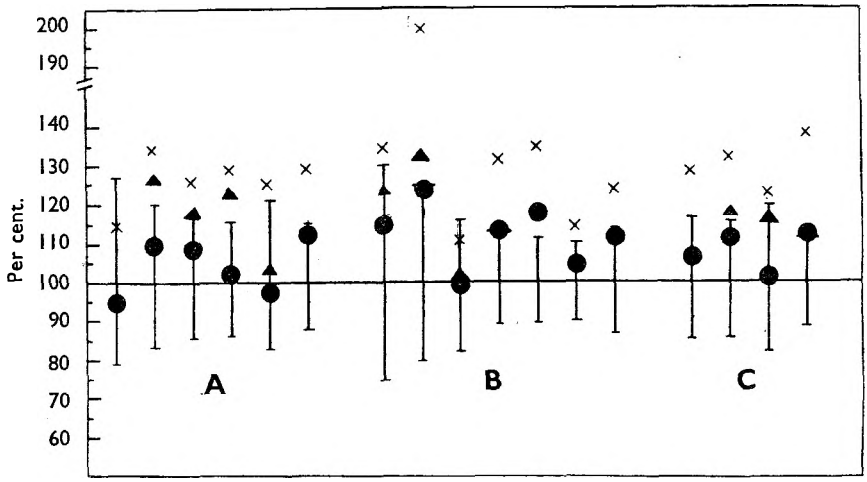

Fig. 1. A comparison of the corrected, uncorrected and antimony trichloride methods with vaginal smear assays of Vitamin A on cod-liver oils, concentrates and halibut-liver oils. Bioassay potency is plotted as 100 per cent.

A. Cod-liver oils.
B. Concentrates.
C. Halibut-liver oils.

I Biological.
● Corrected.
× Uncorrected.
▲ Antimony trichloride.

calculated from the data on 48 to 60 rats. The vaginal smear method was chosen for the majority of the comparisons because of its relatively greater precision, the speed with which an assay could be completed and because several assays could be completed on one group of rats. Calculations of potency in the growth assays were based on figures obtained by considering the weights of rats at each week of test as suggested by Bliss and György[14]. The U.S.P. XIV methods were followed for the corrected spectrophotometric and colorimetric determinations which were

made using a Beckman model D.U. spectrophotometer and Evelyn colorimeter respectively. Uncorrected results were calculated by multiplying the uncorrected E_{325} by 1900, 1600 or 1450 as indicated in the text. The samples used in this study were pharmaceutical grade cod- and halibut-liver oils and concentrates, most of which were retail products obtained from manufacturers.

Experimental Results

Comparison of methods. Samples of cod- and halibut-liver oils and concentrates were assayed in duplicate, spectrophotometrically and colorimetrically. The results were compared with biological potencies as determined by the vaginal smear method. In cases where there was a wide difference between results, the biological assay was also done in duplicate. The results of these assays are shown in Figure 1 and in Table I. Except for one halibut-liver oil and two concentrates, all the corrected spectrophotometric results fall within the fiducial limits of the bioassay. It should be noted that there does not appear to be any tendency for the Morton and Stubbs procedure to overcorrect. In fact, the corrected spectrophotometric results are, on the average, 3·9 per cent. higher than the bioassay for cod-liver oils, 7·9 per cent. higher for halibut-liver oils and 12·3 per cent. higher for concentrates. If the factor of 1820, which has been reported by Cama *et al.*[7] to be more correct for vitamin A alcohol, had been used, the difference would have been about 0, 4 and 8 per cent. respectively.

TABLE I

VITAMIN A CONTENT OF FISH OILS AS DETERMINED BY SEVERAL METHODS

	Potency expressed as I.U./g. or capsule					
	Spectrophotometric		Antimony trichloride	Biological assay (vaginal smear)		
Sample	*E* gross × 1900	*E* corr. × 1900		Potency	Confidence limits (P = 0·05)	
Cod-liver oils ..	2220	1840	—	1940	1530	2470
	2250	1840	2120	1680	1400	2020
	3530	3040	3300	2790	2390	3260
	2370	1860	2260	1830	1590	2120
	1090	850	960	870	720	1050
	2250	1960	—	1750	1530	2010
Concentrates ..	3900	3340	3610	2910	2220	3810
	53,600	32,900	35,400	26,800	21,300	33,600
	55,900	50,500	50,800	50,500	41,700	61,100
	70,400	60,800	—	53,600	47,700	60,500
	215,000	188,000	—	15,900	143,000	177,000
	211,000	187,000	—	177,000	16,100	195,000
	120,000	103,000	—	90,900	79,800	104,000
Halibut-liver oils	5290	4370	—	4100	3520	4800
	66,700	56,000	59,500	50,300	43,400	58,400
	4850	4010	4610	3940	3270	4740
	92,400	75,200	—	66,800	59,400	75,000

The uncorrected results, on the other hand, are outside the limits of the bioassay except in the case of one cod-liver oil. The percentage by which the uncorrected results exceeded the bioassay for cod-liver oils was 26·2 per cent., for halibut-liver oils 30·6 per cent. and for concentrates 36·3 per cent. The antimony trichloride values fell in an intermediate

position and are within the fiducial limits of the bioassay in the case of one cod-liver oil, one halibut-liver oil and two concentrates. The extent to which the colorimetric results over-estimated the biopotencies were, for cod-liver oils 17·6 per cent., for halibut-liver oils 17·7 per cent. and for concentrates 18·9 per cent. The colorimetric results have been shown by an analysis of variance to differ significantly ($P = 0{\cdot}01$) from the corrected spectrophotometric results.

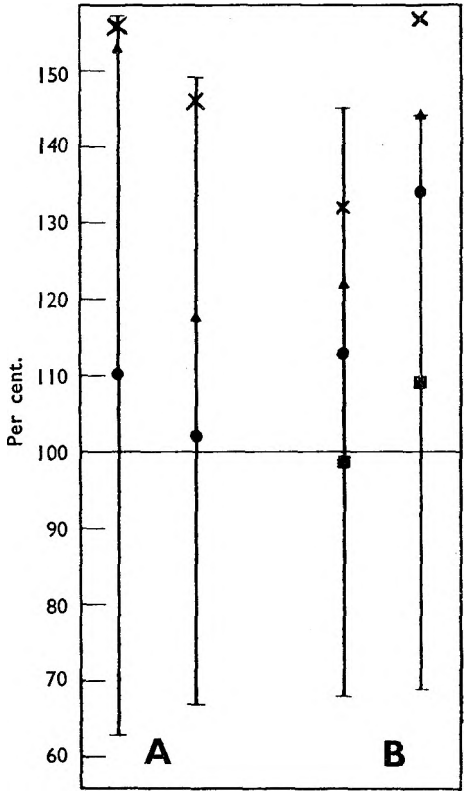

FIG. 2. A comparison of corrected, uncorrected and antimony trichloride methods with bioassays of Vitamin A in cod-liver oils and concentrates. Growth assay potency is plotted as 100 per cent.

A. Cod-liver oils.
B. Concentrates.
I. Biological, growth.
■ Biological, vaginal smear
● Corrected.
× Uncorrected.
▲ Antimony trichloride.

Figure 2 shows the results of similar comparisons in which the rat growth assay was used in place of the vaginal smear method. The first two samples in this comparison were low potency cod-liver oils of the type used for animal feeding. The other two oils were concentrates which had been assayed previously by the vaginal smear method. The relation between potencies as determined by chemical and physical assays and the growth assay agree well with those shown in Figure 1. The corrected values are again the best estimate of biological potency and there is no tendency for the Morton and Stubbs procedure to over-correct. Agreement between potencies estimated by the growth and vaginal smear assays was good.

Potency of neo*vitamin A.* The suggested over-correction by the Morton and Stubbs method has been attributed, in part, to the presence of *neo*vitamin A. Since the results discussed above show no tendency towards over-correction, it was of interest to determine the potency of *neo*vitamin A using the vaginal smear assay. A sample of crystalline *neo*vitamin A acetate in Wesson oil and a sample of crystalline *neo*vitamin A alcohol*

* Samples of *neo*vitamin A kindly supplied by Dr. Norris D. Embree, Director of Research, Distillation Products, Ind., Rochester, N.Y.

were diluted with corn oil and assayed against all-*trans* vitamin A acetate. The results of these assays, shown in Table II, are in good agreement with the values reported by Harris, Ames and Brinkman[15], who found a potency for *neo*vitamin A of 75·6 per cent. of all-*trans* vitamin A when measured by the liver storage method and 83·2 per cent. by the growth assay. The potencies reported here appeared to agree better than would be expected from the confidence limits of the individual assays.

TABLE II

POTENCY OF *neo*VITAMIN A ACETATE ON A PER UNIT *E* VALUE BASIS RELATIVE TO ALL-*trans* VITAMIN A ACETATE

Assay	Sample	Percentage of all-*trans* A	I.U./g.	Limits in per cent. (P = 0·05)
1	Acetate in oil RL 330	71·3	2,377,000	83·5–119·5
2	" "	74·3	2,477,000	84·7–118·0
3	Alcohol crystals 817 B	73·0	2,433,000	89·5–111·7
Mean (logarithmic)	.. .. ..	72·0	2,400,000	91·6–109·1

Presence of oxidised vitamin A. Oxidised vitamin A is one of the constituents most commonly encountered in routine analysis of vitamin A in retail products. It was therefore of interest to determine whether the correction procedure gave reliable results when applied to a partially oxidised oil. For this purpose a sample of cod-liver oil was subjected to progressive oxidation by bubbling oxygen through it at 90° C. As the potency dropped, samples were removed, flushed with nitrogen and refrigerated until biological, spectrophotometric and colorimetric assays were carried out. The results of these assays are shown in Figure 3. It will be noted that there is good agreement between the results of the corrected spectrophotometric assay and those of the biological assay in the two samples to which the correction could be validly applied. The correction could not be applied to the third sample because the absorption maximum had shifted from 325 mμ.

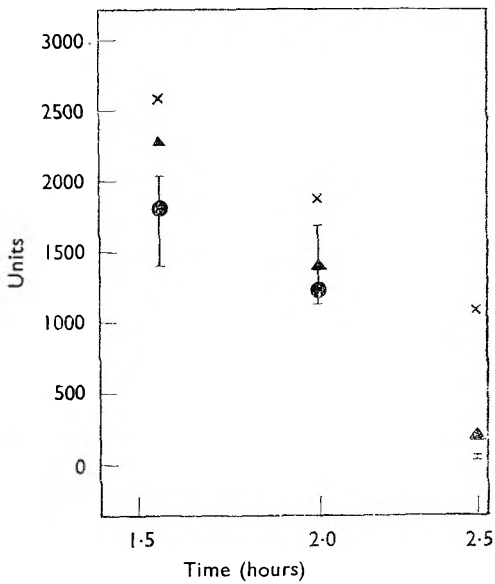

FIG. 3. A comparison of methods for the determination of vitamin A in an oxidised cod-liver oil.
I Biological.
● Corrected.
× Uncorrected.
▲ Antimony trichloride.

The uncorrected results are outside the limits of the bioassay in all

cases, while the antimony trichloride method gave values that fell between the corrected and uncorrected potencies. When the oxidation had reached the point at which the correction could not be applied, neither the antimony trichloride nor the uncorrected spectrophotometric figures was a good estimate of biological potency. This latter observation is supported by unpublished data on other oils in which extensive oxidation had taken place.

Determinations on whole oil solutions. Another of the criticisms of the spectrophotometric method, as it is now used, is lack of adaptability to routine analysis. The elimination of saponification, if practicable, would do much to simplify this method. It was of interest, therefore, to compare results obtained by the U.S.P. XIV method with those obtained by applying the correction directly to solutions of the whole oil. The solvent chosen for this work was *cyclo*hexane, which is used in the method

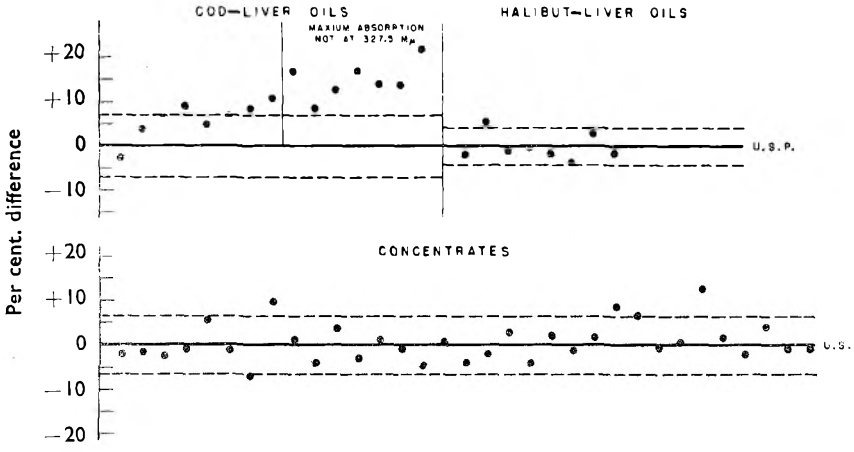

FIG. 4. A comparison of the corrected potency of fish oils determined on the whole oil and non-saponifiable basis. Base line is U.S.P. method non-saponifiable basis.

of the British Pharmacopœia for this determination. The appropriate formula for vitamin A ester was derived from the absorption curve of the Canadian Reference Standard vitamin A acetate. The wavelengths at which absorption was 6/7 maximum were found to be 312·5 and 337·7 mμ with the maximum at 327·5 mμ. These figures agree with those published by Cama *et al.*[7] and it follows that the correction formula is $7(E_{327\cdot5} - 0\cdot405E_{312\cdot5} - 0\cdot595E_{337\cdot7})$. *The E value at 327·5* was found to be 5·11, so that the appropriate conversion factor was 1958 rather than 1900, which was used in determinations of the unsaponifiable fraction in *iso*propanol.

Samples of pharmaceutical grade cod-liver oil, halibut-liver oil and concentrates were assayed in duplicate, on different days, by the application of the appropriate correction formula to absorption readings of unsaponifiable fractions in *iso*propanol, and of whole oil solutions in *cyclo*hexane. The comparison of these results is shown in Figure 4.

The cod-liver oils fell into two categories, those in which the absorption maxima of the whole oil solutions occurred at or near 327·5 mμ and those to which the correction could not be validly applied because maximum absorption occurred between 320 and 325 mμ. It may be noted that using the regular 3-point correction, no distinction would be made between these oils since the absorbancy at 310 mμ in all cases was less than at 327·5 mμ. The absorption maxima of all unsaponifiable fractions occurred at 325 mμ. In neither group was there good agreement between the results of the two methods, the difference between them being significant at P = 0·01. This indicated that it was not possible to apply the correction directly to cod-liver oils. In the case of concentrates and halibut-liver oils there was no significant difference between the results of the two methods. Only 3 samples fell outside the limits of error (2 × S.E. of the difference between duplicate determinations), the maximum deviation being 12·6 per cent. and the average deviation a fraction of 1 per cent. If, as Cama *et al.*[7] have reported, the conversion factor of pure vitamin A acetate in *cyclo*hexane is 1920, the values reported here should be reduced by 2 per cent.

Elimination of correction. The potencies of the same group of oils were also estimated by multiplying gross *E* values by a factor, without first correcting for irrelevant absorption. Since all samples examined contained irrelevant absorption it was obvious that the factor of 1900 was too large. The factor of 1600 which, according to Morton[16] is as good as could be chosen, was therefore used. For cod-liver oils it was soon found that, as judged by the U.S.P. XIV method, the conversion factor of 1600 was too high. Consequently a factor of 1450, obtained by calculating the average factor required to arrive at the corrected potency, was used for cod-liver oils while 1600 was retained for halibut oils and concentrates. The results obtained in this manner were compared with those found by the U.S.P. method and the analysis of variance of the logarithms of the data from this comparison is shown in Table III.

TABLE III

ANALYSIS OF VARIANCE OF VITAMIN A ACTIVITY (IN LOGARITHMS) AS MEASURED BY THE U.S.P. AND GROSS *E* × 1600 BASIS

Source of variation	Halibut-liver oils			Concentrates			Cod-liver oils		
	D.F.	Mean square	F.	D.F.	Mean square	F.	D.F.	Mean square	F.
Methods ..	1	0·000351	3·98	1	0·000249	2·18	1	0·000434	1·38
Samples ..	5	1·850703	20,971*	9	0·755005	6617*	11	0·179199	572*
Method X samples ..	5	0·000237	2·68	9	0·001151	10·08*	11	0·0016100	5·14*
Error ..	12	0·00008825		20	0·0001141		24	0·00031291	

* Significant at P = 0·01.

It will be noted that none of the mean squares for methods was significant which shows that the factors chosen were of the right magnitude. An examination of the mean squares for the method X sample interaction shows that this was significant at P = 0·01 for cod-liver oils and concentrates. This indicated that some cod-liver oils and concentrates were

over-estimated, while others were under-estimated by the elimination of the correction. The halibut-liver oils which were tested were sufficiently uniform in irrelevant absorption that a similar estimate of potency was obtained on the uncorrected and on the corrected basis. This is in agreement with results published by Bagnall and Stock[12].

Discussion

One of the most serious criticisms which has been made of the Morton–Stubbs correction, as applied in the U.S.P. XIV method, is that it under-estimates the true potency of samples. The results of comparisons of methods which have been carried out in this laboratory give no indication of under-estimation of biological activity by the U.S.P. spectrophotometric method.

In the case of cod-liver oils, the geometric correction applied to unsaponifiable fractions gave results which were in excellent agreement with the biological potencies as measured by the vaginal smear method. Uncorrected results on the same oils were, on the average, 26·2 per cent. higher than the potencies determined by biological assays. Similar results were obtained when two low potency cod-liver oils were assayed spectrophotometrically, colorimetrically and by the rat growth assay. These results appear at variance with those of Cama *et al.*[7], who report that, while the correction applied to unsaponifiable extracts leads to erroneously low results, uncorrected results on the same fraction gives results which need only be corrected for the presence of vitamin A_2. Neither do the results reported here agree with those of Swann[9], who reported that the correction applied to unsaponifiable fractions led to low results.

The biological potencies of halibut-liver oils and of concentrates were also in good agreement with the potencies obtained by the U.S.P. XIV method, in fact, almost all samples of this type were slightly over-estimated. These results, too, do not agree with the conclusions of Cama *et al.*[7] More surprisingly they do not appear to agree with the results of Melnick *et al.*[10], who based their conclusion that the U.S.P. XIV method under-estimated the biological potency of concentrates on rat growth assays. However, it should be noted that, of the 3 laboratories which carried out biological assays reported by Melnick *et al.*[10], one laboratory reported results which were, in every case, higher than those found by multiplying gross *E* values by a factor of 1894. The average biological value of 5 oils reported by this laboratory was 11 per cent. greater than the average uncorrected value of the same 5 oils. These results are obviously biased towards high values and should, for purposes of comparison, be disregarded. The biological results from the other 2 laboratories varied above and below the corrected spectrophotometric results, the average biological results were, in one case, exactly equal to the average corrected value, and in the other, 8·8 per cent. higher. No indication of the error of these assays was given, and it must be concluded that, with the exception of the results from one laboratory, the data of Melnick *et al.*[10] are actually in good agreement with those reported here.

They are also in agreement with those found by the U.S.P. Informal Committee[11].

It is interesting to note that the relation reported here between uncorrected spectrophotometric results and bioassay data was in excellent agreement with that found by Chilcote, Guerrant and Ellenberger[17]. They found that the uncorrected spectrophotometric results exceeded the biological potencies of 28 fish oils by an average of 30·6 per cent. The antimony trichloride method gave results 18·8 per cent. higher than biological potencies. These authors measured biological activity by means of the growth assay and did not report corrected spectrophotometric results.

Some of those who have found that the procedure of Morton and Stubbs introduced too great a correction have sought a partial explanation in the fact that *neo*vitamin A is under-estimated whenever the geometric correction is applied. If, as has been assumed, *neo*vitamin A has the same potency as all-*trans* vitamin A, the spectrophotometric over-correction of *neo*vitamin A would amount to 14 per cent. if measured in *cyclo*hexane and 20 per cent. in *iso*propanol. Using the vaginal smear assay, the potency of *neo*vitamin A has been found to be 72 per cent. of that of the all-*trans* form. This is in good agreement with the potency found by Harris *et al.*[15] by the liver storage method, but somewhat lower than the figure of 83·2 per cent. reported by the same authors for the growth assay. If, as now seems likely, the potency of *neo*vitamin A is accepted as being 17 to 28 per cent. less than that of all-*trans* vitamin A, then the need for adjusting results upward by a factor of 1·04 to allow for its presence, as suggested by Cama *et al.*[7], will have disappeared. If any adjustment need be made it would appear to be in the downward direction, especially if the solvent is *cyclo*hexane.

The fact that *neo*vitamin A is evidently not as potent biologically as had been supposed does not fully explain the difference between the results reported here and those of Cama *et al.*[7] and Swann[9]. The reasons for this difference are not at once evident. Obviously the approach to the problem has been made from different angles. While we also find that the correction applied to unsaponifiable fractions gives lower results than any other physical or chemical method, there is not the slightest indication of over-correction in our comparisons as judged by biological assays. The possibility of some unknown factor influencing either or both the chemical and biological assays cannot be excluded. For example, the relative utilisation of various esters is not known precisely. The fact that such a difference exists indicates that very careful consideration should be given to the whole problem of the estimation of the vitamin A assay before the biological assay is discarded completely. Since the unit of measurement is still the International Unit, indicating specific activity, the question seems of considerable importance.

Cama *et al.*[7] have recommended that concentrates and halibut-liver oils be assayed on the whole oil basis, since saponification followed by correction apparently led to low results. In the samples we have tested we have been unable to demonstrate a significant difference between the

results obtained by these methods. Occasionally oils were encountered in which the whole oil corrected values were as much as 12 per cent. higher than the corrected value measured on the unsaponifiable fraction. In our experience, however, these oils appeared to be exceptions to the general picture. When the correction was applied to whole oil solutions of cod-liver oils, the results were significantly higher than the corrected results of unsaponifiable fractions, which have already been shown to be in excellent agreement with biological potencies.

The practice of multiplying gross E values by a factor to obtain biological units appears to be applicable only to halibut-liver oils. Concentrates and cod-liver oils seemed too variable in their content of irrelevant absorption to be handled in this manner. Moreover, the factor of 1600 which has been proposed for this method was too high for the cod-liver oils we have examined, the average factor required by these oils being 1450.

It would appear from these data that similar results can be obtained by applying a correction to either whole oil solutions or unsaponifiable fractions of concentrates or halibut-liver oils. Similar results may also be obtained on halibut-liver oils by multiplying gross E values by 1600. However, for the sake of uniformity, and particularly for control purposes, it is considered preferable to adopt, as far as possible, one method for the routine estimation of vitamin A. The correction procedure applied to the non-saponifiable fraction seems to be most widely applicable.

Summary

1. The Morton and Stubbs correction procedure for vitamin A has been widely adopted as an official method, but few data are available to indicate its validity as determined by direct comparison with the biological method. Accordingly, the results of vitamin A assays by existing physical and chemical methods have been compared with each other and with the results of biological assays.

2. The procedure of Morton and Stubbs, as applied in the U.S.P. XIV method, afforded a more accurate estimation of the biological potency of market samples of fish-liver oils than did either the antimony trichloride method or uncorrected spectrophotometric measurements. The correction also yielded the best estimate of the biological potency of a partially oxidised cod-liver oil.

3. Application of the Morton and Stubbs correction to the optical density of whole oil solutions of halibut-liver oils and concentrates yielded results which were similar to those found on the non-saponifiable basis. When applied to solutions of cod-liver oils, the correction yielded results significantly higher than when applied to non-saponifiable fractions of the same oils. The practice of multiplying uncorrected E values by 1600 did not indicate accurately the vitamin A content of cod-liver oils or concentrates, but appeared to be satisfactory for halibut-liver oils.

4. The potency of *neo*vitamin A has been found to be 72 per cent. of that of all-*trans* vitamin A when assayed by the vaginal smear method.

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THE QUANTITATIVE DETERMINATION OF ALKALOIDAL SALTS USING THE STRONGLY BASIC ANION EXCHANGER DOWEX 2 AND ITS APPLICATION IN THE ASSAY OF TABLETS

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In recent years a number of papers have been published on the determination of alkaloidal salts[1,2,3,4,5] and salts of local anæsthetics[6] by the use of anion exchange resins. In an earlier method aluminium oxide was used for the same purpose[7]. Although the latter method has given good results, anion exchange resins have great advantages over aluminium oxide, primarily because they can easily be regenerated.

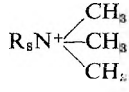
In the use of anion exchangers, the choice of resin is of great importance. Jindra and co-workers[1,2,3] and Levi and Farmilo[5] used the weakly basic Amberlite IR–4B. Baggesgaard Rasmussen, Fuchs and Lundberg[4] preferred to use the strongly basic Amberlite IRA–400, and the same resin was used by Jindra and Rentz[6] for the determination of salts of local anæsthetics.

In some preliminary experiments with the use of Amberlite IR–4B in the carbonate form, as prescribed by Jindra[1,2,3], we got unsatisfactory results in the assay of some alkaloidal salts. We therefore changed to Amberlite IRA–400, and the results were quite satisfactory, but this resin was not found to be suitable for this kind of analyses, as its regeneration was extremely time-consuming. This is a great inconvenience and limits its use in analytical practice.

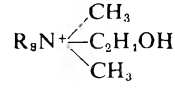
Samuelson and Schramm[8] analysed some inorganic salts using strongly basic anion exchangers. For the reason mentioned above, they found Amberlite IRA–400 to be unsuitable for this special purpose. Dowex 2, on the other hand, was found to be suitable. When we later changed to this resin, we arrived at the same conclusion.

The hydroxyl ion possesses a higher affinity than other anions for a weakly basic anion exchanger[9]. For the strongly basic Amberlite IRA–400, hydroxyl is one of the weakest replacing ions[10]. This is quite analogous to the position of the hydroxonium ion in cation exchange equilibria. Accordingly, the regeneration of a weakly basic exchanger is effected much more easily than the regeneration of a strongly basic exchanger.

Wheaton and Bauman[11] studied the properties of the strongly basic anion exchangers Dowex 1 and Dowex 2, which are polystyrene-divinylbenzene resins, containing quaternary ammonium groups. The formulæ may be written[12]:



Dowex 1



Dowex 2

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Dowex 1 is somewhat more basic than Dowex 2. This is important, as it determines the relative ease of regeneration of the resins into the hydroxide form. The position of the hydroxyl ion in the exchange potential series for Dowex 2 is more favourable than in the series for Dowex 1. Therefore, Dowex 2 seems to be especially suitable for analyses involving the free base form of the resin. It has the same salt-splitting ability as other strongly basic anion exchangers, but its regeneration is effected more easily.

In this connection a list of anion exchange resins is reprinted from a table of equivalent ion-exchange materials[13]. The resins in the same horizontal line are equivalent and generally behave in a similar manner.

TABLE I

EQUIVALENT ANION EXCHANGERS

Manufacturer ..	The Permutit Co., Ltd., London	Rohm and Haas, U.S.A.	Chemical Process Co., U.S.A.	Dow Chemical Co., U.S.A.
Weakly basic exchangers	De-Acidite E	Amberlite IR-4B Amberlite IR-45	Duolite A-2	Dowex 3 (Nalcite WBR)
Strongly basic exchangers	De-Acidite F De-Acidite FF	Amberlite IRA-400 Amberlite IRA-410		Dowex 1 Dowex 2 (Nalcite SAR)
Porous anion exchangers	Decolorite		Duolite S-30	

As will be seen, Amberlite IRA-410 is equivalent to Dowex 2, and therefore is assumed to possess the same favourable relationship between hydroxide and chloride. We have, however, been unable to find any reports on this point.

This paper describes the use of Dowex 2 for the determination of a number of salts of alkaloids and other organic bases and for the assay of some tablets.

EXPERIMENTAL

The apparatus used (Fig. 1) has been described by Samuelson[12]. The tube containing the resin bed is equipped with a funnel and an outlet capillary tube with a stopcock. The opening of the outlet tube is somewhat above the upper level of the resin bed, so that the ion exchanger is always covered with liquid. It is very important that air should never enter the resin bed, since this would cause channelling, which would decrease the efficiency considerably. The diameter of the resin bed is 10 mm. and the height 130 to 140 mm. It is kept in place by two small plugs of glass wool.

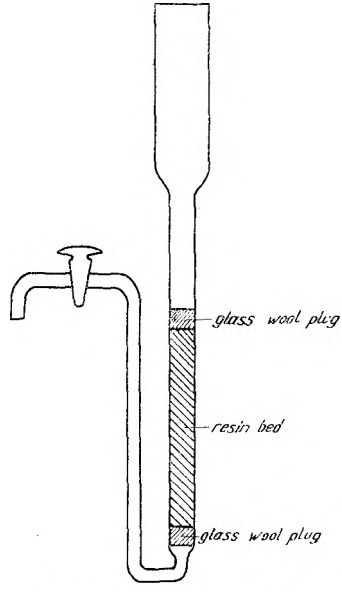

FIG. 1. Apparatus used.

This apparatus possesses the same advantage as that of Levi and Farmilo[5], and is more practical in use than those described by Jindra[1] and Baggesgaard Rasmussen, Fuchs and Lundberg[4].

The resin was used in the commercially available particle size. It is swelled in water and poured into the apparatus. The resin is supplied in chloride form and must be converted to the hydroxide form before use. This is done with about 250 ml. of N sodium hydroxide (until the chloride reaction is negative). The column is then washed with carbon dioxide-free distilled water until the washings are colourless on the addition of phenolphthalein. The water in the column is displaced by ethanol (70 per cent.), and the column is ready for use.

Standard procedure for analysis of alkaloidal salts. The salt (about 0·5 milli-equivalent) is dissolved in 10 ml. of ethanol (70 per cent.) in a small beaker. The solution is introduced into the column, and washing is performed with successive 5-ml. quantities of the same solvent, until a total volume of 50 ml. is used (in some cases more). Flow rate: about 3 ml./minute. The effluent is collected in an Erlenmeyer flask. The opening of the outlet tube is rinsed with a few ml. of solvent, and the contents of the Erlenmeyer flask are titrated with 0·1N hydrochloric acid standardised by titration of borax in ethanol (70 per cent.), using bromophenol blue as indicator.

50 ml. of ethanol (70 per cent.) is percolated through the column and titrated with 0·1N hydrochloric acid (bromophenol blue). This blank is subtracted from the value found by titration of the alkaloidal base. The completeness of the washing may be controlled by determination of the blank value immediately after the analysis. If this value is equal to the standard value for the volume used (0·04 ml. for 50 ml. of ethanol (70 per cent.)), the washing has been complete.

As the column has a total capacity of about 11 milli-equivalents (about 10 ml., capacity 1·1 milli-equivalents per ml.), it can be used several times without regeneration.

The results of a number of analyses of some salts of organic bases are summarised in Table II.

Special precautions are necessary in the analysis of amphetamine sulphate and atropine sulphate. The quantitative elution of the amphetamine base required a greater volume of solvent and a low flow rate. The admixture of 0·1 per cent. of tween 20 (non-ionic surface active agent) to the washing liquid promoted the elution. A smaller resin particle size would also have been advantageous.

For the elution of the atropine base, ethanol (96 per cent.) must be used.

On account of the strong yellow colour of the solution of the mepacrine base, a potentiometric titration was necessary. Christophers[14] has evaluated its dissociation constants. They are recorded as $pK_1 = 3{\cdot}88$, $pK_2 = 6{\cdot}47$, and a possible third value above pK 11·0. The titration curve was therefore expected to have two equivalence points. It had, however, only one sharp potential drop (in ethanol (70 per cent.)). The volume of acid used corresponded to the neutralisation of two basic nitrogen atoms.

TABLE II

DETERMINATION OF SALTS OF ALKALOIDS AND OTHER ORGANIC BASES USING DOWEX 2

About 0·5 milli-equivalent of alkaloidal salt

The base is eluted with 50 ml. of ethanol (70 per cent.) unless otherwise specified

Flow rate: about 3 ml./minute

Salt	Recovered per cent.	Remarks
Amphetamine sulphate	98·3	100 ml. of ethanol (70 per cent.)
	98·7	225 ml.
	99·9 99·7	Dissolved in 3 ml. of water, rinsed with 3 ml. of water and ethanol (70 per cent.) to a total volume of 100 ml. Flow rate: about 0·5 ml./minute
	99·3 98·7	50 ml. of ethanol (70 per cent.) with 0·1 per cent. of tween 20
Atropine sulphate	83·4	
	99·0 99·8	Dissolved in ethanol (70 per cent.) (10 ml.), rinsed with ethanol (96 per cent.) to a total volume of 50 ml.
	98·6 98·7	
Cocaine hydrochloride	99·1 99·6 98·8	
Codeine phosphate	100·0 99·8 100·3	
Diphenhydramine hydrochloride	99·3 99·9 100·3	
	99·8 99·7	
Ephedrine hydrochloride	99·8 99·1 100·8	
	99·2 99·1 99·3	
Hyoscine hydrobromide	101·0 100·3 100·3	
Lobeline hydrochloride	100·4 101·4	
Mepacrine hydrochloride	99·2 99·6 99·9	Potentiometric titration
Methadone hydrochloride	99·4 100·0 98·7	
	98·7	
Pethidine hydrochloride	99·8 100·0 99·8	
Procaine hydrochloride	100·5 100·6 100·6	
	100·5 100·3	
Strychnine nitrate	100·2 100·2 101·8	
	100·1	

Salts of morphine and other phenolic bases cannot be analysed with this resin, as they are retained as anions. This may, however, be utilised for the separation of morphine from other alkaloids. In a single experiment we found that ephedrine hydrochloride (0·2515 g.) could be quantitatively determined in the presence of morphine hydrochloride (0·1505 g.) by percolation through the column.

Owing to its strongly basic character, Dowex 2 cannot be used for the analysis of some bases containing easily hydrolysable groups. We found that neostigmine (synstigmine), pilocarpine and yohimbine were in part retained by the resin. This must be due to the formation of acidic groups as a result of the hydrolysis, which is followed by sorption as anions to the resin.

Like Baggesgaard Rasmussen, Fuchs and Lundberg[4] we found that carbacholine chloride could not be analysed by this method.

Standard procedure for assay of tablets. An amount of pulverised tablets containing about 0·5 milli-equivalent of alkaloidal salt is suspended with 5 to 10 ml. of ethanol (70 per cent.) in a small beaker. The suspension is filtered into the funnel, and beaker, filter and funnel are rinsed with 5-ml. quantities of ethanol (70 per cent.). A total volume of 50 ml. of effluent (in some cases more) is collected and titrated with 0·1N hydrochloric acid (bromophenol blue). The blank is determined.

Four tablet species of the Danish Pharmacopœia have been assayed by the official methods and by ion exchange. The tablet powders were made with an accurately known content of alkaloidal salt. The proportions of starch, lactose, talc and gelatin (powder) were much the same as in the official tablets. The results are given in Table III.

TABLE III

ASSAY OF TABLETS USING DOWEX 2 AND BY OFFICIAL METHODS (PH.D.)

Tablet powder corresponding to about 0·5 milli-equivalent of alkaloidal salt

The base is eluted with 50 ml. of ethanol (70 per cent.) unless otherwise specified

Flow rate: about 3 ml./minute

	Content of salt given per cent.	Found		Remarks
		Ion exchange per cent.	Official method per cent.	
Tablettæ amphetamini Ph.D.	6·39	6·32 6·35 6·36 6·35 6·31 Mean: 6·34	6·22 6·38 6·33 6·53 Mean: 6·37	By the ion exchange method 100 ml. of ethanol (70 per cent.) is used
Tablettæ codeini Ph.D.	30·3	29·7 30·0 29·8 30·3 29·9 30·3 Mean: 30·0	28·2 29·0 27·5 28·3 28·8 29·3 Mean: 28·5	
Tablettæ ephedrini Ph.D.	25·1	25·2 25·1 25·2 25·1 25·4 Mean: 25·2	26·1 26·1 26·0 26·0 26·0 Mean: 26·0	
Tablettæ pethidini Ph.D.	11·11	11·13 11·19 11·09 11·11 11·14 Mean: 11·13	11·02 11·02 11·18 11·12 Mean: 11·09	

DISCUSSION

Our work on ion exchange analysis of pure salts confirms the results found earlier by others[1,2,3,4,5]. Two salts, lobeline hydrochloride and mepacrine hydrochloride (atebrin), seem not to have been analysed in this way previously. This series of papers illustrates the usefulness of the ion exchange method in pharmaceutical analysis.

Some limitations of the method have been mentioned in the experimental part. Strongly basic anion exchangers cannot be used for the analysis of salts of phenolic bases or of bases containing easily hydrolysable groups, with formation of acidic groups by hydrolysis.

It must be assumed that atropine and hyoscine are also hydrolysed to a certain degree. In these cases, however, the moiety containing the basic nitrogen atom has no acidic groups and is consequently not retained by the resin, and the result of the analysis is not affected.

Neither Baggesgaard Rasmussen and co-workers[4] nor ourselves met with any complications in the analysis of cocaine hydrochloride. This is a little surprising, as the product of hydrolysis, ecgonine, contains a carboxylic acid group besides the nitrogen atom, and it should therefore be retained by the resin.

The adsorption of the amphetamine base must be due to the intervention of van der Waals' forces. The irregular behaviour of aromatic compounds

on ion exchange resins, which are predominantly aromatic in character, is well known from the work of others (*e.g.*, Partridge[15]). The elution of ephedrine, however, caused no difficulties, although the constitution of this aromatic base is very similar to that of amphetamine.

The 4 tablet species chosen are assayed in 4 different ways according to the Danish Pharmacopœia. The methods are as follows.

Amphetamine sulphate tablets. The tablet powder is boiled with hydrochloric acid for half an hour. The mixture is cooled and transferred to a separatory funnel. An excess of sodium hydroxide is added and the base shaken out with chloroform 5 times. The chloroform solution is shaken with 0·1N hydrochloric acid and the excess of acid titrated with 0·1N sodium hydroxide.

Codeine phosphate tablets. The tablet powder is boiled with hydrochloric acid, and after having been made alkaline, the mixture is shaken 4 times with chloroform. The chloroform solution is evaporated to dryness. The base is dissolved in ethanol and titrated with 0·1N hydrochloric acid.

Ephedrine hydrochloride tablets. The tablet powder is suspended in water, starch solution and fluorescein indicator are added, and chloride ion is titrated with 0·1N silver nitrate.

Pethidine hydrochloride tablets. To the tablet powder nitric acid and 0·1N silver nitrate are added. The mixture is boiled, water and ferric ammonium sulphate are added, the mixture is filtered, and the excess of silver nitrate in an aliquot is titrated with 0·1N ammonium thiocyanate.

As will be seen from Table III, these tablet powders are accurately assayed by the ion exchange method. The results correspond very well to those found by the official methods.

Summary

1. A number of alkaloidal salts and salts of other organic bases have been assayed by use of the anion exchange resin Dowex 2. This resin is especially suitable for analyses of this kind.

2. As examples of the application of the method for the assay of alkaloid-containing galenicals, 4 tablet species have been assayed. The results agree very well with the values found by official methods. The ion exchange method should be of general value for the assay of tablets containing alkaloidal salts, as other salts as a rule are not present. It is in general more accurate, rapid and convenient than older methods.

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THE ADSORPTION ANALYSIS OF ALKALOIDS

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In a previous communication[1] it was shown that tropa alkaloids are quantitatively taken up by the synthetic zeolite, decalso, and that they are fully desorbed by moderately strong solutions of acids or salts. In this paper it is proved that the same applies to a number of other alkaloids, viz. to morphine hydrochloride, codeine phosphate, papaverine hydrochloride, berberine sulphate, hydrastinine hydrochloride, neostigmine bromide, *d*-tubocuraine chloride and *dl*-α-(4-hydroxyphenyl)-β-methylaminoethanol tartrate.

As with the tropa alkaloids both tertiary and quaternary salts behave similarly, and it is believed that the sorption and desorption processes are generally valid for salts of nitrogenous bases, thus offering a convenient method of isolating them.

Experimental

General procedure. Pour an aqueous solution of the alkaloidal salt on a column of activated decalso. Here 0·7 to 5 mg. of the substance and 0·25 to 0·6 g. of decalso have been used (5 to 20 micro-equivalents per g. of decalso) in a tube 4 to 5 mm. wide. Wash with water *ad libitum* and elute the base in the column with a sufficient quantity of 0·2N hydrochloric, sulphuric or acetic acid or with 25 per cent. potassium chloride or sodium chloride solution. (Further details are given in our previous paper[1].) Determine the substance in the eluate by a suitable method.

If not otherwise stated, the final estimation of the alkaloid was performed in the following way. The eluate was diluted to 50·0 ml. with the eluant acid or, when salt had been used as the eluant, with water and hydrochloric acid to make the solutions 0·2N in hydrochloric acid. In this solution the amount of the alkaloid was determined by measuring the extinction at the maximum of the absorption curve in a Hilger spectrophotometer, model Uvispek.

As blank values are often obtained when the assays are performed at wavelengths below 300 mμ the following device was used. The same amount of alkaloid as in the test was put into a 50-ml. flask. Into this flask the same amount of eluant as in the test was allowed to flow through a blank column and the resultant mixture diluted as in the test. The extinction of this solution was considered to be the quantity of alkaloid taken. This method is correct only if Beer's law is valid, which was ascertained in each case.

It should be pointed out that elution with acids will dissolve iron from the decalso if too large eluates are taken. Thus, with 0·4 g. of decalso, iron will appear after about 10 ml. of eluate, when 0·2N hydrochloric or sulphuric acid is used as eluant, and after about 20 ml. when 0·2N

acetic acid is used. More decalso delays the break-through of iron, and the reverse.

If the spectrophotometric assay is performed at wavelengths below 300 mμ, mere traces of iron will render the analysis impossible. As will be seen from the results in those cases where iron might have been eluted, the measurements are made at high wavelengths where the interference is negligible.

Results. The results for codeine phosphate, papaverine hydrochloride, hydrastinine hydrochloride, *d*-tubocurarine chloride and *dl*-α-(4-hydroxyphenyl)-β-methylaminoethanol tartrate are given in Tables I, II, III, IV and V.

TABLE I

CODEINE PHOSPHATE

Sample: Ph. Sv. Ed. XI. Assayed at 284·0 mμ

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with							
		Water 10 to 30 ml.	0·2N hydrochloric acid		0·2N acetic acid		25 per cent. potassium chloride	
			5 ml.	10 ml.	5 ml.	10 ml.	5 ml.	10 ml.
5	0·6	0	99·1	100·7	79·1	99·4	97·3	98·6
		0	99·4	101·4	79·9	99·9	98·3	102·2

TABLE II

PAPAVERINE HYDROCHLORIDE

Sample: Ph. Sv. Ed. XI. Assayed at 308·5 mμ

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with					
		Water 10 to 30 ml.	0·2N hydrochloric acid		0·2N acetic acid		
			10 ml.	15 ml.	First 15 ml.	Second 15 ml.	Total
1·2	0·6	0		100·1	97·7	2·9	100·6
		0		100·9	98·3	3·1	101·4
		0	98·8		98·6	1·0	99·6
1·8		0	97·8		98·3	0·0	98·3

TABLE III

HYDRASTININE HYDROCHLORIDE

Sample: A product from Merck, Germany, recrystallised 3 times from ethanol and ether. Assayed at 306·5 and 364·0 mμ. The recoveries are means of values obtained at the two wavelengths. The elution could be followed in ultra-violet light

Quantity of alkaloid g.	Decalso g.	Percentage recovery after elution with						
		Water 10 to 30 ml.	0·2N hydrochloric acid			0·2N acetic acid		
			First 10 ml.	Second 10 ml.	Total	First 10 ml.	Second 10 ml.	Total
0·7	0·4	0	99·5	0	99·5	100·3	0·6	100·9
		0	97·0	2·4	99·4	95·9	0·6	97·5
		0	98·3	2·0	100·3			
		0	97·9	4·8	102·7			
	0·6	0	100·0	3·0	103·0			
		0	97·4	0·8	98·2			

TABLE IV

d-TUBOCURARINE CHLORIDE

U.S.P. XIV sample. Assayed at 280·5 mμ

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with: Water 10 to 30 ml.	0·2N hydrochloric acid 5 ml.	0·2N acetic acid: First 5 ml.	Second 5 ml.	Third 5 ml.	Total	0·2N acetic acid: First 15 ml.	Second 15 ml.	Total	0·2N sulphuric acid: First 5 ml.	Second 5 ml.	Total
2·0	0·4	0	98·5					93·9	5·9	99·8			
		0	95·9					94·1	5·9	100·0			
		0	97·1										
		0	100·7										
		0	98·6*										
		0	100·0*										
				27·4	45·6	18·6	91·6				92·0	7·0	99·0
4·0	0·4	0		35·9	39·6	14·9	90·4				92·3	5·0	97·3

* Second eluate nil.

TABLE V

dl-α-(4-HYDROXYPHENYL)-β-METHYLAMINOETHANOL TARTRATE

Sample: Oxedrini tartras, Ph. Sv. Ed. XI. Assayed at 273·5 mμ

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with: Water 10 to 30 ml.	0·2N hydrochloric acid 5 ml.	0·2N sulphuric acid 5 ml.	0·2N acetic acid 5 ml.
4	0·4	0	99·1	98·8	99·8
		0	99·8	99·8	100·2
		0	100·5		
		0	99·3		

Morphine hydrochloride. A sample complying with the Swedish Pharmacopœia, Ed. XI, was used. The morphine was assayed photometrically by a modified nitrosation method[2,3]. As the intensity of the colour developed varied with the concentration of salts, acids, etc., in the reaction mixture, a new standard curve had to be made for each case. To get conditions identical in the standard curve solutions and in the eluates, decalso blank columns were eluted with the specified amount of a certain eluant, a known amount of morphine was added to the eluate and the determination was performed in the following way. The solution was mixed with 0·2 g. of sodium citrate* and sufficient water and hydrochloric acid to make about 20 ml. of solution approximately 0·5N in hydrochloric acid. 8·0 ml. of a 1 per cent. sodium nitrite solution was added and after exactly 5 minutes, 10 ml. of 5N ammonia and water to 50·0 ml. The extinction was measured at 470 mμ in 1-cm. cells. For results see Table VI.

Berberine sulphate. Preliminary experiments gave somewhat low recoveries and paper chromatography performed by Rutter's method[4], with *iso*butanol saturated with 0·2N acetic acid as the developer and ultra-violet light to visualise the process, revealed that the substance

* The citrate is necessary to keep dissolved aluminium in solution after the addition of ammonia.

TABLE VI

MORPHINE HYDROCHLORIDE

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with: Water 10 to 30 ml.	0·2N hydrochloric acid 5 ml.	0·2N acetic acid 5 ml.	0·2N acetic acid 10 ml.	0·2N acetic acid 15 ml.	25 per cent. sodium chloride First 5 ml.	25 per cent. sodium chloride Second 5 ml.	25 per cent. sodium chloride Total
4	0·5	0	101·3	86·4	95·5	99·8	90·2	10·1	100·3
4	0·5	0	101·3	83·5	95·0	100·4	90·9	8·6	99·5
4	0·5	0	100·2						
4	0·5	0	97·9						

contained an impurity with an R_F value about half as great as that of the berberine itself. Therefore, the sample was recrystallised 3 times from ethanol and ether and was then chromatographically pure. It was easy to follow the elution process visually, as berberine exhibits a strong fluorescence in ultra-violet light.

TABLE VII

BERBERINE SULPHATE

Sample: See text. Assayed at 421·0 mμ

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with: Water 10 to 30 ml.	0·2N sulphuric acid First 15 ml.	0·2N sulphuric acid Second 15 ml.	0·2N sulphuric acid Total	0·2N acetic acid 20 ml.	0·2N acetic acid 25 ml.	0·2N acetic acid 20 ml.	0·2N acetic acid 25 ml.
1·5	0·6	0	96·4	4·0	100·4				99·9
		0	96·4	1·5	97·9				99·4
2·1	0·6	0				97·7*		95·7*	
		0				98·0*		100·7*	
1·5	0·25	0					99·9		99·3

* Second eluate nil.

TABLE VIII

STEPWISE ELUTION OF BERBERINE SULPHATE

Quantity of alkaloid mg.	Decalso g.	Elution with	Percentage recovery First 5 ml.	Second 5 ml.	Third 5 ml.	Total
1·2	0·25	0·2N Sulphuric acid	88·8	9·2	1·9	99·9
		0·2N Acetic acid	75·7	24·6	1·9	102·2

TABLE IX

NEOSTIGMINE BROMIDE

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with: Water 10 to 30 ml.	0·2N hydrochloric acid 5 ml.	0·2N acetic acid 10 ml.
2·5	0·4	0	100·5	100·0
		0	100·7	99·6
		0	98·5	
		0	101·4	

Neostigmine bromide. A sample of prostigmine bromide, of U.S.P. XIV quality, was used. For the assay the substance was hydrolysed with 1N sodium hydroxide on the water bath for 15 minutes. Experiments gave 95 per cent. hydrolysis after 5 minutes, 100 per cent. after 10 minutes, and 100 per cent. after 30 minutes. The product was assayed in 0·2N sodium hydroxide at 293·5 mμ. (Cf. Mørch[5] and Spross[6].)

Discussion

The results show that all substances tested were recovered quantitatively by the proposed procedure. There appears to be no significant difference between the eluability of tertiary and quaternary bases. Generally speaking, the bases are more easily eluted by the strong acids than by acetic acid, although the difference is very slight for some alkaloids.

Of the compounds tested berberine was by far the most difficult one to elute. In this case hydrochloric acid is not suitable, as the chloride is very slightly soluble in hydrochloric acid. Even with 30 ml. of 0·2N hydrochloric acid only 90 per cent. of the base was eluted from 0·6 g. of decalso.

It may be mentioned that narcotine hydrochloride could not be recovered quantitatively from decalso. The reason was proved to be that the alkaloid was partly decomposed in the column. It was, however, completely adsorbed by the decalso.

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STUDIES ON LOCAL ANÆSTHETIC DRUGS

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Many workers have produced indirect evidence that acetylcholine may be concerned in transmission at sensory nerve endings (Harvey, Lilienthal and Talbot[1], Gray[2]). A group of drugs of widely different chemical nature yet all possessing local anæsthetic activity has been shown by Elio[3] (1948) to antagonise the action of acetylcholine on different tissues. Local anæsthetic drugs also antagonise the actions of nicotine and potassium chloride on different tissues and abolish the peristaltic reflex in the guinea-pig ileum (Dawes[4], Feldberg and Lin). It was decided, therefore, to study in detail the relative antagonism of a series of 6 well-known local anæsthetic drugs on the responses of skeletal and plain muscle to various stimulants including acetylcholine, nicotine and potassium.

Methods

The isolated rectus abdominis muscle of the frog, suspended in a bath of volume 2 ml., has been used for experiments on skeletal muscle. The muscle was immersed in a solution of tetraethyl pyrophosphate (3·5 μg./ml.) for 30 minutes to inhibit cholinesterase, and then maintained in a concentration of 0·02 μg./ml. (Hobbiger[6], 1950). Pieces of rabbit and guinea-pig ileum were suspended in an isolated organ bath (volume 15 ml.) containing oxygenated Tyrode's solution at 37° C. A larger bath (volume 50 ml.) was necessary for studying the peristaltic reflex (Feldberg and Lin[5]). Isolated tracheal preparations of the cat and guinea-pig and preparations of the human bronchus (obtained from the post-mortem room) were suspended in a 15 ml. bath (Akcasu[7]).

The local anæsthetic drugs were used in the form of hydrochlorides and the amounts indicated in the text and tables refer to their corresponding salts. In all cases, the values quoted in the tables are the geometric mean of at least 4 results based on a 50 per cent. inhibition of the contraction produced by the stimulant drug.

Other drugs used were acetylcholine chloride, benzoylcholine chloride, succinylcholine bis-iodide, *d*-tubocurarine chloride, nicotinic acid tartrate and histamine acid phosphate.

Results

Frog rectus abdominis muscle

Low concentrations of local anæsthetic drugs (5×10^{-5} to $2{\cdot}5 \times 10^{-6}$) inhibit the acetylcholine and succinylcholine responses on the rectus

*This work was carried out at the Department of Pharmacology and Therapeutics, University of St. Andrews Medical School, Dundee, while the author was in receipt of a grant from the Inspector General of Civil Hospitals, Patna.

muscle and there is good agreement between relative activities based on these actions and their local anæsthetic potencies (Table I). Whereas procaine and lignocaine are easily washed out from this tissue, it is difficult to remove cinchocaine. Procaine and lignocaine in equal concentrations ($2{\cdot}5 \times 10^{-5}$) also inhibit the stimulant action of benzoylcholine (2×10^{-6}). Local anæsthetic drugs also antagonise the stimulant

TABLE I

FROG RECTUS ABDOMINIS MUSCLE

RELATIVE POTENCY OF LOCAL ANÆSTHETIC DRUGS (PROCAINE = 1) IN ANTAGONISING ACTIONS OF VARIOUS STIMULANTS

Drug	Stimulant			Relative local anæsthetic potency (Elio[3])
	Acetyl choline (2×10^{-7})	Succinyl choline (10^{-7})	Potassium chloride (10^{-3})	
Procaine	1	1	1	1
Lignocaine	0·5	1	1	1·2*
Cocaine	4·2	4·1	4·2	7·4
Cinchocaine	10	10·4	8	10
Concentration of procaine to produce 50 per cent. antagonism ..	$2{\cdot}5 \times 10^{-6}$	$2{\cdot}5 \times 10^{-5}$	10^{-4}	*Data of Doubleday[8]

action of potassium chloride on the frog rectus muscle but the concentrations needed are about 4 times those required to antagonise the acetylcholine response (Table I).

Higher concentrations of the local anæsthetic drugs (10^{-3}) produce contraction of the rectus muscle, but whereas the stimulant actions of procaine, lignocaine and cocaine are repeatable, that of cinchocaine is not. Tetraethyl pyrophosphate potentiates the stimulant action of acetylcholine 10 to 20 times but has no action on that of the local anæsthetic drugs. Similarly, tubocurarine has little or no effect on the stimulant action of the local anæsthetic drugs.

Isolated rabbit ileum

The contractions produced by acetylcholine are inhibited by all the local anæsthetic drugs. Procaine, lignocaine, cocaine and amethocaine however are equally potent, whilst amylocaine is twice, and cinchocaine 5 times, as potent as procaine (Table II), results which are in line with those reported by Elio[3] using rabbit duodenum. The local anæsthetic drugs inhibit the peristaltic reflex and the nicotine-induced contractions of the rabbit ileum, and in these actions there is better agreement between their relative activities and local anæsthetic potencies than when acetylcholine is the stimulant (Table II). With the exception of procaine, the concentrations required to antagonise these latter responses are ineffective against the acetylcholine response. The local anæsthetic drugs also inhibit the contractions induced by potassium chloride (Table II). Low concentrations of lignocaine and cocaine (and sometimes procaine) increase the normal pendular movements of the rabbit ileum, making quantitative measurements often difficult.

TABLE II

RABBIT ILEUM

RELATIVE POTENCY OF LOCAL ANÆSTHETIC DRUGS (PROCAINE = 1) IN ANTAGONISING ACTIONS OF VARIOUS STIMULANTS AND THE PERISTALIC REFLEX

Drug	Stimulant			Peristalic reflex	Relative local anæsthetic potency
	Acetyl choline (3×10^{-8})	Potassium chloride (10^{-3})	Nicotine (10^{-6})		
Procaine	1	1	1	1	1
Lignocaine	1	1·6	1·4	1·2	1·2
Cocaine	1	2·5	2·4	2·5	7·4
Amylocaine	2·2	10	2·2	2·8	8
Amethocaine	1	10·5	12·5	12·5	8
Cinchocaine	5·1	12·5	20	25·6	10
Concentration of procaine to produce 50 per cent. antagonism	10^{-5}	5×10^{-5}	5×10^{-6}	5×10^{-8}	—

Guinea-pig ileum

In this preparation, procaine regularly produces inhibition of the contraction induced by acetylcholine but the other local anæsthetics often produce variable inhibition. Cocaine usually has about one ninth and lignocaine one fourth the activity of procaine in this respect. Amylocaine is equally potent, whereas amethocaine and cinchocaine are slightly more active than procaine (Table III). Smaller concentrations of the local anæsthetic drugs are needed to inhibit the peristaltic reflex and the contraction induced by nicotine, and there is fair agreement between their relative activities based on these antagonisms and local anæsthetic potencies. When potassium chloride is the stimulant drug, still better agreement is obtained, although higher concentrations of the local anæsthetic drugs are necessary.

Recently, Euler[9] has shown that many nerves contain histamine, and this substance may play some part in nervous conduction. Since the antihistamine drugs possess local anæsthetic activity, it was of importance to study the antihistamine action of local anæsthetic drugs. The dose of procaine needed to inhibit the histamine-induced contraction of the guinea-pig ileum is twice that needed to produce a similar inhibition of the acetylcholine contraction. Cocaine has half, and lignocaine about one sixth, of the activity of procaine in this test; on the other hand, amylocaine, amethocaine and cinchocaine are more active than procaine (Table III).

Isolated cat tracheal chain preparation

The trachea of the cat, like that of the rabbit, is sensitive to acetylcholine but insensitive to histamine (Akcasu[7]). Procaine is a very effective antagonist to acetylcholine, being the most active of the local anæsthetic drugs tested (Table IV). Non-effective doses of lignocaine and cocaine even potentiate the acetylcholine response.

The concentration of procaine which reduces the acetylcholine response does not affect the potassium chloride contraction, and it has to be

TABLE III

GUINEA-PIG ILEUM

RELATIVE POTENCY OF LOCAL ANÆSTHETIC DRUGS (PROCAINE = 1) IN ANTAGONISING ACTIONS OF VARIOUS STIMULANTS AND THE PERISTALTIC REFLEX

Drug	Stimulant				Peristaltic reflex	Relative local anæsthetic potency
	Acetyl choline (4×10^{-6})	Potassium chloride (10^{-3})	Histamine (4×10^{-6})	Nicotine (10^{-6})		
Procaine	1	1	1	1	1	1
Lignocaine	0·2	2	0·2	0·6	0·6	1·2
Cocaine	0·1	2·6	0·5	1	1	7·4
Amylocaine	1	4	2·8	2	2·2	8
Amethocaine	1·2	5·4	3·5	5·4	5·4	8
Cinchocaine	1·6	10·2	4	8·9	6·6	10
Concentration of procaine to produce 50 per cent. antagonism	5×10^{-6}	4×10^{-5}	10^{-5}	10^{-6}	2×10^{-6}	—

TABLE IV

CAT TRACHEA PREPARATION

RELATIVE POTENCY OF LOCAL ANÆSTHETIC DRUGS (PROCAINE = 1) IN ANTAGONISING STIMULANT ACTIONS OF ACETYLCHOLINE AND POTASSIUM CHLORIDE

Drug	Stimulant		Relative local anæsthetic potency
	Acetylcholine (10^{-5})	Potassium chloride (10^{-3})	
Procaine	1	1	1
Lignocaine	0·1	1·3	1·2
Cocaine	0·1	4	7·4
Amylocaine	0·2	5·4	8
Amethocaine	0·3	6·1	8
Cinchocaine	0·2	10·3	10
Concentration of procaine to produce 50 per cent. antagonism	10^{-5}	2×10^{-4}	—

increased 20 to 40 times to give a similar inhibition. However, the same concentrations of the other local anæsthetic drugs are usually equally effective against both acetylcholine and potassium chloride, so that there is good agreement between their relative activities based on the antagonism of the potassium response and their local anæsthetic potencies.

Similar results to those found on the cat trachea have also been obtained on the isolated tracheal chain of the guinea-pig and the isolated human bronchus.

DISCUSSION

Local anæsthetic drugs have been shown to possess at least 3 actions on the frog rectus abdominis muscle. Firstly, they antagonise the nicotinic action of various choline derivatives, and there is good agreement between their relative potencies based on this property and their local anæsthetic potencies. This confirms the results of Elio[3] using acetylcholine as the stimulant drug. Secondly, in somewhat higher concentrations, they antagonise the stimulation produced by potassium

chloride, and again there is good agreement between their relative potencies and their local anæsthetic activities. Such an antagonism has already been shown on the sartorius muscle of the toad (Guarino[10]) and the rectus abdominis muscle of the frog (Blavier, Lecomte, Osterrieth and Vanremoortere[11]), although no quantitative studies were undertaken by these workers. Thirdly, in higher concentrations still, they stimulate the muscle (Zipf[12]) and tetraethyl pyrophosphate and tubocurarine are without effect on this stimulation. As Fleckenstein, Wagner and Googel[13] have already indicated, it may be due to depolarization of the muscle fibres.

Elio[3] stressed the similarity in action between the local anæsthetic drugs and atropine, but a detailed comparison of the atropine-like property of these drugs indicates that in both rabbit and guinea-pig ileum preparations there is little correlation between these actions and the relative local anæsthetic potencies. Similarly, there is no correlation between the antihistamine and local anæsthetic potencies, a fact already reported for the antihistamine drugs by Halpern, Perrin and Dews[14] and Reuse[15].

The relative activities of the local anæsthetic drugs, based on their ability to reduce nicotine response and the peristaltic reflex in the gut agree well with their local anæsthetic activities. Whereas the concentrations of procaine which are effective against the contractions produced by acetylcholine and by nicotine in the gut do not differ widely, those of other local anæsthetic drugs for antagonising the nicotine response are much lower. Greeff[16] has recently reported similar results using procaine and cocaine. The activities of local anæsthetic drugs in reducing potassium chloride responses on the gut are also in line with their local anæsthetic potencies. This may be surprising since in the rabbit ileum potassium chloride produces contraction mainly by ganglionic action (Feldberg[17]). This result supports the hypothesis of Fleckenstein[18,19] that these substances are "anelectronic" and antagonise the responses of catelectronic drugs (e.g. potassium chloride).

The results on the trachea preparations are comparable with those found on the gut, so that there is better agreement between the ability of the local anæsthetic drugs to reduce the potassium chloride response and their relative local anæsthetic potencies than with their ability to reduce that of acetylcholine. Whereas the concentrations of lignocaine, cocaine, amylocaine, amethocaine and cinchocaine are almost the same for both the antagonisms, those of procaine differ widely. For example, the dose of procaine needed to reduce the acetylcholine response has to be increased 20 to 40 times to produce a similar reduction of the potassium chloride response. Nicotine cannot be used as a stimulant in this preparation since it is not effective (Akcasu[7]).

Summary

1. On the frog rectus abdominis muscle, local anæsthetic drugs antagonise the actions of acetylcholine, succinylcholine and potassium chloride. Relative activities based on these properties and their local anæsthetic potencies follow the same trend.

2. The stimulant action of high concentrations of local anæsthetic drugs on the frog rectus muscle has been confirmed. It is little affected by tetraethyl pyrophosphate and tubocurarine.

3. Local anæsthetic drugs antagonise the responses of nicotine and potassium chloride on the rabbit and guinea-pig ileum and abolish the peristaltic reflex. They also antagonise the potassium chloride response on the cat trachea. There is good agreement between their relative activities based on these antagonisms and their local anæsthetic potencies.

4. The atropine-like and antihistamine activities of local anæsthetic drugs on smooth muscle preparations appear to be unrelated to their local anæsthetic potencies.

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AN EXAMINATION OF THE MUCILAGE-CONTAINING SEEDS OF *ALYSSUM CAMPESTRE* L.

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The seeds known in Iran as "Ghoddumeh" are derived from *Alyssum campestre* L. (family Cruciferae)[1,2], a small annual herb, distributed throughout most parts of Iran. 3 botanical varieties have been described[1] and 2 kinds of these seeds are known in the Teheran market, one "Ghoddumeh shahri" (G. of town) and the other "Ghoddumeh shirazi" (G. of Shiraz). The latter are the most esteemed and are used especially as a mild expectorant.

The present investigation was undertaken to obtain information concerning the constituents of the seeds and to ascertain if they compared favourably, for pharmaceutical purposes, with other mucilage-containing seeds such as those derived from species of *Plantago.*

Material

Samples of seed used for the investigation were obtained in Teheran and consisted of ripe seeds with a small proportion of unripe seeds and about 2·5 per cent. of foreign seeds and other organic matter.

Characters of the Seeds

Ripe seeds are pinkish-brown with a lighter margin, whilst the unripe seeds are olive-green with a margin of varying shades of yellowish-brown. Their size is very uniform varying from about 0·02 to 2·19 mm. in length and from 1·55 to 1·76 mm. in breadth. The seeds are very much flattened and broadly ovate to pear-shaped in outline. At the pointed end, the remains of a funicle are often present. A transparent V-shaped cleft occurs below the hilum to which runs an oblique groove, present on both sides of the seed. The testa is minutely pitted. The average weight of 100 seeds, selected at random, was 0·135 g., whereas that of 100 ripe seeds, selected by hand, was 0.150 g. On soaking a seed for a few moments in water the epidermis of the testa swells up and a thick mucilage soon surrounds it like a halo. When observed under a lens this mucilage, which is at first transparent, is seen to lose its transparency gradually while remaining translucent. Unripe seeds produce a smaller mucilage-halo than ripe seeds. Both the whole and powdered seeds are odourless and when chewed have a bland mucilaginous taste.

The powders produced from the ripe and unripe seeds are yellowish-brown and yellowish-green respectively. Small starch grains are abundant in the unripe seeds, but rare in those that have ripened. The mucilage stains with ruthenium red and with methylene blue, both stains being retained on washing with water. A pigment layer is present in the testa and preliminary experiments indicate its chief constituent to be an

anthocyanin glycoside. The guaiacum resin and benzidine tests show a cold water extract of the powder to contain a peroxidase enzyme but no oxydase enzyme. On boiling the seeds with a solution of sodium hydroxide, a deep orange colour is produced.

EXPERIMENTAL

Swelling Factor. Mucilage-containing seeds are often evaluated by their swelling factors and previous work[3,4] has shown that careful standardisation of the experimental conditions is essential if comparable results are to be obtained. In this case the following procedure was found to give satisfactory results. 1 g., accurately weighed, of seeds was placed in a 25 ml. graduated cylinder of diameter 1·5 cm. The water, or other aqueous solution, was added to the 20 ml. level and a plastic stopper inserted. The cylinder was vigorously shaken at the beginning of the experiment and again after 1, 2 and 20 hours. Immediately prior to each shaking the cylinder was inverted and gently tapped. The volumes of the swollen seeds were finally read at the end of 24 hours. Other determinations in which the cylinder was inverted once at the beginning of the experiment and in which subsequent shakings were omitted, were also carried out. The results are recorded in Table I.

TABLE I

SWELLING FACTORS UNDER DIFFERENT CONDITIONS AND USING DIFFERENT SOLUTIONS

Liquid	Swelling Factors	
	With shaking	Without shaking
Distilled water	17·2—17·7—18·10	10·5
Chloroform water	17·0—17·5—18·0	9·5
Soft tap water	16·2—16·6—17·0	—
0·5 per cent. of sodium chloride	17·5	—
1·0 per cent. of sodium chloride	17·0	8·2
2·0 per cent. of sodium chloride	16·5	—
0·5 per cent. of potassium chloride	15·0	—
1·0 per cent. of potassium chloride	14·5	8·0
2·0 per cent. of potassium chloride	12·5	—
0·5 per cent. of calcium chloride	14·0	—
1·0 per cent. of calcium chloride	13·5	5·7
2·0 per cent. of calcium chloride	9·0	—

Isolation of the Mucilage. The complete extraction of the mucilage from the seeds presented problems which have not yet been completely solved. After many experiments with different presses and types of filter, the following general method was adopted.

The seeds, with 30 times their weight of distilled water were shaken frequently during 24 hours. The viscous product was poured, a little at a time into a linen cloth and the mucilage squeezed out by hand. The residual seeds were soaked for about 30 minutes in distilled water and the pressing repeated. This procedure was repeated 4 times. The mucilage from each pressing was precipitated separately by the addition of ethanol (95 per cent.) (6 ml. for every 5 ml. of mucilage) and stirred gently with a glass rod. In some instances, dilute hydrochloric acid or 1 per cent. sodium chloride solution was added to the mucilage prior to precipitation. The coagulated mucilage, which formed a mass floating on the dilute

ethanol, was transferred to a linen filter, allowed to drain and then pressed to remove all the dilute ethanol. It was dried at a temperature just below 100° C. and weighed. The product from the first extraction was white and in typical experiments represented 8·2 to 12·0 per cent. of the original weight of the seeds; the products of later extractions were dirty-white and formed 9·2 to 10·0 per cent. of the total seed weight. Two subsequent hydrations of the discoloured material in distilled water followed by precipitation with ethanol failed to yield an absolutely white product. When hydrochloric acid was used and the coagulated mucilage washed with ethanol and ether, white products with a fibrous appearance were obtained. The results are recorded in Table II.

TABLE II

EXTRACTION OF MUCILAGE FROM *Alyssum campestre*

Experiment	Weight of Seeds, g.	Volume of extracted mucilage, ml.	Volume of mucilage used for coagulation ml.	Dilute hydrochloric acid, ml.	1 per cent. sodium chloride solution, ml.	Ethanol (95 per cent.), ml.	Yield when dried in oven at 96° to 100° C., per cent.
I ..	5·0	420	420	—	—	505	17·4
II ..	7·5	580	580	1·5	—	695	19·8
III* ..	10·0	765	765	—	—	—	25·4
IV ..	5·0	450	100 100 100 100	0·5	—	120 120 150 200	15·3 18·9 15·5 16·3
V ..	2·0	200	100 100	— —	— 1	120 120	15·1 18·0
VI ..	3·0	240	75 75 75	— — —	— 1 2	90 90 90	18·1 19·3 20·3

* In this experiment the total mucilage was evaporated and dried to constant weight at 100° C. The product was brown and brittle.

For the tests recorded below, the dried white mucilage was further purified by hydrating in the minimum quantity of distilled water on a water bath for 1 hour, cooling and adding the ethanol all at once with gentle stirring. To avoid any possible hydrolysis by heat or long standing[5], the coagulated mucilage was immediately collected and dried *in vacuo*.

Properties of the Precipitated, Dried Mucilage. With water the precipitated and dried mucilage gives a neutral, slightly turbid, colloidal solution which is viscous in concentrations of 0·5 per cent. This solution does not reduce Fehling's solution until after hydrolysis with mineral acids. Addition of a solution of lead subacetate causes precipitation with the formation of a white gel on warming. There is no reaction with a borax solution.

The reconstituted mucilage requires more ethanol for its reprecipitation than originally. If the ethanol in the continuous phase is not sufficient, coagulation may occur at first, but later form a clear liquid on shaking. With a concentrated mucilage and a high proportion of ethanol, the coagulate is in a form which can be separated from the aqueous ethanol. The presence of small quantities of hydrochloric acid, sodium chloride,

potassium chloride, calcium chloride and sodium sulphate make the mucilage more sensitive to coagulation by ethanol. The results of adding 1 ml. of a number of solutions to 5 ml. quantities of a 0·1 or 0·25 per cent. reconstituted mucilage are recorded in Table III.

TABLE III

ACTION OF REAGENTS ON MUCILAGE OF *Alyssum campestre*

Solution added, ml.	Strength of mucilage, per cent.	Result
Concentrated sulphuric acid	0·1	No coagulation
Sulphuric acid 10 per cent.	0·1	No coagulation
Sodium hydroxide 20 per cent.	0·1	Coagulation and no dispersal by shaking and standing
Sodium chloride 1 per cent. and 10 per cent.	0·1or 0·25	No coagulation
Potassium chloride 1 per cent. and 10 per cent.	0·1 or 0·25	No coagulation
Calcium chloride 1 per cent. and 10 per cent.	0·1 or 0·25	No coagulation

Quantitative Determination of Mucilage by Greenberg's Method. The mucilage content of the seeds was determined by the method of Greenberg[4] for seeds of *Plantago* spp. A weighed quantity of seeds were soaked in a definite volume of distilled water for 24 hours with vigorous agitation and transferred to the press. A seed:water ratio of 1:20 or 1:30 as employed with psyllium seeds, presented certain difficulties due to clogging of the sieve, incomplete removal of mucilage from the seeds and leakage of mucilage past the piston. These effects were largely overcome by reducing the seed:water ratio to 1:50 and by placing a small piece of linen over the sieve. A determination involving a reduced period of soaking with constant shaking was also made. The results, together with some of Greenberg's for comparison are shown in Table IV.

TABLE IV

MUCILAGE CONTENT OF THE SEEDS OF *Alyssum campestre* COMPARED

SEEDS	PERCENTAGE OF MUCILAGE			
	Seed : water ratio 1 :20	Seed : water ratio 1 :30	Seed : water ratio 1 :50	Seed : water ratio 1 : 30. Soaking time 15 to 20 minutes
Alyssum campestre ..	13·00	16·00	19·50 ×	14 to 16
Plantago ovata	16·00	18·00	—	—
,, *arenaria*	* 7·66	* 7·36	—	—
,, *ovata*	*22·30	*21·70	—	—
,, *wrightiana* ..	*20·50	*23·00	—	—
,, *rhodosperma* ..	*16·00 (+)	*15·11 (+)	—	—

× In this case the soaked seeds were transferred to the press in two portions.
(+) Greenberg found that after further soaking and expression this species yielded about 18 to 20 per cent of mucilage.
* Figures obtained by Greenberg.

Miscellaneous Determinations on the Powdered Seeds

Moisture content—loss on drying at 100° C., 7·2 per cent.

Total ash—4·26 per cent., almost completely soluble in dilute hydrochloric acid.

Continuous extraction—with ether yielded 13·97 per cent. of extract.

Total nitrogen on the powder dried at 100° C., 3·06 per cent.

Sulphur determined by the U.S. Dispensatory (23rd edition) method 0·096 per cent.

Discussion and Conclusions

Table I indicates that chloroform water gives practically the same value for the swelling factor of *Alyssum campestre* as distilled water, but that low figures are obtained with salt-containing solutions and even with public water supplies. The swelling of the seeds is markedly reduced by the presence of sodium, potassium and particularly calcium ions and is inversely proportional to the concentration of the salt solutions. The need for the careful standardisation of the experimental conditions, if comparable results are to be obtained, is also confirmed.

The swelling factor of *Alyssum campestre* compares very favourably with the published figures for other seeds (Table V).

TABLE V

Swelling factor of *Alyssum campestre* compared with other seeds, (G) indicates Greenberg's figures.[4]

Seeds	Swelling Factor	
	With shaking	Without shaking
Alyssum campestre	17·7	10·5
Plantago arenaria	17·0 (G)	8·0 (G)
„ *ovata*	11·0 (G)	9·5 (G)
„ *wrightiana*	20·0 (G)	14·5 (G)

To obtain the maximum yield of coagulated mucilage the minimum amount of water should be used for extraction and sufficient ethanol added to attain an alcoholic strength exceeding 50 per cent. (Table II). The yield of precipitated mucilage can also be increased by about 3·5 per cent. as a result of adding 2 ml. of 1 per cent. sodium chloride solution to each 100 ml. of mucilage. This increased sensitivity to ethanol is also obtainable by the addition of dilute hydrochloric acid.

Prolonged soaking of the seeds with successive quantities of water extracts other principles and renders the dried precipitated mucilage an off-white colour and difficult to purify. Naturally seeds containing earthy matter make purification of the mucilage still more difficult.

The quantitative determination of the mucilage content of the seeds by Greenberg's method[4] is not entirely suitable since it is impossible to press out all the mucilage from the seeds after one soaking in water. Hence the mucilage values by this method were lower than the yield obtained by successive soakings with water and precipitation with ethanol. (Tables II

and IV.) However, the mucilage content of the seeds, when determined by this method compares favourably with similar determinations on species of *Plantago*.

It would therefore appear that the seeds of *Alyssum campestre* contain sufficient mucilage to be of commercial value and in another publication[6] the pharmaceutical applications have been investigated.

Summary

1. The seeds of *Alyssum campestre* have been briefly described.
2. The extraction of the mucilage from the seeds and the properties of the mucilage have been investigated.
3. The proportion of mucilage in the seeds compares favourably with that in various species of *Plantago*.

This work was undertaken in the Department of Pharmacy, University of Nottingham and I desire to thank Mr. G. E. Trease for his help and encouragement.

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THE BACTERICIDAL ACTIVITY OF PHENOLS IN AQUEOUS SOLUTIONS OF SOAP

PART III. THE BACTERICIDAL ACTIVITY OF CHLOROXYLENOL IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE

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IN a previous paper[1] it was shown that the sparingly-water-soluble compound 5-chloro-2-hydroxydiphenylmethane (benzylchlorophenol) may be solubilised by aqueous solutions of potassium laurate when the latter are sufficiently concentrated to be micellar. The bactericidal activity of such solutions was found to be related to the concentration of the benzylchlorophenol in the micelles, and not to its overall concentration in the system as a whole.

The present communication deals with the solubility and bactericidal activity in aqueous solutions of potassium laurate of 2-chloro-5-hydroxy-1:3-dimethylbenzene (chloroxylenol), a phenol of somewhat greater water-solubility than benzylchlorophenol. This compound was selected for further study because, although more water-soluble than benzylchlorophenol, it is not so soluble that appreciable concentrations obtain in the aqueous phase when it is dissolved in aqueous solutions of potassium laurate. Compounds which are markedly soluble in water were avoided, since the bactericidal activity of systems containing such compounds dissolved in aqueous potassium laurate solutions, must be the summation of the activity of the aqueous phase and of the activity possessed by the micelles containing the compound. Such a condition would introduce difficulties in the interpretation of bactericidal measurements.

1. *The Solubility of Chloroxylenol in Aqueous Solutions of Potassium Laurate.*

EXPERIMENTAL

The solubility of chloroxylenol in aqueous solutions of potassium laurate was determined by the method described in an earlier paper[1]. Essentially, it consisted of diluting a series of solutions of chloroxylenol and potassium laurate with water, until crystals of chloroxylenol deposited on standing at 20° C. for about twenty-four hours.

For convenience, we prepared initially a concentrated aqueous solution of 0·3 M potassium laurate saturated with chloroxylenol. This solution could be diluted several-fold with water without deposition of crystals of chloroxylenol. The addition of water beyond a certain critical

volume, however, did result in the deposition of chloroxylenol crystals on standing. A solution containing slightly less than this critical volume of water was used as the basic working solution throughout the experiments. Its composition was as follows:—

Chloroxylenol ..	..	10·20g. or 0.265 M
Potassium Laurate	..	31·931 g. or 0·134 M
Distilled water to	..	1,000·00 ml.

The relative molar proportions of chloroxylenol and potassium laurate were approximately 1:2.

The addition of a volume of 0·1 M potassium laurate solution to the working solution, reduced the molar proportion of chloroxylenol to potassium laurate in the system, and increased the volume of water that could be added before the chloroxylenol crystallised out. Systems containing gradually increasing volumes of 0·1 M potassium laurate per unit volume of working solution were prepared until one was obtained which could be diluted with an infinite volume of water without crystallisation of chloroxylenol occurring on standing.

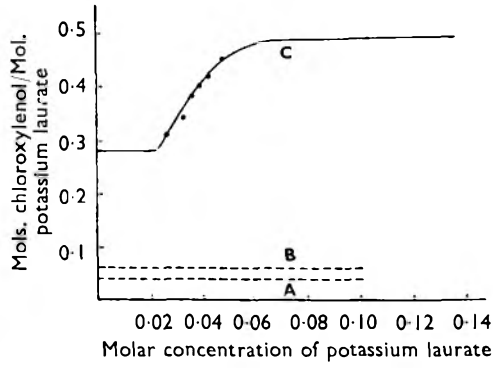

FIG. 1. The solubility of chloroxylenol in potassium laurate solution. The composition of the solutions examined for bactericidal activity is represented by points along lines A and B.

A. $\frac{0{\cdot}046 \text{ mol. chloroxylenol}}{\text{mol. potassium laurate}}$

B. $\frac{0{\cdot}061 \text{ mol. chloroxylenol}}{\text{mol. potassium laurate}}$

C. Solubility of chloroxylenol.

The composition of the systems prepared and examined is shown in Table I, together with the concentration of potassium laurate at which crystallisation occurred. The solubility curve of chloroxylenol in potassium laurate is shown in Figure 1, where the solubility is expressed as mols of chloroxylenol solubilised per mol. of potassium laurate at different concentrations of the latter.

The diagram shows that the solubility of chloroxylenol per mol. of potassium laurate remains constant for concentrations of potassium laurate up to about 0·02 M. As the concentration of the latter is increased from this value to about 0·04 M the relative solubility of chloroxylenol increases very rapidly. A further increase in the potassium laurate concentration from about 0·04 M to about 0·06 M produces a much less rapid increase in the solubility of the chloroxylenol. In excess of about 0·06 M potassium laurate there is negligible increase in the solubility of chloroxylenol per molecule of potassium laurate, even for a five-fold increase in the concentration of the latter.

Discussion

The overall sigmoidal shape of the solubility curve for chloroxylenol in potassium laurate closely resembles that described in a previous paper for the solubility of benzylchlorophenol in potassium laurate[1], except that the inflection in the curve is less marked. The increase in solubility of chloroxylenol with increase in the concentration of potassium laurate occurs over very approximately the same potassium laurate concentration range as did the increase in solubility of benzylchlorophenol. The solubility curve is similar to that obtained by McBain, Merrill and Vinograd[2] for the solubilisation of a water-insoluble dye by solutions of

TABLE I

The solubility of chloroxylenol in solutions of potassium laurate

Solutions, — 6	Volume of working solution, ml.	Weight of chloroxylenol in working solution, g.	Weight of potassium laurate in working solution, g.	Volume of 0·1 M potassium laurate added, ml.	Weight of potassium laurate added, g.	Total weight of potassium laurate in final solution, g.	Mols. chloroxylenol/mol. soap	Maximum volume of water that may be added before crystals deposit, ml	Total volume of final solution, ml.	Molar concentration of potassium laurate at end-point
A	1·0	0·0102	0·0319	nil	nil	0·0319	0·485	trace	1·0	0·134
B	1·0	0·0102	0·0319	0·10	0·0024	0·0343	0·451	2·0	3·1	0·046
C	1·0	0·0102	0·0319	0·20	0·0048	0·0367	0·416	2·5	3·7	0·042
D	1·0	0·0102	0·0319	0·30	0·0071	0·0390	0·397	3·0	4·3	0·038
E	1·0	0·0102	0·0319	0·40	0·0095	0·0404	0·377	3·5	4·9	0·035
F	1·0	0·0102	0·0319	0·60	0·0143	0·0462	0·336	4·5	6·1	0·032
G	1·0	0·0102	0·0319	0·80	0·0191	0·0510	0·030	6·5	8·3	0·026
H	1·0	0·0102	0·0319	1·00	0·0238	0·0556	0·277	∞	∞	$1/\infty = 0$

sodium lauryl sulphonate. It represents the formation of micellar material in aqueous solutions of potassium laurate. As the amount of micellar material per unit volume increases with increase in the concentration of the potassium laurate, so the solubility of chloroxylenol per molecule of potassium laurate increases until the micelles attain their maximal size. This occurs between 0·05 and 0·06 M potassium laurate. Further increase in the concentration of potassium laurate produces no further increase in the size of the micelles, with the result that there is no further increase in the solubility of chloroxylenol per molecule of potassium laurate. This is in agreement with the observations of McBain *et al.*[2].

It is interesting to note that the apparent concentration of chloroxylenol in the aqueous phase of saturated systems containing slightly less than the critical concentration of potassium laurate is greater than its published water-solubility (1 in 3,000). A similar anomalous solubility was noted with benzylchlorophenol in solutions of potassium laurate of similar concentration, and has been observed by other workers employing different systems[3,4].

2. *The Bactericidal Activity Against* Bacterium coli *of Chloroxylenol in Aqueous Solutions of Potassium Laurate*

Experimental

Two series of solutions containing chloroxylenol and potassium laurate were prepared. Each series contained an arbitrary proportion of chloroxylenol to potassium laurate, and was prepared by taking a fixed volume of a concentrated solution containing the arbitrary proportion of chloroxylenol to potassium laurate and distributing it into stoppered glass bottles. Increasing volumes of carbon dioxide free water were then added to each of the containers of a series, so that the solutions thus produced consisted of increasing dilutions of the concentrated parent solution. The composition of the individual solutions may be represented by points along two horizontal lines drawn on the solubility curve at the 0·046 and 0·061 molecules of chloroxylenol/molecule of potassium laurate levels respectively (Fig. 1), and is shown in Table II.

TABLE II

Mean death-times of *Bacterium coli* in solutions of chloroxylenol in aqueous potassium laurate

Concentration of potassium laurate	Mean death-times of *Bacterium coli* when mol. chloroxylenol / mol. potassium laurate = 0·061	Mean death-times of *Bacterium coli* when mol. chloroxylenol / mol. potassium laurate = 0·046
0·0113 M	213·0 minutes	—
0·0130 M	55·8 ,,	>240·0 minutes
0·0146 M	35·0 ,,	—
0·0162 M	7·2 ,,	16·3 ,,
0·0194 M	3·3 ,,	5·3 ,,
0·0227 M	2·6 ,,	—
0·0259 M	1·9 ,,	3·6 ,,
0·0292 M	3·0 ,,	—
0·0324 M	4·2 ,,	7·8 ,,
0·0389 M	4·3 ,,	11·3 ,,
0·0438 M	4·3 ,,	—
0·0454 M	—	11·6 ,,
0·0518 M	5·6 ,,	15·0 ,,
0·0648 M	6·2 ,,	10·0 ,,
0·0778 M	5·6 ,,	6·0 ,,
0·0907 M	3·2 ,,	4·6 ,,
0·1037 M	3·3 ,,	—

The bactericidal activity of the solutions against *Bacterium coli* was determined by adding 0·2 ml. of a 24-hour culture of the organisms to 5·0 ml. of the solution being assayed. Immediately after mixing, small volumes of the reaction mixture were transferred to sterile glass tubes maintained at 20°C, where the reaction proceeded for a known time, after which it was quenched by the addition of sterile broth. Details of the method were given in our previous paper[5].

The death-times of *Bacterium coli* in the different solutions are recorded in Table II. They are shown superimposed on the solubility curve for chloroxylenol in potassium laurate in Figure 2. The upper line represents the death-times of *Bacterium coli* in solutions containing 0·046 mol. chloroxylenol/mol. potassium laurate, and the lower line the death-times in solutions containing 0·061 mol. chloroxylenol/mol. potassium

laurate. The general shape of the death-time curves closely resembles that obtained with solutions containing benzylchlorophenol solubilised by potassium laurate.

The weakest solutions examined in each series contained about 0·01 M potassium laurate and had low activity. As the concentration of the solutions was increased with respect to both the chloroxylenol and the potassium laurate, the death-times of the organisms fell sharply, reaching a minimum in solutions containing about 0·025 M potassium laurate. As the concentration of the potassium laurate was increased beyond about 0·025 M and that of the chloroxylenol increased by the same proportion, the death-times began to increase again in both series of solutions. These increases in the death-times continued with increase in the concentration of both components of the solutions, until the soap concentration approximated 0·05 M, when a minimum of bactericidal activity was observed. A second increase in activity with increase in soap concentration was observed in solutions containing potassium laurate in excess of about 0·05 M.

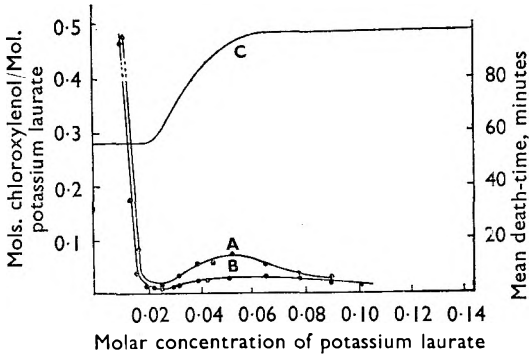

FIG. 2. The bactericidal activity against *Bact. coli* of solutions with constant chloroxylenol-potassium laurate ratio and increasing potassium laurate concentration, and the relation of the activity to the solubility of chloroxylenol.

A. $\frac{\text{0·046 mol. chloroxylenol}}{\text{mol. potassium laurate}}$

B. $\frac{\text{0·061 mol. chloroxylenol}}{\text{mol. potassium laurate}}$

C. Solubility of chloroxylenol.

DISCUSSION

The experiments showed that the changes in bactericidal activity that are associated with changes in the concentration of chloroxylenol and potassium laurate in aqueous systems closely resemble those previously reported in systems containing benzylchlorophenol and potassium laurate[5].

The very rapid decrease in the death-times that was observed as the concentration of both the chloroxylenol and the potassium laurate was increased by the same proportion, over the range 0·01 to 0·02 M potassium laurate, can be attributed to the increase in the concentration of both components of the system.

When the concentration of the potassium laurate reaches 0·02 M micelles begin to form in the solution. That is, as the concentration of potassium laurate is increased from below 0·02 M to above 0·02 M, a transition takes place from a true solution to a colloidal solution containing

micelles. This change in the physical state of the solution is accompanied by an increase in the death-times, even though the concentration of both the chloroxylenol and the potassium laurate has been increased. The increase in the death-times continued until the potassium laurate concentration was about 0·05 M and parallelled that observed in the experiments with benzylchlorophenol[5]. It was shown in the latter experiments that as the concentration of potassium laurate was increased from about 0·02 M to about 0·04 M and that of benzylchlorophenol by the same proportion, the concentration of soap in the micellar state increased much more rapidly than did the concentration of the benzylchlorophenol. That is, as the concentration of both soap and benzylchlorophenol increased over the soap range 0·02 M to 0·04 M, the concentration of benzylchlorophenol in the micelles decreased.

TABLE III

THE RELATION BETWEEN THE PERCENTAGE SATURATION OF POTASSIUM LAURATE MICELLES BY CHLOROXYLENOL AND THEIR BACTERICIDAL ACTIVITY AS INDICATED BY THE DEATH-TIME OF *Bacterium coli*

Mol. chloroxylenol / Mol. potassium laurate	Molar concentration of potassium laurate	Mols. chloroxylenol / Mol. pot. laurate to saturate solution	Percentage saturation of micelles	Log. of percentage saturation of micelles	Death-time of *Bacterium coli*	Log. of death-time of *Bacterium coli*
0·0457	0·026	0·304	$\frac{0·0457 \times 100}{0·304} = 15·03$	1·1770	3·6 min.	0·5563
0·0457	0·032	0·354	$\frac{0·0457 \times 100}{0·354} = 12·91$	1·1109	7·8 ,,	0·8921
0.0457	0·039	0·405	$\frac{0·0457 \times 100}{0·405} = 11·28$	1·0525	11·3 ,,	1·0531
0·0457	0·045	0·440	$\frac{0·0457 \times 100}{0·440} = 10·39$	1·0165	11·6 ,,	1·0645
0·0457	0·052	0·476	$\frac{0·0457 \times 100}{0·476} = 9.60$	0·9823	15·0 ,,	1·1761
0·0609	0·026	0·304	$\frac{0·0609 \times 100}{0·304} = 20·00$	1·3017	1·9 ,,	0·2787
0·0609	0·029	0·335	$\frac{0·0609 \times 100}{0·335} = 18·18$	1·2596	3·0 ,,	0·4771
0·0609	0·032	0·354	$\frac{0·0609 \times 100}{0·354} = 17·20$	1·2356	4·2 ,,	0·6232
0·0609	0·039	0·405	$\frac{0·0609 \times 100}{0·405} = 15·04$	1·1772	4·3 ,,	0·6335
0·0609	0·044	0·436	$\frac{0·0609 \times 100}{0·436} = 13·97$	1·1451	4·3 ,,	0·6335
0·0609	0·052	0·476	$\frac{0·0609 \times 100}{0·476} = 12·79$	1·1070	5·6 ,,	0·7482

In order to determine whether the same explanation applied in the case of the present experiments with chloroxylenol and potassium laurate, the percentage saturation of the micelles by chloroxylenol has been calculated for both series of solutions for the potassium laurate range 0·02 M to 0·05 M. The values are shown in Table III, where the death-times of *Bacterium coli* in the systems is also recorded. Reference to Figure 3 will show that the death-time of *Bacterium coli* in the solutions

is related to the logarithm of the percentage saturation of the micelles by chloroxylenol. It is independent of the concentration of chloroxylenol in the systems as a whole.

The second increase in activity observed as the concentration of the potassium laurate is increased from about 0·05 M cannot be due to an increase in the concentration of the chloroxylenol in the micelles, since at this concentration they reach their maximal size. A similar phenomenon was reported in the experiments with benzylchlorophenol, and we offered the suggestion that this second increase in activity was due to an increase in the number of micelles per bacterium introduced into the system. Preliminary experiments have indicated the possible validity of this explanation and experiments are in progress to substantiate it.

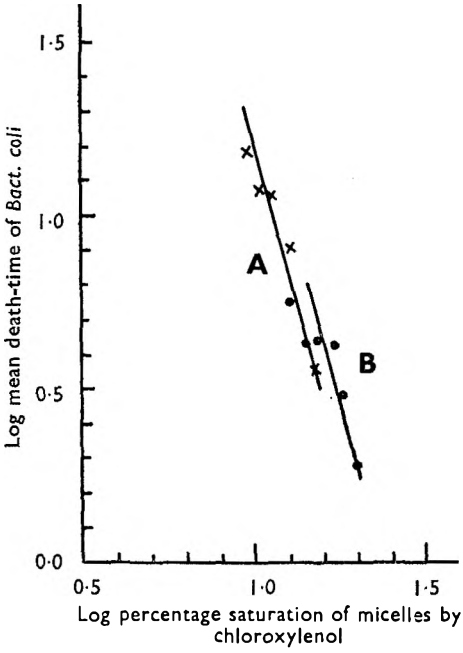

FIG. 3. Relation between the percentage saturation of micelles by chloroxylenol and the log mean death-time of *Bact. coli*.

A. $\frac{\text{0·046 mol. chloroxylenol}}{\text{mol. potassium laurate}}$

B. $\frac{\text{0·061 mol. chloroxylenol}}{\text{mol. potassium laurate}}$

SUMMARY

1. The slightly water-soluble compound, chloroxylenol, is solubilised by aqueous solutions of potassium laurate.

2. It is solubilised by internal solution in the micelles which form in potassium laurate solution.

3. The bactericidal activity of systems containing chloroxylenol solubilised by potassium laurate is independent of the concentration of chloroxylenol in the system.

4. The bactericidal activity of the system is a function of the concentration of chloroxylenol in the soap micelles.

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CHEMISTRY

ANALYTICAL

Amino-acids, Acetous Perchloric Acid Titration of. P. Ekeblad. (*Svensk. farm. Tidskr.*, 1953, **57**, 185.) The acetous perchloric acid titration of amino-acids has been tested on glycine and asparagine. It was found that glycine as well as *l*-asparagine monohydrate can be dissolved directly in acetic acid but that anhydrous asparagine requires an excess of perchloric acid. In all cases heating was necessary to obtain complete solution within a reasonable time. Heating without perchloric acid caused low and irregular results; apparently the amino-acids are partially acetylated. For glycine the acetylation is avoided if excess of perchloric acid is added. Heating of asparagine with excess of perchloric acid causes high results; probably the amido linkage in the asparagine molecule is broken down. The use of formic acid permits rapid dissolving of amino-acids without heating. The formic acid should contain as little water as possible and the final solution should contain not more than 2 per cent. of formic acid. The following method is proposed for the titration of amino-acids not soluble in acetic acid at room temperature. 1 milli-equivalent of the finely divided amino-acid is dissolved in 0·5 ml. of formic acid (97 to 100 per cent.) and the solution diluted with 25 ml. of anhydrous acetic acid. After the addition of Blue-BZL solution, the solution is titrated with 0·1N acetous perchloric acid to a red colour. S. L. W.

Ammonia in the Presence of Hydrazine, Determination of. W. Puch and W. K. Heyns. (*Analyst*, 1953, **78**, 177.) Procedures are described for the determination of ammonia in mixtures of ammonium and hydrazine salts. Known weights of hydrazine hydrochloride were added to known volumes of the standard ammonium salt solutions and, after oxidation of the hydrazine, the ammonia was distilled. For the oxidation 3 procedures using iodic acid, bromine, and alkaline permanganate were used. Equally good results were obtained with all 3 methods and a distillation time of 9 to 10 minutes was sufficient. R. E. S.

Antimony in Aromatic Organic Compounds, Determination of. N. T. Wilkinson. (*Analyst*, 1953, **78**, 165.) Methods were investigated for determining antimony in organic compounds that also contain nitrogen and chlorine. Decomposition of the organic compound by fusion with sodium peroxide, cane sugar and potassium nitrate in a Parr bomb was not satisfactory, as an antimony compound was formed that was insoluble in dilute nitric acid or dilute hydrochloric acid. In the recommended process the organic matter was destroyed by wet digestion with nitric and sulphuric acids, and the antimony compound formed was dissolved in a mixture of hydrobromic acid and bromine; the antimony was then reduced by sulphurous acid, the excess of sulphur dioxide being removed by boiling, and was determined volumetrically by titration with standard potassium bromate solution. In an alternative procedure the antimony was precipitated as the sulphide, separated by filtration, brought into solution and then titrated with potassium bromate solution. Satisfactory antimony recovery experiments are recorded in which aliquots of potassium

antimony tartrate solution were taken and known weights of *p*-chloraniline, aniline hydrochloride, and pyridine hydrochloride were added. The chlorostibinate of diphenylamine was also analysed by this method. R. E. S.

Cadmium, Microdetermination of. B. E. Saltzman. (*Analyt. Chem.*, 1953, **25**, 493.) A procedure applicable to the determination of micro quantities of cadmium in samples containing as much as 5 to 10 mg. of the common interfering metals is described. An improved dithizone separation is used with cyanide as a suppressing agent in two extractions from strongly alkaline solution, and using tartaric acid as the stripping medium. Stable colours are obtained and losses due to decomposition are controlled by using hydroxylamine in the extractions and by reducing the time of contact of the chloroform with the alkali. Working details of the method are given by which cadmium can be determined spectrophotometrically in a volume of 15 ml. with a sensitivity of 0·05 μg. The separation of thallium is made possible by the development of a special procedure which transposes the dithizonate of thallium with cobalt. Satisfactory results for the recovery of cadmium from urine, water, and spelter are quoted. R. E. S.

Carbonyl Compounds, Determination of. A. J. Feuell and J. H. Skellon. (*Analyst*, 1953, **78**, 135.) A volumetric method of determining aldehydes and ketones with semi-carbazide has been devised. The carbonyl compound was dissolved in water and the semi-carbazide reagent added; after filtering the precipitate and washing, hydrochloric acid and potassium cyanide were added to the filtrate which was then titrated with standard potassium iodate solution. Experiments with salicylaldehyde and benzaldehyde showed that useful results could be attained with a precipitation time of only 10 minutes and a 20 to 60 per cent. excess of reagent. The application of the semi-carbazide method to oxidised oils possessing marked reducing properties was disappointing, as semi-carbazones could not be precipitated and the method consequently failed. For estimating the carbonyl groups in the products of oxidation of fatty acids and esters by gaseous oxygen, the method of Maltby and Primavesi (*Analyst*, 1949, **74**, 498) was modified by the use of two reagent solutions instead of a single one, so that interference from colour inherent in the sample was eliminated and variations from neutrality in the sample were simultaneously compensated; the matching procedure was also adapted to daylight or artificial light. The method has been found useful for studies of the carbonyl content of the various fractions obtained in the separation of the complex end-products of oxidised fatty-acid esters. It has also been used to follow the steady decrease in carbonyl content occurring during the thermal catalytic autoxidation of ketohydroxystearic acid. R. E. S.

Copper, New Colorimetric Reagent for. G. F. Smith and D. H. Wilkins. (*Analyt. Chem.*, 1953, **25**, 510.) A new complexing reagent specific for copper, 2:9-dimethyl-4:7-diphenyl-1:10-phenanthroline, is proposed which has the highest molecular extinction coefficient in its application to the cuproine reaction of any previously known reactant in this category of the phenanthroline-type products. Spectrophotometric curves for a series of tests are shown, the absorption bands having sharp peaks with small absorption over the 500 to 700 mμ wavelength range. Beer's law applies over the range of copper concentrations 1 to 10 p.p.m. The copper complex cations are not entirely free from the effects of air oxidation (0.05 per cent. per hour under ordinary laboratory conditions). Commonly occurring ions such as chloride, nitrate, perchlorate, and phosphate as well as sulphate and citrate do not interfere. Metal

ions should not interfere but the application of the reagent to the determination of copper in biological, medicinal and food products would require further investigation. R. E. S.

Cyanide and Thiocyanate, Colorimetric Determination of. J. M. Kruse and M. G. Mellon. (*Analyt. Chem.*, 1953, **25**, 446.) A study was made of methods available for the determination of free and combined cyanide and thiocyanate in industrial wastes. Two procedures were examined based on the use of a pyridine-benzidine or pyridine-pyrazolone reagent to react with cyanogen halide formed by treating the sample with bromine or chloramine-T. A comparison of the two methods showed that the pyrididine-pyrazolone reagent formed a more stable colour with cyanide and this was finally chosen because of its greater precision and sensitivity. The main problem was the treatment of the sample necessary to permit the application of the reagent and 3 separate procedures were developed for the determination of free cyanide, total cyanide, and cyanide in the presence of thiocyanate. Work is outlined which led to the publication (*Sewage and Ind. Wastes*, 1951, **23**, 1402) of step-by-step directions for the separation and subsequent colour development for sewage analysis. Tests on sewage containing known amounts of various metals and the desired constituents showed the procedures to have satisfactory workability, sensitivity, and precision for the industrial application intended. R. E. S.

3-5-Diiodothyronine, Colorimetric Determination of. W. H. C. Shaw. (*Analyst*, 1953, **78**, 253.) An examination was made of the application of Millon's reagent to the determination of 3:5 diiodo-L-thyronine occurring as an impurity in L-thyroxine, since the reagent gives reddish colours with tyrosine and mono-iodotyrosine, but not with 2:6-substituted phenols, such as diiodotyrosine and thyroxine. The procedure of Roche and Michel (*Biochim. Biophys. Acta*, 1947, **1**, 335) was not directly applicable to the determination, since solutions were invariably turbid and the heat treatment prescribed (30 minutes at 60° C.) was insufficient to develop more than a trace of colour, this being adsorbed on the precipitate. The addition of the surface active agents sodium lauryl sulphate and trimethylcetylammonium bromide at concentrations of about 0·02 per cent. w/v proved effective, although the former was slightly better. In conjunction with 0·7 N sulphuric acid, sodium lauryl sulphate successfully prevented precipitation with up to 1 mg. of diiodothyronine. With larger quantities, opalescence sufficient to cause marked deviations from linear colour response developed. Increases in the concentration of surface-active agents failed to secure any improvement with more than 1 mg. R. E. S.

Fatty Acids, Determination of, by Potentiometric Titration. B. W. Grunbaum, F. L. Schaffer and P. L. Kiri. (*Analyt. Chem.*, 1953, 25, 480.) A titration assembly is described which provides isolation from atmospheric carbon dioxide and uses commercially available electrodes and *p*H meter. Using a rotating magnetic stirrer, ethanolic solutions of fatty acids were titrated with carbonate-free aqueous potassium hydroxide of constant normality provided by an ion exchange column. The method was applied to pure fatty acids in quantities of 0·005 to 0·025 microequivalent and to the fatty acids from liver digests. The *p*H readings of the meter were used empirically, the end-point with fatty acids being approximately 9·0. Replicate titrations were carried out with pure stearic and palmitic acids and with a mixture of stearic, palmitic, myristic, and lauric acids with satisfactory results. R. E. S.

Gamma Benzene Hexachloride, Determination of. I. Hornstein and W. N. Sullivan. (*Analyt. Chem.*, 1953, **25**, 496.) The procedure used involves the dechlorination of gamma benzene hexachloride to benzene and its subsequent nitration to *m*-dinitrobenzene, which after extraction is allowed to react with methyl ethyl ketone in the presence of strong alkali; the violet-red colour that develops is measured photometrically. In the estimation of gamma benzene hexachloride in air the air sample is drawn through gas-washing bottles containing acetic acid, and the amount absorbed in the acetic acid is determined; as an alternative the air sample is taken through an alumina adsorption column, the adsorbed gamma benzene hexachloride being washed off the column with acetic acid. The vapour is readily adsorbed, especially by rubber, and no material other than glass should precede the adsorbents. As a check on the methods, analyses were made in a closed cabinet, in which the walls had been treated with gamma benzene hexachloride and equilibrium attained in the enclosed air space; using both acetic acid and alumina adsorption, results corresponding to a "relative lindane saturation' of about 91 per cent. based on the theoretical value calculated from vapour pressure measurements obtained. The precision of the method appeared to be about ± 2 per cent., the results obtained showing good agreement with theoretical values calculated from known vapour pressure measurements. Details of procedure are given. R. E. S.

Glycerol in Fermentation Solutions, Chromatographic Determination of. A. F. Williams. (*Nature, Lond.*, 1953, **171**, 655.) A rapid and simple chromatographic procedure has been developed for the determination of glycerol in solutions obtained by the sulphite fermentation process of Cuban blackstrap molasses. The method involves the preparation of a column of coarse-grade cellulose powder (2·5 g.) which acts as a support for chromatographic alumina (5 g.). Sufficient of the sample is taken to give up to about 0·5 g. of glycerol and containing about 3 ml. of water, its volume being 5 ml. (approximately). After addition of sodium sulphite (0·5 g.), sodium acetate (1 g.) and acetic acid (0·1 ml.) to the sample, the resulting solution is mixed with alumina (15 g.) and the mixture then transferred to the prepared column. The glycerol is eluted with 250 ml. of solvent (acetone containing 5 per cent. v/v of water and 0·05 per cent. v/v of glacial acetic acid). Results obtained from recovery experiments are given. R. E. S.

Iodine Titration with Thiosulphate, End-point in. G. Knowles and G. F. Lowden. (*Analyst*, 1953, **78**, 159.) 5 methods were tried to determine the most suitable method for the detection of the end-point in the titration of iodine with thiosulphate; 2 depended on a colour change, the indicators being starch and sodium starch glycollate, and 3 were electrical: an amperometric method, the dead-stop end-point method, and a derivative polarographic method. The sensitivity of sodium starch glycollate appeared to be rather less than that of starch. Soluble starch indicator could lead to errors, on the low side, of the order of 20 to 40 μg. of iodine in volumes of 50 to 200 ml., and sodium starch glycollate was even less accurate. Comparisons of the derivative polarographic titration showed that although not so precise as the amperometric, it was capable of giving an end-point with a possible error of ± 0·01 ml. of 0·0025 N iodine solution (± 3 μg. of iodine). Results obtained with the dead-stop end-point method varied with the applied potential difference; the method also gave a slightly less sensitive indication of the presence of

iodine than the amperometric method. Of the 5 methods tried, the amperometric is the most satisfactory. The simple circuit described, incorporating a pointer micro-ammeter, detects one μg. of iodine in 40 ml. of solution.

R. E. S.

Magnesium, Colorimetric Determination of. A. E. Harvey, J. M. Komarmy and G. M. Wyatt. (*Analyt. Chem.*, 1953, **25**, 498.) A spectrophotometric method is given for the determination of magnesium using the reagent Eriochrome Black T, 1-(1-hydroxy-2-naphthylazo)-2-hydroxy-5-nitro-4-naphthalenesulphonic acid; at *p*H values from 7 to 10 this reagent forms a slightly dissociated soluble red complex with magnesium. Absorption curves are given for the dye, the magnesium, and the calcium complexes at a number of *p*H values; the light absorption of both magnesium and calcium complexes decreases as the *p*H decreases, but only the calcium complex absorption curve becomes identical with the reagent absorption curve at *p*H 7·75, suggesting the possibility of determining magnesium in the presence of calcium by arithmetical calculation using absorption values over a range of *p*H. Owing to the likelihood of errors, however, it is recommended that calcium is removed by precipitation as sulphate from a 90 per cent. methanol solution. Analyses of water samples by the proposed method agreed with gravimetric determinations within the usual error encountered in water analysis. R. E. S.

ESSENTIAL OILS

***Eucalyptus eudesmioides* Bark, Essential Oil of.** A. Blumann, M. Michael and D. E. White. (*J. chem Soc.*, 1953, 788.) An essential oil was obtained by the extraction of the finely-ground bark of *Eucalyptus eudesmioides* F. Muell. with light petroleum and ether followed by steam distillation of this extract. The volatile oil was then fractionated and yielded cineole, *d*-borneol (isolated as the 3:5-dinitrobenzoate), an uncharacterised hydrocarbon fraction, and globulol (isolated as the 3:5-dinitrobenzoate), a sesquiterpene alcohol previously obtained from *E. globulus* oil. A. H. B.

ORGANIC CHEMISTRY

Phenol, Purification of, for Chromatography. P. H. Mars. (*Pharm. Weekbl.*, 1953, **88**, 319.) Crystalline phenol (50 g.) is melted and dissolved in 100 ml. of light petroleum (40° to 60° C.) by warming to 50° to 55° C. Water (1 to 2 ml.) is added, the mixture is shaken thoroughly, and allowed to separate. The light petroleum layer is then poured off and allowed to crystallise. The light petroleum may be used again, while the phenol is dried in a current of air, and finally in a desiccator. To avoid drying, the phenol may be dissolved by the addition of a little water, the concentration of phenol determined by taking the refractive index, and the product diluted to the required strength.

G. M.

Steroid Sapogenins, Infra-red Absorption Spectra of. R. N. Jones, E. Katzenellenbogen and K. Dobriner. (*J. Amer. chem. Soc.*, 1953, **75**, 158.) The infra-red absorption spectra of 35 steroid sapogenins and derivatives were investigated and the band intensities compared on a molecular extinction coefficient basis. The steroid sapogenins are of considerable interest because they are the starting materials for the bulk synthesis of steroid hormones. Many absorption spectra are recorded and the position of the bands and their correlations with molecular structure are discussed. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Amino-acid Constituents of Normal Urine, Chromatographic Investigation of. W. H. Stein. (*J. biol. Chem.*, 1953, **201**, 45.) A separation of amino-acid constituents of normal urine has been effected using columns of the ion exchange resin Dowex 50 and a range of phosphate buffer solutions of varying *p*H. Substances responsible for various peaks in the effluent are identified by the addition of known amino-acids to the urine, the designated peak rising without loss of symmetry. Special colour tests and paper chromatography were also used as supplementary aids to identification. The absence in freshly voided urine of significant quantities of aspartic acid, citrulline, proline, glucosamine, hydroxylysine and ornithine has been indicated in this way. The presence of appreciable quantities of glutamine, reported by Archibald, could not be confirmed, as the substance decomposes during chromatography at *p*H 3. The presence of a number of unidentified substances has also been revealed; many of these substances are labile to acid hydrolysis. Asparagine, not hitherto reported as a constituent of normal human urine, was identified as an assymmetry on the trailing edge of the serine peak. It is much more readily detected in the urine of patients suffering from Wilson's disease. Quantitative data on a number of amino-acids has been made available for the first time by this method. It is also revealed that only about 10 mg. of cystine is excreted as such per day, whereas previous microbiological and polarographic methods had indicated that this figure was between 70 and 100 mg. It is suggested that these latter methods may actually determine peptides, such as a glutathione as cystine, giving rise to erroneous results. The glutamic acid content increases on standing, indicating that much of the conjugated glutamic acid is present in a labile form. The quantity of almost all the amino-acids rises after acid hydrolysis and gives an indication of the extent to which they are present as conjugates. Relatively large amounts of glycine, glutamic acid and aspartic acid are liberated by acid hydrolysis; only a small portion of the conjugated form of these amino-acids can be accounted for as hippuric acid, glutamine and asparagine. The amount of proline, cystine, serine, threonine, valine and tyrosine is also markedly increased by hydrolysis. Little taurine, leucine, methylhistidine or arginine is excreted in the conjugated form. J. B. S.

Insulin, Reversible Dissociation of. F. Fredericq. (*Nature, Lond.*, 1953, **171**, 570.) The sedimentation and diffusion constants of insulin in moderately alkaline solutions were determined respectively by a "Spinco" analytical ultracentrifuge and by the Lamm's scale method. A figure is given showing the effect of *p*H on the sedimentation constant of insulin (corrected to 20° C. and water) at a constant concentration of 0·25 per cent. of protein and ionic strength 0·1. Another figure shows the effect of protein concentration on the sedimentation constant of insulin at *p*H 10·1 and ionic strength 0·1 and 0·2 (buffer potassium chloride-glycine). The sedimentation constant rises when the concentration decreases from 1 to 0·7 per cent. in accordance with the ordinary charge effect, but goes down below this point because the dissociation becomes the overwhelming factor. At both buffer concentrations, the values tend to become identical and point towards a minimum at around 1·2. Similar conclusions can be drawn from diffusion data. The extrapolation of sedimentation and diffusion constants at zero protein concentration gives a molecular weight around 6000 for the insulin submolecule. In neutral or alkaline media, the dissociation is not influenced by the nature of the anions present. A. H. B.

Suxamethonium (Succinylcholine) Iodide. L. E. Tammelin. (*Acta chem. Scand.*, 1953, **7**, 185.) The synthesis of phenylsuccinylcholine from phenylsuccinic acid is described. The latter substance is converted to the corresponding acid chloride which is condensed in dioxan solution with choline chloride to yield phenylsuccinylcholine chloride; the latter is then converted to the crystalline iodide, m.pt. 250° C. (decomp.) by treatment with potassium iodide. Succinylcholine has a relatively short-lived curare-like action, and like *d*-tubocurarine it paralyses the skeletal muscles, the effect with both substances starting with the eye and pharyngeal muscles and ending with the diaphragm. Phenylsuccinylcholine is considerably less powerful than succinylcholine though it is able to produce a severe paralysis of the extremities. The hydrolysis of both succinylcholine and phenylsuccinylcholine has been investigated under varying *p*H and temperature conditions using Hestrin's method for the photometric estimation of choline ester. The hydrolysis is catalysed by the esterase in cobra venom. The hydrolysis of the two substances by enzyme preparations from various organs of the rabbit has also been studied. The results indicate that succinylcholine is hydrolysed more slowly than acetylcholine, decomposition occurring at much the same rate in blood, liver, voluntary and smooth muscle. Experiments with the electric organ of the electric ray *Torpedo*, which can be regarded as a huge neuromuscular end-plate, suggest that the cholinesterases in end-plates strongly affect the rate of succinylcholine hydrolysis. The relationship between succinyl choline hydrolysis and the number of equivalents of acid liberated in the reaction and the rôle of cholinesterases in changing the clinical picture after injection of succinylcholine is discussed. J. B. S.

Vitamin E in Cod-liver and Other Fish-liver Oils. F. Brown. (*Nature, Lond.*, 1953, **171**, 790.) The tocopherol content of samples of cod-liver oil was determined by the Emmerie-Engel method. Since this is not specific for tocopherols, the following check was carried out. Unsaponifiable material obtained after alkaline hydrolysis in the presence of pyrogallol was freed from carotenoids, vitamin A and sterols and chromatographed on "Vaseline" coated or silicone-impregnated paper using a variety of developing solvents. On spraying with $\alpha\alpha'$-dipyridyl and ferric chloride, a substance having the same R_F value as α-tocopherol was found, and no other substance reducing ferric chloride was detected. The following quantities were found in fish-liver oils: cod, 25·6, 30·2, 32·2, angler fish, 31·9, blue skate, 25·3, haddock, 18·0, ling, 27·2 and turbot, 45·0 mg./100 g. The typical signs of vitamin E deficiency in animals whose diet includes the oils may be due to the presence of the highly unsaturated acids of the oil. G. B.

BIOCHEMICAL ANALYSIS

Adrenaline and Noradrenaline in Suprarenals of Guinea-pigs, Colorimetric and Biological Estimation of. U. S. Von Euler and B. Hökfelt. (*Brit. J. Pharmacol.*, 1953, **8**, 66.) Biological assays of adrenaline and noradrenaline have been compared with the results obtained by the colorimetric method of Euler and Hamburg. The catechol amines were extracted from guinea-pig suprarenals and estimated biologically on the cat's blood pressure and fowl's rectal cæcum. The results obtained by the colorimetric method were in good agreement with the biological estimations, as distinct from the results of Shepherd and West, who previously had reported that the colorimetric method gave erroneous results. G. F. S.

Amino-acids, Chromatography of. A. L. Levy and D. Chung. (*Analyt. Chem.*, 1953, **25**, 396.) A new two-dimensional system for the qualitative analysis of amino-acid mixtures is recorded. Butanol-acetic acid-water (4 : 1 : 5) proved to be the most generally satisfactory solvent for one-dimensional papers; however chromatograms showed that the pairs threonine-glutamic acid, methionine-valine, *iso*leucine-phenylalanine, and to a lesser extent glycine-serine, were inseparable in butanol-acetic acid. To separate these four pairs of amino-acids the aqueous buffer procedure of McFarren (*Analyt. Chem.*, 1951, **23**, 168) was modified by the omission of potassium chloride and by the use of suitable mixtures of phenol and *m*-cresol. The most satisfactory two-dimensional system was found to be butanol-acetic acid-water, followed by 1 : 1 *m*-cresol-phenol, *p*H 9·3 borate buffer, run on Whatman No. 52 paper (an acid-washed paper, with considerable wet strength). The experimental procedures for protein hydrolysis and for the preparation of the two-dimensional chromatogram are given. R. E. S.

Caffeine, Fate in Man and Evaluation in Biological Material. J. Axelrod and J. Reichenthal. (*J. Pharmacol.*, 1953, **107**, 519.) A simple and sensitive method is described for the estimation of caffeine in biological materials and the results of a study of its absorption, excretion, distribution and rate of biotransformation in man. The estimation is based on the method of Ishler *et al.* 1 to 5 ml. of plasma is shaken with benzene and sodium chloride and an aliquot quantity of the benzene phase removed and shaken with 5N hydrochloric acid. An aliquot quantity of the acid extract is transferred to a quartz cuvet and the optical density at 273 mμ determined in a spectrophotometer. Comparisons are made with standard solutions of caffeine. Urine and tissue homogenates may also be extracted by a slightly modified technique. Experiments in man showed that caffeine was rapidly and completely absorbed from the gastro-intestinal tract, but only 1 per cent. was excreted in the urine, indicating almost complete biotransformation. The rate of biotransformation was 15 per cent. per hour. In dogs, caffeine was distributed in various tissues in proportion to their water content. There was a considerable accumulation of caffeine in the body after repeated coffee drinking. G. F. S.

PHARMACY

DISPENSING

Invert Sugar Solution for Injection, Preparation of. J. C. de Jong and W. A. Moeys. (*Pharm. Weekbl.*, 1953, **88**, 317.) As a source of energy, invert sugar given by injection is much more satisfactory than glucose. It is quicker in action, there is less danger of thrombophlebitis, and the excretion in the urine is much lower. A suitable, and colourless, solution may be prepared as follows. Dissolve 950 g. of sucrose in 4·5 l. of freshly distilled water and add 5 ml. of N hydrochloric acid. Heat for 1 hour at 100° C., cool off and adjust to a *p*H of about 6. Shake with asbestos, filter, fill into infusion bottles, and sterilise for 1 hour at 100° C. G. M.

GALENICAL PHARMACY

Liquorice; Effect of Trace-metal Content on Colour of Liquid Extract. S. Collett. (*Mfg. Chem.*, 1953, **24**, 124.) 3 samples of liquid extract of liquorice, all from the same batch, and each having a volume of about 3 fl. oz. were taken. One was kept as a control sample; a second was kept in contact

with a few fragments of granulated zinc for 2 months, the container being shaken once a day and the cork momentarily removed; and the third kept for the same period in contact with a few pieces of metallic lead, the container being shaken daily. The samples were then centrifuged and each portion examined with a view to determining if any metal had been taken up, if any change had occurred in the colour value owing to the presence of the metal, and what effect the metal had on the glycyrrhizin content of the extract. A 1–20 dilution of each sample was prepared, using 25 per cent. ethanol as diluent, and the colour assessed by determining absorption in the E.E.L. photoelectric colorimeter. It was shown that contamination with lead results in an increase and with zinc a decrease in the colour value of liquid extract of liquorice; contamination with lead, moreover, accentuates the red component. It seems probable that the metals exert their effect by combining with the glycyrrhizin; lead, by forming a highly-coloured salt, and zinc by precipitating the glycyrrhizin as the zinc salt. S. L. W.

NOTES AND FORMULÆ

Bacitracin, Stability of Solutions of. V. Würtzen. (*Farm. Tid. Kbh.*, 1953, **63**, 280.) A solution of bacitracin of 2000 units/ml. retains a satisfactory activity after 3 weeks storage at ordinary temperature, and is reduced to half strength in about 50 days. It may be kept for 3 months in a refrigerator. The addition of a buffer salt (pH = 6·0) decreases the stability. G. M.

Disulfiram (Antabuse). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **151**, 1408.) Disulfiram is bis(diethylthiocarbamyl) disulphide, and occurs as a white to light grey, odourless, almost tasteless powder, m.pt. 72° to 73° C., soluble in about 25 parts of ethanol, 14 parts of ether, and 5000 parts of water. When dissolved in methanol and treated with a methanolic solution of cupric chloride, a green colour develops, which changes rapidly from yellow-green to deep green. It contains not more than 400 p.p.m. of heavy metals and yields nor more than 0·5 per cent of ash. The loss in weight on drying in a vacuum oven at 60° C. for 4 hours does not exceed 0·5 per cent. Disulfiram contains 9·21 to 9·68 per cent. of nitrogen (determined by semimicro Kjeldahl), and 42·2 to 44·3 per cent. of sulphur. The sulphur is determined by fusing the disulfiram in a Parr bomb with sodium peroxide, potassium nitrate, sucrose, and potassium chlorate, and estimating the resulting sulphate by precipitation with barium chloride. Disulfiram is used in the treatment of alcoholism.

G. R. K.

Metharbital (Gemonil). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **151**, 1000.) Metharbital is 5:5-diethyl-l-methylbarbituric acid and occurs as a white, crystalline, faintly aromatic powder, m.pt. 151° to 155° C., soluble in about 23 parts of ethanol, 38 parts of ether, and 830 parts of water; a saturated solution has pH 5·6 to 5·7. A solution in sodium hydroxide yields a white precipitate, soluble in ammonia, with silver nitrate and with mercuric chloride. A 0·001 per cent. solution in 0·1 N sodium hydroxide exhibits an ultra-violet absorption maximum at about 244 mμ ($E^{1\,\text{per cent.}}_{1\,\text{cm.}}$, abour 440). Metharbital contains not more than 400 p.p.m. of sulphate and yields not more than 0·1 per cent. of ash. The loss in weight on drying at 105° C. for 4 hours does not exceed 1·0 per cent. It contains 13·85 to 14·42 per cent. of nitrogen, determined by semimicro Kjeldahl, and 95·0 to 105·0 per cent. of metharbital when assayed by measuring the absorption at 244 mμ of a 0·001 per cent. solution in 0·1 N sodium hydroxide. Metharbital is used in the treatment of epilepsy. G. R. K.

Sulfisoxazole (Gantrisin). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1953, **151**, 739.) Sulfisoxazole is N^1-3:4-dimethyl-5-isoxazolylsulphanilamide and occurs as a white, odourless, tasteless, crystalline powder, m.pt. 192° to 195° C., freely soluble in diluted hydrochloric acid and soluble in ethanol. A solution in sodium hydroxide becomes green and yields a greenish-blue precipitate on the addition of copper sulphate (distinction from sulphathiazole). A cold solution in diluted hydrochloric acid becomes yellow on the addition of sodium nitrite (distinction from sulphanilamide) and yields an orange-red precipitate on the subsequent addition of β-naphthol in alkaline solution. Sulfisoxazole loses not more than 0·5 per cent. of its weight when dried at 105° C. for 4 hours, and yields not more than 0·1 per cent. of sulphated ash; it complies with limit tests for chloride and heavy metals. It contains 95·0 to 105·0 per cent. of sulfisoxazole when assayed by solution in excess sodium hydroxide and titration with sulphuric acid, and 97·0 to 103·0 per cent. when assayed by measuring the absorption at 5400 Å. of a solution prepared by dissolving the sample in sodium hydroxide, diazotising, and treating with ammonium sulphamate and *N*-(1-naphthyl)ethylenediamine dihydrochloride.

G. R. K.

PHARMACOGNOSY

***Datura innoxia*, Alkaloids of.** W. C. Evans and M. W. Partridge. (*Nature, Lond.*, 1953, **171**, 656.) 3 samples of *Datura innoxia* Miller, one from the 1950 crop and the others from the 1952 crop, were analysed by the method described previously (*J. Pharm. Pharmacol.*, 1949, **1**, 593; 1952, **4**, 769), and the presence of hyoscine (0·24, 0·30 and 0·37 per cent.) was confirmed. The fraction at first considered to be hyoscyamine (0·035, 0·062 and 0·073 per cent.) afforded a picrate of melting point considerably below that of hyoscyamine picrate; fractional crystallisation of this picrate from water gave two picrates, one which was hyoscyamine picrate and the other which proved to be metaloidine picrate. The simplest conclusions with respect to *D. innoxia* are that the main site of alkaloidal syntheses are the roots for hyoscine and the aerial parts for hyoscyamine.

R. E. S.

PHARMACOLOGY AND THERAPEUTICS

Antibiotics, Actions on Intestinal Absorption. R. Ferrando, J. Bost and Denise Brenot. (*C.R. Acad. Sci. Paris.*, 1953, **232**, 1618.) A comparison of the absorption of casein hydrolysates in isolated segments of the ileum of the anæsthetised rat *in vivo* has shown that aureomycin and procaine penicillin facilitated their absorption. Dilutions of the hydrolysates containing known amounts of nitrogen were introduced for 15 to 25 minutes into the intestinal segment and then removed and the nitrogen depletion measured. Solutions with and without the addition of the antibiotics could be compared in the same segment of ileum.

G. F. S.

***Aspidosperma oblongum*, A.DC., Pharmacology of the Alkaloids of.** J. J. Banerjee and J. J. Lewis. (*Nature, Lond.*, 1953, **171**, 802.) Experiments with a 6 per cent. solution of the alkaloids of *Aspidosperma oblongum* A.DC. in isotonic saline solution at *p*H 6·8 indicate the presence of a potent cardioactive substance which antagonises acetylcholine or is associated with anti-acetylcholinergic substances. The alkaloids antagonise the action of acetylcholine and barium chloride in the isolated rat or rabbit ileum and guinea-pig,

rat or rabbit uterus. They inhibit the action of histamine and acetylcholine in the guinea-pig ileum and adrenaline on the isolated rabbit uterus. Inhibition of the action of acetylcholine in the frog rectus abdominis, curare-like neuromuscular blocking in the frog gastrocnemius-sciatic preparation and graded local anæsthetic action by the frog plexus method have been observed. A small dose (0·06 mg.) reduces the rate and amplitude of contractions in the isolated and *in situ* frog heart, and 0·6 mg. causes irreversible stoppage. In Langendorff heart preparations of the cat, rabbit and guinea-pig, the alkaloids cause reduction in rate and amplitude, followed by reversible auriculo-ventricular block, unmodified by previous atropinisation. The effects of lethal and sub-lethal doses in frogs and mice are described. G. B.

Benzoyltropine and Benzoyl-ψ-tropine, Quaternary Derivatives of, with Anticholinergic and Local Anæsthetic Properties. L. Gyermek. (*Nature, Lond.*, 1953, **171**, 788.) The methyl, ethyl, propyl, butyl and benzyl quaternary derivatives were prepared from benzoyltropine and benzoyl-ψ-tropine and examined for antimuscarine effect (isolated intestine of rabbit and guinea-pig), ganglion-blocking action (nictitating membrane of cat), curare-like effect in frog, infiltration anæsthesia (skin of rat abdomen) and conduction anæsthesia (frog plexus). Special study was given to the relationship between stereochemical structure and pharmacological effect. The aralkyl and higher alkyl quaternary derivatives showed activities similar to the tertiary compounds but the methyl derivatives were relatively inactive. Therefore the generally accepted view that the local anæsthetic action of amines is confined to secondary or tertiary compounds is not valid. G. B.

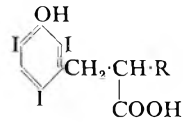
Benzylcholine, Methacholine and Acetylcholine, Correlation of Pharmacological Responses to Activity of Cholinesterases. T. Koppanyi, A. G. Karczmar and G. C. Sheatz. (*J. Pharmacol.*, 1953, **107**, 482.) This paper reports the pharmacological responses to cholinergic agents before and after the administration of anticholinesterase drugs in rabbits and dogs. They were initiated to elucidate the rôle of "effector" and "transport" cholinesterases in controlling the responses to cholinergic agents. Advantage was taken of the different pharmacological properties of acetylcholine, methacholine and benzoylcholine in studying this problem. Methacholine is hydrolysed *in vitro* only by true or acetylcholinesterase while benzylcholine is only affected by pseudocholinesterase. *In vivo* benzoylcholine causes only limited vasodepression and stimulation of gastro-intestinal motility and with doses larger than 1 mg. nicotinic actions entirely predominate, vasopressor effects due to stimulation of the sympathetic ganglia, and fasciculations and paralysis of voluntary muscles. The vasopressor effects were abolished by ganglionic blocking agents but not by adrenalectomy. Methacholine had only vasodepressor effects, largely due to vasodilation, which were blocked by atropine. It had no nicotinic actions. Acetylcholine showed predominantly muscarinic effects and atropinisation and employment of large doses were necessary for nicotinic effects. Di*iso*propyl phosphorofluoridate and other cholinesterase inhibitors converted the muscarinic effects of small doses of benzoylcholine to nicotinic effects and potentiated the nicotinic effects of larger doses. The muscarinic effects of methacholine were not potentiated by di*iso*propyl phosphorofluoridate. The degree of benzoylcholine potentiation by di*iso*propyl phosphorofluoridate followed closely the inhibition of cholinesterase activity in the blood and maximum potentiation was obtained with doses of di*iso*propyl phosphorofluoridate which completely inhibited cholinesterase but not acetylcholinesterase,

while responses to acetylcholine were first potentiated by doses of di*iso*propyl phosphorofluoridate which inhibited cholinesterase and acetylcholinesterase. Blood cholinesterase was shown to be the factor controlling the fate and the intensity of the pharmacological responses of benzoylcholine. This investigation has shown the importance of cholinesterase and acetylcholinesterase outside the neuroeffectors ("transport" cholinesterases) in controlling the fate of certain cholinergic agents prior to their reaching the neuro-effectors.

G. F. S.

***dl-, l-,* and *d*-Berbine, Determination of the Sympatholytic Activity of.** R. Hamet. (*C.R.Acad. Sci. Paris*, 1953, **256**, 1616.) Berbine, a synthetic compound which differs from yohimbine by the absence of both the OH group and the carboxymethoxyl from the pyrrol nucleus, has a sympatholytic action in the dog. A comparison of the *dl-*, *l-* and *d*-isomers showed the hypertensive effects of adrenaline to be abolished and ultimately reversed by 4·44 mg./kg. of the *dl-*, 3·24 mg./kg. of the *l-*, and 37·86 mg./kg. of the *d-* isomers, compared with 4·52 mg./kg. of the natural alkaloid corynanthine. 1 to 2 mg./kg. of berbine reduced the arterial pressure, while 2 to 4 mg./kg. increased a little the frequency and amplitude of the respiratory movements. G. F. S.

Cholecystographic Agents. D. Papa, H. F. Ginsberg, I. Lederman and V. DeCamp. (*J. Amer. chem. Soc.*, 1953, **75**, 1107.) The preparation and preliminary pharmacological evaluation of a new series of iodinated compounds of general formula (I) in which R = H, $-CH_3$, $-C_2H_5$, n-C_3H_7, n-C_4H_9, is described. Also compounds were prepared in which the phenolic –OH of compound I was replaced by –H or –I and R = $-C_2H_5$ (compounds II and III respectively). The compounds (I, R = C_2H_5 and R = n-C_3H_7) were outstanding in the quality of gall-bladder contrast and complete absence of side effects and residual medium in the colon. Compounds II and III were ineffective.



(I)

A. H. B.

Hydantoins as Anticonvulsants; 5-R-5-(2-Thienyl)-hydantoins. J. J. Spurlock. (*J. Amer. chem. Soc.*, 1953, **75**, 1115.) The synthesis of nineteen 5-substituted-5-(2-thienyl)-hydantoins and nine 3-alkyl- or 1:3-dialkyl-5-substituted-5-(2-thienyl)-hydantoins is described. The results of the anticonvulsant tests using the electroshock method with cats, with 5:5-diphenylhydantoin as standard are recorded. Only a few of the compounds were as active as 5:5-diphenylhydantoin.

A. H. B.

Isoniazid, Absorption, Distribution and Excretion of. B. Rubin and J. C. Burke. (*J. Pharmacol.*, 1953, **107**, 219.) After single or repeated oral doses of isoniazid in dogs it was found that peak plasma levels of the drug usually occurred within 30 minutes and only occasionally as late as 2 hours. The average 30-minute plasma concentrations were strictly proportional to and, when expressed as μg./ml., nearly identical with the oral dose in mg./kg. of body weight. The average plasma disappearance rate of the drug was 19 per cent. per hour. There was found to be relatively uniform distribution of isoniazid in plasma, spinal fluid, stomach, brain, liver, lung, spleen and intestine after the attainment of peak plasma levels; highest drug concentrations were obtained in the kidney after the first few hours, and lowest concentrations in the heart and omental fat. There was no chemically detectable cumulation of the drug in body

fluids or tissues with chronic doses tolerated for 2 to 8 months. Nearly one-half to four-fifths of an oral dose is excreted in the urine in 24 hours as unchanged drug plus *iso*nicotinic acid. With the evidence available to date it appears that absorption, distribution and excretion characteristics in the dog are similar to those in man. S. L. W.

Liquorice and Cortisone in Addison's and Simmond's Disease. J. G. G. Borst, S. P. ten Holt, L. A. Vries and J. A. Molhuysen. (*Lancet*, 1953, **264**, 657.) A synergistic effect between liquorice and cortisone was found in the treatment of 3 cases of Addison's disease. Liquorice alone did not exhibit the deoxycortone-like effect seen in normal people, but one of the patients, with a short period of suprarenal insufficiency before treatment, reacted well to liquorice at first. The effect of liquorice on the mineral metabolism was partially restored by giving 2·5 mg. of cortisone daily, and completely restored by 10 mg. daily. 2 of the patients were also treated with liquorice and adrenocorticotrophic hormone, but failed to respond, while a patient with Simmonds-Sheehan disease reacted favourably to liquorice and cortisone, liquorice and adrenocorticotrophic hormone and liquorice alone. It is considered possible that the favourable response of some Addisonian patients to liquorice is due to the presence of remnants of funtioning suprarenal tissue. The explanation of the activity of liquorice is discussed. J. R. F.

Liquorice and Glycyrrhetinic Acid, Effects of, on Salt and Water Metabolism. W. I. Card, W. Mitchell, J. A. Strong, N. R. W. Taylor, S. L. Tompsett and J. M. G. Wilson. (*Lancet*, 1953, **264**, 663.) The effects of liquorice and its derivatives on salt and water balance have been investigated. The preparation from crude extract of liquorice, block juice, was found to be capable of producing water, sodium and chloride retention in normal individuals. Similar results were also obtained by using glycyrrhetinic acid, which was also shown to have effects on the weight and electrolytes of an Addisonian patient, resembling those of deoxycortone and cortisone. The survival time of adrenalectomised rats, which is prolonged by deoxycortone, was not, however, lengthened by liquorice and its derivative. The mechanism of action of the liquorice is obscure, but it is suggested that the results are most readily explained on the assumption that its action on the kidney is similar to that of adrenal cortical hormones. J. R. F.

Menadione Sodium Bisulphite and Vitamin K_1, Comparative Effects on the Hypoprothrombinæmia Induced by Dicoumarol. W. G. Bannon, C. A. Owen and N. W. Barker. (*J. Lab. clin. Med.*, 1953, **41**, 393.) The counteracting effects of menadione sodium bisulphite and vitamin K_1 in prothrombin deficiency induced by dicoumarol have been compared in patients. Prothrombin times were determined by the method of Quick *et al.* Comparisons were made within the same patients, in paired patients and in other patients with varying degrees of hypothrombinæmia and dicoumarol administration. Vitamin K_1 was definitely more effective than menadione sodium bisulphite and 250 mg. of vitamin K_1 by mouth nearly always restored the prothrombin time to normal within 24 hours, whatever the height of the prothrombin time or the duration of the dicoumarol therapy. There was no refractoriness to renewed administration of dicoumarol afterwards. G. F. S.

Phenylbutazone, Toxic Effects of. J. C. Leonard. (*Brit. med.* J., 1953, **1**, 1311.) In 1526 patients treated with phenylbutazone, 22 per cent. showed toxic reactions. The most frequent complications were œdema, nausea, vomiting and diarrhœa, although more serious effects such as reactivation of peptic ulcer, hæmatemesis and melæna were observed. Skin rashes were fairly common and jaundice was noted in 6 patients. The effects on the blood included depression of any or all of the cellular elements. There were 11 cases of agranulocytosis and several of granulocytopenia. The incidence of reactions does not seem to be related to dosage, reactions occurring commonly with small doses. A fatal case of aplastic anæmia and one of agranulocytosis are described described in detail. G. B.

***iso*Propylnoradrenaline, Pharmacological Action of.** A. Lindner and C. Stumpf. (*Scientia Pharm.*, 1953, **21**, 1.) A comparative examination of the pharmacological actions of the *laevo* and *dextro* isomers of *iso*propylnoradrenaline showed that the toxicity of the former, for mice, is about 1·3 times that of the latter, when administered perorally or intravenously. With subcutaneous application this proportion is increased to 2·2. In pharmacological activity the *lævo* form is always the more active, the ratio of the activities of the two forms being as follows: broncholytic action, 5·4; blood pressure effect, 11·8; action on heart frequency, 8·7; spasmolytic effect, 7·9. The effect of *l-iso*propylnoradrenaline in lowering blood pressure is especially noticeable, as it is of the same order as that of acetylcholine: 4 μg. produces a mean drop in blood pressure, with chloralosed cats, of about 50 mm. of mercury. G. M.

Quaternary Compounds possessing Lasting Local Anæsthetic Action. K. Nádor, F. Herr, G. Pataky and J. Borsy. (*Nature, Lond.*, 1953, **171**, 788). When tertiary nitrogen compounds with local anæsthetic action are converted into alkyl or aralkyl quaternary derivatives, the resulting compounds show a delayed local anæsthetic effect of long duration. This effect usually develops after 30 minutes but frequently it is delayed 12 to 16 hours. The bromobenzylate of procaine was obtained by reaction of procaine with benzyl bromide in benzene or acetone, care being taken to exclude even traces of humidity. Similarly, amethocaine bromobenzylate was prepared in methanol-ether and cinchocaine bromobenzylate in ethanol-ether. The methyl quaternary derivatives were made in the same way. Generally the methyl derivatives were less active, but some of the benzyl quaternary derivatives were more effective local anæsthetics than the tertiary compounds. G. B.

Suxamethonium, Pharmacology of. G. F. Somers. (*Brit. J. Pharmacol.*, 1953, **8**, 19.) The actions of suxamethonium, a short-acting neuromuscular relaxant, have been studied in the cat. Suxamethonium acted like decamethonium first potentiating the twitches of the soleus and tibialis anticus muscles before producing a brief period of paralysis. Neostigmine potentiated and prolonged the paralysing action. Although closely related chemically to acetylcholine, suxamethonium showed no muscarine-like actions on the parasympathetic nervous system. In large doses it showed nicotine-like actions causing a rise in blood pressure through stimulation of the sympathetic ganglia which was prevented by ganglionic blocking drugs. G. F. S.

(ABSTRACTS *continued on p. 656.*)

BOOK REVIEWS

TEXTBOOK OF QUANTITATIVE ANALYSIS. Third Edition. *By I. M. Kolthoff and E. B. Sandell.* Pp. xv + 727 and Index. Macmillan and Co. Ltd., London. 1952. 30s.

The textbook of Quantitative Inorganic Analysis by Kolthoff and Sandell has become well-established because of the first two editions. In this third edition, the contents have been brought up to date and the survey of physical and physicochemical analytical methods has been rewritten and enlarged. Other changes include the complete revision of the chapter dealing with quantitative separation and the inclusion of a qualitative and quantitative discussion of the Brönsted theory of acids and bases. As in the previous editions, in order to facilitate the use of the book for instruction purposes, the authors have made use of three different types of print, namely, the finest to indicate informative material, an intermediate type to indicate advanced work, and the largest for material suitable for an elementary course. A number of problems are included at the end of each chapter.

The book is divided into four principal sections—gravimetric analysis, volumetric analysis, physical and physicochemical methods of analysis, and analysis of complex materials. The sections on gravimetric and volumetric analysis, which comprise more than three-quarters of the book, are subdivided into a treatment of the theoretical principles, a discussion of the practical work (including the apparatus and technique) and finally an adequate description of many types of determinations. These two main sections can be highly recommended for students of chemistry and pharmaceutical chemistry. The authors stated in their preface to the first edition that it seemed desirable to have available a book that could be used as an introductory text and which in addition would have the more or less comprehensive character of an advanced textbook, so that it would be suitable for use in beginning and advanced courses in analytical chemistry. In this edition, as in the previous ones, the authors have certainly achieved the object in the main sections of the book. The section on physical and physicochemical methods of analysis can only be regarded as an introduction to instrumental analysis, and this is the intention of the authors.

In these days of expensive books, many of which seem to be priced out of the students' financial reach, it is a pleasure to commend the publishers and the authors upon this excellently produced book at the reasonable price of thirty shillings.

A. H. BECKETT.

ANTIBIOTICS: A Survey of their Properties and Uses. Second Edition. Pp. xii + 290 (including 24 illustrations and Index). The Pharmaceutical Press, 17, Bloomsbury Square, London, W.C.1. 1952. 25s.

The 6 years which have elapsed since the earlier edition of this book (*Penicillin: Its Properties, Uses, and Preparations*) have seen a continuous and intensive research campaign directed towards the fuller exploitation of existing antibiotics and to the discovery of new. The surveying of the present position of these drugs must have been a correspondingly formidable task and the authors are to be congratulated on the readable manner of their presentation, which avoids the pitfalls of the "scientific digest" and yet confines the information within less than 300 pages. The original method of approach to the subject has been retained and there is very little modification to the sequence of chapters and their titles. The reader is first brought up-to-date on penicillin and then

given a similar but briefer account of other antibiotics, chiefly streptomycin, chloramphenicol, aureomycin, and terramycin. The most notable expansion is the chapter on Standards and Methods of Assay, now twice its original length, and one wonders whether this may lead to an increased interest in antibiotics on the part of public analysts. Veterinary practice, which was only mentioned briefly in the first edition, now has a complete chapter.

The pharmacist will find two uses for this book; firstly, as an aid to his own work in the compounding, packing and storing of antibiotic formulations, and secondly, as a source of up-to-date general information on the basic properties of the drugs and their clinical use. For the former, a pharmacist (himself in retail practice) describes the setting-up of an aseptic room and the extemporaneous preparation of various formulations in common use. Attention is drawn to the lack of precise information on the stability of many of these and one would have wished to have seen a little more on the effect of the container on shelf-life. A further small point of criticism is the list of commercial products which is hardly worthy of the title "Chapter II", and might better be included as an Appendix. These are, of course, only minor matters and the practising pharmacist will be well advised to have this book convenient to the dispensing counter even though he may not wish to consult any of the 841 references to scientific papers. Certainly the student who has mastered its contents need have no fear of the examiner. A. G. FISHBURN.

A STUDY OF ANTIMETABOLITES, by D. W. Woolley. Pp. xiii + 269 (including Index). Chapman and Hall, London. 1952. 40s.

The principle of drugs competing with natural metabolites has become important in the study of drug action. It was first conceived by Ehrlich, during his experiments on immunity and developed by Michaelis and his co-workers and by Quastel and Wooldbridge in their work on enzymes. Clark propounded the idea of "receptors" and recognized the competitive antagonism between structurally related drugs such as atropine and acetylcholine. Further impetus was given to the idea by the important observations of Wood, who found that the bacteriostatic action of sulphonamide drugs was reversed completely and competitively by *p*-aminobenzoic acid, which is regarded as the natural metabolite. Now, many other examples of drug antagonism can be explained by this form of interference and the hypothesis of antimetabolites provides a fundamental theory for explaining modes of drug action. This book, written by Dr. Woolley of the Rockefeller Institute, provides a critical survey of the discovery and principles of antimetabolites; and from a close examination of experimental evidence summarises our factual knowledge in developing the theory and applying it in physiological processes, biochemistry, chemotherapy and pharmacology. The relationship of structurally related drugs is discussed and an attempt is made to explain why some drugs, having quite dissimilar structures, may have similar effects. Among the important drugs which are dealt with are folic acid, vitamin K, the antihistamines, antithyroids, sex hormones and antibiotics. Finally the book presents practical suggestions for the synthesis and testing of antimetabolites and a most thorough bibliography. While Dr. Woolley has presented the essential facts up to 1950, he has rightly emphasised that there are still many difficulties in accepting the theory, and it will require considerable modification as time goes on and further factual knowledge is obtained. This book should be closely studied by all those who are engaged in producing or examining the actions of new chemical compounds. G. F. SOMERS.

HALE-WHITE'S MATERIA MEDICA, PHARMACOLOGY AND THERAPEUTICS, 29th Edition by A. H. Douthwaite. Pp. viii + 512 (including Index). J. and A. Churchill, Ltd. 1952. 20s.

The first edition of this book was published 60 years ago and the present author has been responsible for the 10 editions which have appeared during the past 21 years. It is no mean task to undertake revision of a textbook on a subject which frequently changes and rapidly extends its frontiers. The continued success of this enterprise is well reflected in the appearance of the 29th edition—a fitting tribute to the popularity and usefulness of this type of textbook. In previous editions much information was given about the character and nature of drugs, but in the new edition, this part of the text has been considerably revised and condensed to permit more extensive discussion of the actions and uses of drugs. This pharmacological bias has added greatly to the value of the book, which now includes an account of most of the recent work on the subject. The contents are arranged in three major sections. The first deals with definitions, pharmaceutical matters and general principles of prescribing; the second with substances which are used chiefly for their local action, and in the third section are the substances used chiefly for their general action. This arrangement of the subject-matter is convenient for describing the materia medica but is less well adapted for pharmacological description. For example, it might be anticipated that the analgesics drugs would be described in one section but the only reference in the index leads to page 286 where the antipyretic drugs, acetanilide, phenazone, phenacetin and amidopyrin are discussed, and these are separated from the salicylates by a chapter on cinchona and the drugs used in the treatment of malaria, whilst the more potent analgesics are discussed in the section on opiates on pages 199 to 211. The disadvantages of this arrangement are also apparent in the description of veratrine under local anæsthetic drugs; *iso*nicotinyl hydrazide in the vitamin chapter is pharmacologically isolated from the other tuberculostatic drugs described under antibiotics. A method of presenting the subject in a completely logical fashion however has not yet been described, and this is therefore only a minor criticism of a textbook which continues to maintain its reputation for its clarity of exposition.

ANDREW WILSON.

(ABSTRACTS *continued from p. 653.*)

Tetanus Toxoid, Rapid Specific Preventive Action of. M. Raynaud and E. A. Wright. (*Nature, Lond.*, 1953, **171**, 797.) The injection of 500 Lf doses of tetanus toxoid into mice completely prevented local tetanus and death from 1 MLD of tetanus toxin given subcutaneously 24 hours later. Toxoid from another laboratory was used, administered intravenously to ensure that the protection was not a local effect or dependent on the toxin and toxoid being made from the same strain of organism. Protection lasted only a short time, no protection being observed 6 days after injection of the toxoid. Since no antibodies have been detected in the first days after the administration of toxoid, the most probable explanation is that the toxoid acts either by prior blocking of the hypothetical receptor substance in the central nervous system or by competitive inhibition of the action of the toxin. G. B.

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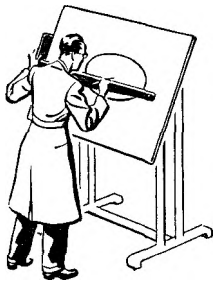

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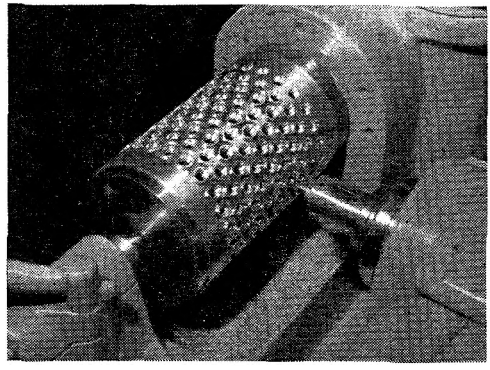

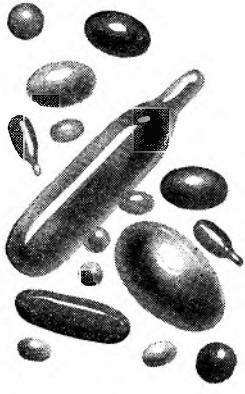

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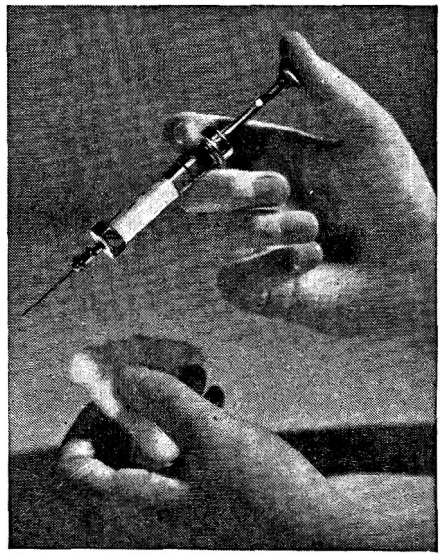

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