ION EXCHANGE*

TECHNICAL APPLICATIONS OF ION EXCHANGE

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Reaction and Technical Methods of Ion Exchange

If the principle of ion exchange is reviewed it is seen that the exchange reactions have been exploited in many chemical and pharmaceutical procedures. Fig. 1 gives a survey of the various kinds of ionic exchange reactions distinguished according to the types of electrolyte participating[1].

In the first reaction equation, ion exchange is illustrated by the example of cation exchangers. Here, the exchangeable ions are small; the cations of a molecular dispersion are exchanged for the cations held on the cation exchanger. This type is probably the most widely used technically. The second reaction reproduces the conditions prevailing in an exchange system in which filamentary polyions—of, for example, colloidal structure and having a charge of the same sign as the ion exchanger itself—are present in the external solution. In this particular instance the exchangeable ions are able to distribute themselves between the resin and the solution without involving a Donnan effect[2]. The third reaction equation reproduces the conditions in a so-called "contact exchange". This consists of an exchange of ions between solid material containing electrolyte and the ion exchanger, as used by us in, for example, the isolation of the total alkaloids of cinchona bark[3]. The reaction equilibria of the fourth and fifth reactions are usually displaced far to the left because, for steric reasons, no more than a superficial adsorption of the soluble polyelectrolytes occurs and the internal charged particles of the ion exchanger are generally inaccessible to the polyions. As is the case in a mixed bed, nothing more occurs in a cation exchanger of H-form and in an anion exchanger of OH-form, for example, than a superficial adhesion between the two ion exchangers, so that they also can neutralise each other only superficially. The last two reactions have accordingly acquired no great significance in chemical and pharmaceutical elaboration. The use of cation exchangers and anion exchangers in the mixed-bed method is based on the circumstance that the two exchangers exert practically no influence on one another.

As ion exchangers do not act in accordance with the old dictum "Corpora non agunt, nisi soluta," but participate in the solid phase in exchange reactions and, when a desired type of ion has been fixed, can be mechanically separated together with the latter from the solution treated, they can be used for ionic separation.

The techniques of ion exchange at present used industrially do not differ in principle from the method used in the laboratory for analytical

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purposes. The following techniques are in use for bringing an ion exchanger into contact with the electrolyte to be treated.

The Batch Technique

After suitable pre-treatment, a specific quantity of the granular ionic exchange resin is agitated with the electrolyte solution until equilibrium of exchange between the ions adsorbable from the solution and the non-adsorbable ions has been established. In the conditions prevailing in the batch method, this process does not take place quantitatively. The extent to which ions from the solution are replaced by the ions fixed in the resin depends on the selectivity of the resin in the prevailing conditions of equilibrium. Where selectivity is not favourable, only a small portion of the ions from the solution can be exchanged for ions from the exchanger resins. When the ionic reaction is completed, the exchanger resin is separated by filtration, washed and processed. This specific technique is used where reactions are required in a closed system and where for technical reasons there is no possibility of supplying new substance to be treated. The batch method is used in the titrimetric determination of exchange capacity, in so-called "contact exchange" and in cases where ion exchangers are used in catalysis.

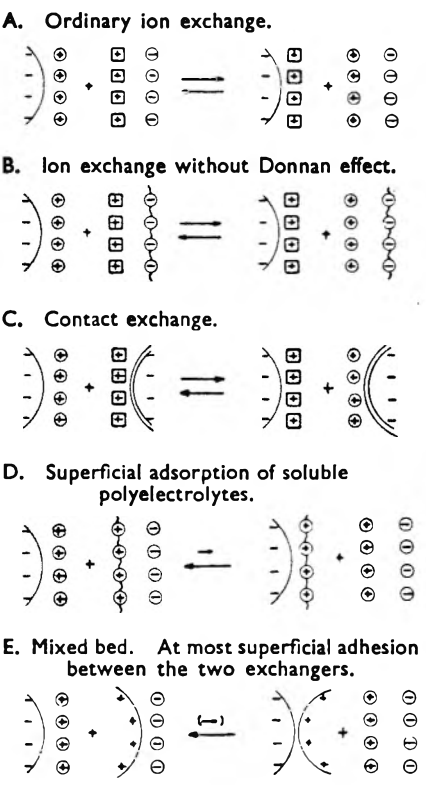

FIG. 1

The Column Technique

When it is desired to achieve the complete exchange of one ion for another, the batch method is unsuitable. The column method has been developed to achieve this aim. The electrolyte solution to be treated passes down a column charged with the ion exchanger. The upper layers of ion exchanger are thus always brought into contact with fresh, untreated electrolyte solution. As the electrolyte solution passes down through the column, undergoing exchange until a specific equilibrium is established, it comes into contact with fresh ion exchanger, through whose exchange action the equilibrium of the reaction is progressively displaced further to the right, i.e. in the direction of a quantitative ionic exchange.

The effect achieved by the column technique is comparable with that obtained by a large number of successive batch methods.

According to the difference in affinity between two substances to be separated, the column method can be carried out in different ways. The ordinary exchange method is suitable for separating oppositely-charged ions. The ions in the solution flowing through are exchanged for those in the ion exchanger, while foreign ions pass through the column. The elution method enables ions of like charge to be separated. This method combines the chromatographic principles of operation with those of ion exchange on columns[4]. Here the existence of small differences in exchange potential between related ions is exploited. The ions adsorbed at the top of a relatively long ion exchange column are eluted through a continuous interplay of adsorption and desorption and by the fact that a separation of the different ions into more or less clearly separate layers occurs because of slight differences between the equilibria of exchange in the middle and lower parts of the column. If elution is continued, these layers begin to appear in specific fractions of the effluent. A method of separating amino-acids by this principle has been developed by Stein and Moore[5], who were able to recover thirty-eight different amino-acids by this method.

The variants of the elution method include gradient elution, in which the concentration of the eluent is steadily increased and improved separation effects are achieved, and the displacement method, in which the eluent contains an ion whose affinity with the ion exchanger lies between those of the two ions to be separated. Further methods for the use of the ion exchange column will be found in the literature[6,7].

The membrane technique

Ion exchangers can also be regarded as ion filters of selective permeability. Thus in the ideal case a cation exchanger is permeable only to cations, while an anion exchanger is permeable only to anions. These properties were exploited in order to produce semipermeable membranes on the basis of ion exchangers.

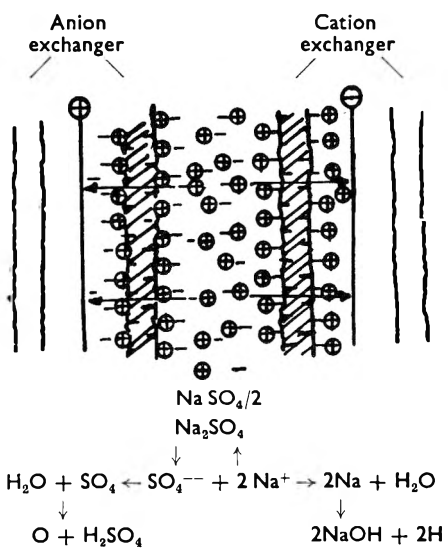

FIG. 2. Ion exchange membranes.

Figure 2 reproduces schematically a cation and an ion exchanger membrane. Negatively and positively charged groups, respectively, are present on their two outer surfaces. The intermediate cell contains an electrolyte solution (e.g. sodium sulphate). If a potential gradient is

applied to the two sides of the ion exchanger membranes, the cations (Na^+) in the electrolyte cell are able to migrate through the cation exchanger membrane into the cathode chamber. The OH^- occurring at the cathode cannot leave the cathode chamber because the cathode exchanger membranes sealing the chamber are impermeable to anions. Accordingly, soda lye is formed here. The anion (SO_4^{--}) present in the electrolyte cell passes through the anode exchanger membrane enclosing the anode chamber to the anode and there causes the formation of sulphuric acid, because the anode exchanger membranes are impermeable to cations. Thus such cells permit the production of free bases or acids from their salts[8] and also the demineralisation of organic compounds[9]. This principle has made possible the economical demineralisation of sea water for the purpose of producing drinking water, using multiple-cell units[10].

The membrane method, proposed in 1952, has a promising future, especially in electrodialysis.

Prospects for the Technical Utilisation of Ion Exchangers

Ionic separation according to the ion exchange principle enables: (*a*) undesired foreign ions or electrolytes to be removed from a preparation, (*b*) certain ions or electrolytes to be elaborated as desirable end-products, or (*c*) certain ions in the form of ion exchangers to be used as catalysers of chemical reactions. These applications will now be discussed by reference to a few examples.

Elimination of undesired foreign ions

If in the course of purifying and elaborating a pharmaceutical preparation it is desired to eliminate contaminating foreign ions, these ions are generally fixed on the ion exchanger by an ion displacement reaction, while the solution of the purified substance is separated from the ion exchanger. The adsorbed product is thus not the desired preparation, but an impurity or by-product originating from accessory substances or produced through side-reactions. A classical example is the softening of hard water, which constitutes the first technical application of ion exchange. The purpose here is to eliminate from the water the hardness-producing ions (Ca^{++}, Mg^{++}, Fe^{++} and Fe^{+++}, Mn^{++}) which are often detrimental. The sodium forms of cation exchangers, such as the permutites and, more recently, the synthetic exchanger resins, are used for this purpose. The hardness-producing cations are exchanged for the Na^+ ions of the cation exchanger according to the following equation (R = exchanger):

$$2[R^-\cdot Na^+] + [Ca^{++}\cdot 2Cl^-] \rightarrow [2R^-\cdot Ca^{++}] + 2(Na^+\cdot Cl^-).$$

Since the introduction of the new high-capacity exchangers based on polystyrol, the technique of water softening has been further improved. Advantages afforded by these new exchangers are higher capacities, improved utilisation of the regenerating agents, a higher speed of reaction and a longer life of the ion exchangers[6].

In the electro-plating industry, chromic acid baths have to be renewed

from time to time because metallic ions such as Cu^{++}, Fe^{+++}, Mn^{+++} and Al^{+++} accumulate in them. While regeneration of the chromic acid by chemical methods does not pay, it can, after dilution, be passed through a column containing a strongly acidic cation exchanger in the H-form, when the metallic impurities are almost completely held by the exchanger resin, while the chromic acid anion passes without hindrance through the column. Chromic acid solutions so purified can then be returned to the chromium-plating bath. The introduction of cation exchangers of the polystyrol type, which are not attacked by a 10 per cent. chromic acid solution, was necessary before this method of purification could be attempted.

Simple exchange reactions are also used for converting the potassium salt of penicillin into the sodium salt. For this purpose an aqueous solution of the potassium salt is passed through a cation exchanger in the Na-form:

$$[R^{-}\cdot Na^{+}] + [Pen.^{-}\cdot K^{+}] \rightarrow [R^{-}\cdot K^{+}] + [Pen.^{-}\cdot Na^{+}]$$
$$2[R^{-}\cdot Na^{+}] + C^{++}(M) \rightarrow [2R^{-}\cdot Ca^{++}] + 2Na^{+}(M).$$

Similar reactions are also carried out in the elaboration of dietetic milk preparations. For example, calcium can be removed from cow's milk by treating the latter with a cation exchanger in the Na-form. Such low-calcium milk forms a flocculent, readily digestible, coagulated milk in the infant's stomach. In the preparation of another kind of dietetic milk the sodium in cow's milk is replaced by potassium.

The removal of undesired anions is likewise frequently resorted to in industry. According to Christy and Lembcke[11], a formic acid-free formaldehyde can be obtained cheaply by ion exchange. By de-acidifying excessively acid fruit juices and wines by the use of anion exchangers, more pleasant-tasting and better-tolerated beverages can be prepared. In these, a part of the wine is treated with a weakly basic anion exchanger and, after passage through the exchanger, mixed with a sufficient quantity of the untreated wine, since de-acidification, if carried too far, spoils the flavour and adversely affects the keeping properties. The ion exchange method is also industrially used in Switzerland for the partial de-acidification and demineralisation of fruit-juice concentrates[12].

The elimination of cations and anions which are impurities in or harmful ingredients of preparations is known as total demineralisation, and is often done. If the cation exchanger is brought into action in the H-form and the anion exchanger in the OH-form, the electrolyte solution being passed first through the one exchanger and then through the other, complete elimination of the electrolytes is achieved. This de-ionisation or demineralisation of sodium chloride, as a simple example of an electrolyte, may be formulated thus:

$$[R^{-}\cdot H^{+}] + [Na^{+}\cdot Cl^{-}] \rightarrow [R^{-}\cdot Na^{+}] + [H^{+}\cdot Cl^{-}]$$
$$[R^{+}\cdot OH^{-}] + [H^{+}\cdot Cl^{-}] \rightarrow [R^{+}\cdot Cl^{-}] + H_2O.$$

This technique is used for the demineralisation of water and other electrolyte solutions and for purifying colloidal solutions, gels, and so

forth. In many instances the method replaces dialysis and electrodialysis with satisfactory results.

The problem of the production of unobjectionable demineralised water from potable water arose from the needs of pharmaceutical practice. In addition to freedom from chemical and bacteriological contamination, the pharmaceutical industry requires a technique which, while being as cheap as possible, is able to deliver large quantities of purified water at short notice. In view of the rapid deterioration of distilled water through picking up foreign matter from the air, containers and contamination by micro-organisms, the pharmacy ought to have at its disposal a method by which it can itself rapidly manufacture purified water to meet its own requirements.

In the pharmaceutical literature, Harrison, Myers and Herr[13] were the first, in 1943, to consider this question. The first investigations in Europe were carried out by Bütikofer and Ammann[14], and valuable contributions have since been made by Saunders[15], and Fischer[16]. At about the same time Büchi and Soliva[17], at Zurich, reported their experience in the manufacture of demineralised water. These latter investigations were made for the Swiss Federal Pharmacopœia Commission to determine whether "aqua demineralisata" could be accepted on an equal footing with "aqua destillata" for official purposes. To be free from criticism, demineralised water should comply at least with the specifications laid down in the pharmacopœias for distilled water. In the ideal case demineralised water could also, if necessary, subject to certain safety regulations, be used for the preparation of eye-drops, eye lotions and injection solutions.

The experience of Saunders[15], and our own observations[17], have shown that freedom from odour and colour can be satisfied by prepared demineralised water. A complete elimination of mineral substances can be achieved by collecting an effluent water having a specific resistance of not less than 1 million ohms per cm. If the quality of the exchanger resins is as it should be, if they are correctly prepared for ion exchange, and if the deionisation unit is properly operated, it is, as Saunders[15], Fischer[16] and Büchi and Soliva[17] have shown, possible to comply with the requirement that there should be no organic impurities in the demineralised water. Fischer[16] and Büchi and Soliva[17] have confirmed by bacteriological tests of their deionised products that if the exchangers are of suitable quality and the plant is correctly operated, without protracted standby periods, sterile demineralised water can be collected, but there is no guarantee that the effluents will be invariably sterile. The ever-present possibility of a "break-through" of pyrogens in the demineralised water renders the method uncertain. In our opinion, therefore, a demineralised water ought not to be used for the manufacture of collyria or injection solutions unless it has been tested for freedom from pyrogens.

Since the ion exchange process has been technically mastered, it is possible to manufacture unobjectionable purified water which complies with the strict purity specifications prescribed for distilled water in the pharmacopœias, and in view of the fact that increasing quantities of demineralised

water are being used in the pharmaceutical industry, hospital dispensaries and larger pharmaceutical laboratories for preparing medicines, the demineralisation method ought—as Saunders[15] also proposes—to be officially permitted by all pharmacopœias. In both the British and the United States pharmacopœias purified water is now official, and the Swiss Federal Pharmacopœia Commission has also decided to adopt the method while requiring certain safeguards.

Demineralisation is also employed in the sugar industry. The dry residue of molasses from sugar-beet consists of about 91 per cent. sugar, the remainder consisting of inorganic salts and organic impurities which add to the difficulty of crystallisation and reduce the yield of crystalline sugar. By demineralising sugar juices, the yield of crystallisable sugar can be increased by about 10 per cent.

The 10 to 12 per cent. glycerol waters which accumulate in the Twitchell and autoclave processes for hydrolysing fats, contain many inorganic and organic impurities. While the pre-purification and distillation of glycerol by the old methods are very expensive recovery processes, demineralisation by means of ion exchange is much cheaper. By a combined treatment in multiple-bed and mixed-bed plants a glycerol complying with pharmacopœial specifications can be obtained. The product elaborated in this way is cheaper and purer than distilled glycerol[18,19]. A detailed investigation by Rentschler and Tanner[20] shows that faulty brandies can in many cases be elaborated into up-to-standard products by treatment with suitable ion exchangers. Acetic acid, sulphurous acid and mercaptans can be eliminated with anion exchangers, Fe^{+++}, Cu^{++} and Zn^{++} with cation exchangers and excessively high gravities by demineralisation.

Ion exchange is also being used with increasing frequency for purifying high-grade biological preparations. Müller[21], for example, reports the successful elimination of proteolysis products from viruses. In addition, ion exchange is being increasingly resorted to in place of dialysis and electrodialysis for the purification of enzymes.

Isolation of dissociating end-products

In this ionic exchange technique the desired product is adsorbed on a suitable exchanger, there enriched in purified form and, in a final phase, liberated again from the ion exchanger and worked up. This technique is particularly suitable for the extraction of small quantities of substance from very dilute solutions. The desired substance can be concentrated and isolated quickly, without injury, and cheaply without further extraction and evaporation. Due to a high selectivity for specific ions, it is also possible to closely separate related ions through anion exchange. The special properties of the exchanger resins and specific exchange techniques are applicable here.

The recovery of heavy-metal ions from industrial waste liquids is becoming increasingly important. It is not only a question of purifying the waste liquids, some of which are toxic, but also of increasing the economy of the manufacturing processes by recovering accessory substances. The last-mentioned consideration is particularly important in

the manufacture of copper-acetate rayon, for example. In order to improve its economy, the practice of recovering copper with the aid of ion exchange has been adopted. The alkaline water from the first wash is passed through a weakly acid cation exchanger, such as Amberlite IRC-50, the copper being almost quantitatively retained on the exchanger resin. Regeneration is made with sulphuric acid and the copper recovered by electrolysis. In the rayon industry zinc is also now being successfully recovered from the washing water. The wash water containing zinc and sodium is passed through a strongly acid cation exchanger in the H-form. The zinc held on the exchanger is liberated by the use of sulphuric acid as a regenerating agent. The resultant zinc sulphate solution can, after concentration, be returned to the manufacturing cycle[12].

Large quantities of chromic acid are apt to be lost with the washing water in electroplating shops. The practice has recently been adopted in many places of collecting this water and treating it with a strongly basic anion exchanger, such as Amberlite IRA-410 or, better still, IRA-401. After passage through the exchanger column, the column is regenerated with sodium sulphate. The regenerating fluid must subsequently be passed through a strong cation exchanger for the recovery of the free chromic acid.

The preparative extraction of organic bases and the manufacture of their salts have been studied at length by many authors. We shall refer later in detail to the use of ion exchange for the isolation and elaboration of alkaloids.

A very important application of ion exchange in pharmaceutical chemistry is in the elaboration of free organic acids and their salts. Strongly contaminated acids which accumulate as dissolved salts in a chemical or biochemical reaction can be passed over suitable anion exchangers in the hydroxyl form and fixed on the exchangers. The elution of the free acids from the column is effected with a mineral acid.

$$[R^{+}\cdot OH^{-}] + [RCOO^{-}\cdot Na^{+}] \rightleftharpoons [R^{+}\cdot RCOO^{-}] + [Na^{+}OH^{-}]$$
$$[R^{-}\cdot H^{+}] + [RCOO^{-}\cdot Na^{+}] \rightleftharpoons [R^{-}\cdot Na^{+}] + RCOOH.$$

The salts of organic acids can also be passed over a cation exchanger in the H form, whereupon the solution of the free organic acid will appear in the effluent. This acid may have to be eluted with an organic solvent if it is insufficiently soluble in water. Carbonic acids, amino-acids, oxycarbonic acids and the like can be elaborated in this way. This technique is also used, for example, in the manufacture of complexones.

A procedure of the utmost importance in pharmacy is the isolation of alkaloids from drug extracts and the like for the purpose of determining the vegetable base content or of extracting the vegetable bases for purposes of elaboration. The underlying principle is that the alkaloidal salts dissociate in aqueous solution, alkaloidal cations being formed. Ion exchange in the case of alkaloidal salts can, in principle, be carried out by two different techniques, i.e. either by the use of cation or anion exchangers.

In the technique I (Fig. 3) a cation exchanger in the H-form is used. The alkaloidal cation displaces the H^+ from the exchanger and is fixed on the cation exchanger like a salt. In the favourable case the colouring matters and other ballast substances of the drug extract pass through the column without being adsorbed on the exchanger, and can be separated with the effluent. The alkaloid base can then be liberated from the resin through the treatment of the latter with an alkali; ammonia is most suitable for this purpose. The poorly soluble alkaloid base which separates is then dissolved in an organic solvent such as ethanol, which is used as an eluent.

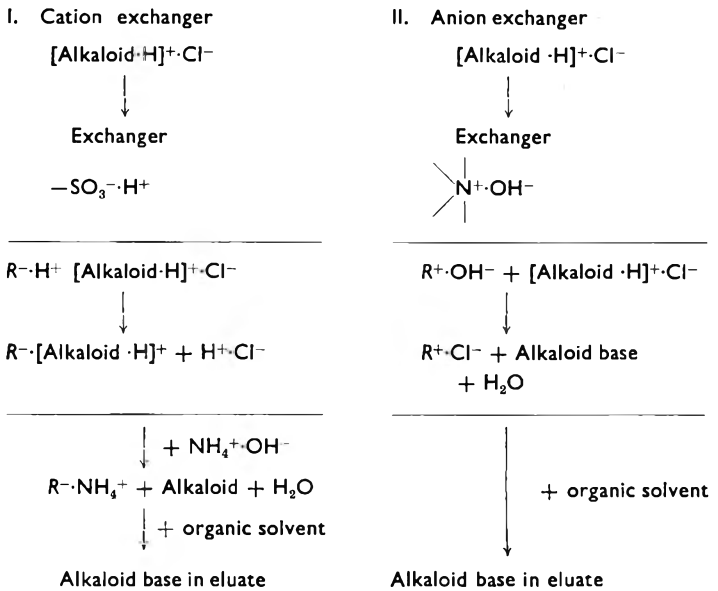

FIG. 3. Isolation of alkaloids. R = exchanger.

The liberation and elution of the alkaloid base can be carried out in one operation if separation and elution are performed with an ammoniacal ethanol.

In the technique II an anion exchanger in its OH-form is used. The exchanger extracts the salt formed from the alkaloid salt and the poorly-soluble alkaloid bases are precipitated in the column. The base is then eluted from the column with the aid of an organic solvent.

Technique I has been successfully used preparatively by Applezweig[22] for the isolation of the alkaloids of cinchona and solanaceæ. Many other authors have since attempted isolation with permutites, carboxylic and sulpho-acid resins. Nicotine can be obtained from tobacco juices and scopolamine concentrated from highly diluted fluid extracts by this technique, which is suitable both for elaborating the pure total alkaloids of a drug and for extracting individual alkaloids in the pure state.

Technique II has been proposed by Jindra[23] for determining the content of alkaloidal drug preparations. It has, however, the great drawback

that the separation of the bases from the vegetable colouring matters is inadequate, with the result that the eluates are usually strongly coloured and in many cases can be titrated only electrometrically. If in addition other salts, such as sodium chloride, are present in the alkaloid solution, they appear as alkalis in the eluate and prevent direct titration.

Ion exchangers were early applied to preparative elaboration from drug extracts. In particular, the development of ion exchangers on a resin basis gave impetus to this pharmaceutical application. Nachod[24] and Applezweig[22,25] were the first to institute experiments with a view to the incorporation of ion exchangers in the process of extracting alkaloids. It is to them that we owe the first studies on the extraction of cinchona alkaloids, and atropine. Stimulated by our earlier experience in the percolation of cinchona bark and the manufacture of cinchona dry extracts[26], in which field, as is commonly known, the problem of the quantitative extraction of the alkaloids and the elimination of the ballast substances without loss of alkaloids has not yet been solved, we set ourselves the task of studying the isolation of the total alkaloids of cinchona bark and their elaboration into totaquine-like preparations on a laboratory scale by the use of a suitable cation exchanger. We were able to base our work on our own analytical studies[3] of cinchona bark and extract. Those studies elicited the fact that, among cation exchangers of different degrees of acidity, Duolite C–10 ($-SO_3^-\cdot H^+$ and $-O^-\cdot H^+$) is best suited to the purpose of adsorbing quinine quantitatively from an acid ethanolic solution such as is available after drug extraction. We knew, moreover, that it was preferable to use a cation exchanger rather than an anion exchanger because the colouring matter of cinchona bark can be much better separated by the former method. A part of the coloured accompanying substances is adsorbed and a part passes through. The colouring matter retained in the column can be dissolved with 10 per cent. ammonia, while the alkaloids remain held in the resin. The alkaloid bases are then liberated with 10 per cent. spirit of ammonia and can be eluted quantitatively.

It seemed logical to us to apply this principle of exchange and elution to the continuous percolation method, the menstruum then being used continuously in the circulation process, thereby achieving an economy. The alkaloid yields in Table I show that if these yields are compared, for example, with the figures obtained in the manufacture of cinchona extract according to Ph. Helv. V (88 per cent. of the alkaloids are yielded to the percolates, 55 per cent. appear in the finished dry extract), the results achieved by ion exchange can be regarded as very satisfactory. There are practically no alkaloid losses, during ion exchange and elution, and the total yield achieved, i.e. 85 per cent. of the alkaloids, is very favourable.

Ion exchange has also been successfully resorted to in the extraction of vitamins. B_1 and B_2, for example, can be easily and perfectly separated by this method. The procedure used in the case of these B-complex vitamins is that yeast is extracted with dilute acidic ethanol and the weakly basic aneurine is adsorbed from this solution with the help of a strongly

acid cation exchanger. Riboflavine is retained in the solution and is recovered therefrom. Aneurine is then separated from the exchanger resin with a strong acid, and processed[27]. Since with this method there is a danger of the decomposition of aneurine, isolation is preferably carried out with a weakly acid ion exchanger such as Amberlite IRC-50, as elution then takes place much more gently. Ascorbic acid, folic acid and vitamin B_{12} have also been extracted and purified by ion exchange[28].

TABLE I

ALKALOID YIELDS OF CINCHONA BARK USING EXCHANGE AND ELUTION

Working phases	Bark I 11·75 per cent.	Bark II 4·21 per cent.
(*a*) Extracted by percolation with 0·1N sulphuric acid ..	88·9 "	86·25 "
Loss during extraction	11·1 "	13·75 "
(*b*) Adsorbed on Dowex C–10 from the percolate by ionic exchange without loss	0 "	0 "
(*c*) (i) Lost in the elution of the colouring matter and ballast substances	0·05 "	0·05 "
(ii) Lost in the elution of the alkaloids (readsorption, etc.)	1·34 "	0·95 "
Total losses determined	12·6 "	14·75 "
(*d*) The following were obtained on crystallisation of the alkaloids		
1st crystallisation	43·05 "	43·0 "
2nd crystallisation	34·5 "	32·8 "
3rd crystallisation (or mother liquid)	9·95 "	9·4 "
Total yields of alkaloids	87·5 "	84·2 "

Suitable exchange techniques are being used in the manufacture of antibiotics. Through its adsorption on anion exchangers, penicillin can be separated from raw culture filtrates and freed from accompanying toxic matter. These latter substances pass through the exchanger column together with other impurities, and the penicillin is subsequently liberated from the exchanger column in the form of the sodium or potassium salt. Streptomycin was formerly isolated from the culture filtrates through adsorption on animal charcoal and other adsorption agents. The yields achieved by this means were, however, unsatisfactory, amounting to only about 30 per cent. Streptomycin can be much more successfully adsorbed from the filtered culture liquid directly on a weakly acid carboxyl exchanger such as IRC-50. When the exchanger column charged with streptomycin is regenerated with mineral acids, the antibiotic is quantitatively liberated and processed. Various other antibiotics are now being elaborated and purified by ion exchange[29–32].

Ion exchange has also proved serviceable in the separation of chemical compounds which are relatively closely related. The basis of this application is that, while the selectivity of the exchangeable ions differs widely according to the sign of their charge, their size, form, charge and specific structure, very slight changes in the structure and in the constitution of the exchanger (particle size and shape, chemical constitution of the ionogenic groups, degree of wetting, exchange capacity, etc.) are able to greatly modify the pattern of selectivity. If it is further borne in mind that

external factors also (nature of the solvent, nature and quantity of additives such as chelate formers, reaction temperature and reaction time) play a part, it will be appreciated that there must be possibilities of selectively separating dissociating substances which are closely related chemically. Accordingly, it was possible to simplify very considerably the separation of the amino-acids, after the hydrolytic cleavage of the proteins, by the use of exchange resins. It is principally to Kunin and Winters[33] that credit is due for having exploited the high efficiency of the synthetic exchanger resins for the preparative separation of the amino-acids into the principal groups of the acid, neutral and basic representatives, and in the case of the latter group into arginine, lysine and histidine.

Application of ion exchangers as catalysers in elaborative chemistry

A large number of chemical reactions in the fluid phase are catalysed by H^+, OH^- and other ions. The necessary ions were formerly added to the reaction system exclusively in the form of soluble electrolytes. Shortly before the last World War a systematic investigation was undertaken for the first time to ascertain whether exchange resins could also be used for this purpose. In principle, an ion exchanger is suitable for catalytic reactions if it contains the catalytically active ion as the opposed ion. The catalysed reactions take place mainly in the pores of the exchanger compound. No exchange of ions takes place so that it is not necessary to regenerate the resins. The catalytic power of an ionic exchange resin depends fundamentally on the number of active groups, which in their turn condition the exchange capacity. Given equal capacity, a carboxylic acid exchanger is substantially less effective than a sulphonic acid exchanger for the purposes of a reaction catalysed by H^+. Ion exchangers saturated with H^+, OH^-, CN^-, CH_3COO^- or Hg^{++} have already been used with great success in the catalysis of many reactions, such as hydrolysis and saponification, condensation, addition, dehydration, alcoholysis, interchange of ester radicals and so forth. The following processes have been performed technically on a considerable scale: the hydrolysis of ethyl acetate, galacturonic acid ethyl ester, cane sugar, maltose, polyvinyl acetate, esterification of acetic acid, oxalic acid and benzoic acid with methanol, butanol and even with higher alcohols[34]. The advantage of the exchanger catalysers resides in the fact that they are readily separated from the reaction product because they are solid and the catalytically active ions are fixed on the resin. Interfering side-reactions can also often be avoided, which may lead to improved yields and purer products than those obtained by the conventional methods. Thus the hydrolysis of proteins, such as casein, can be effected without the formation of humic acids[35]. Many condensations with aldehydes can be performed without the formation of resinous polymerisation products[36]. Deuel[37] was able to show, moreover, that with the aid of ion exchange catalysers it is possible to hydrolyse substances selectively. Maltose, for example, can be decomposed in the presence of glycogen without the glycogen being attacked. The macromolecules of the glycogen cannot

penetrate the fine-pored ion exchangers that are suitable for this reaction, and are therefore practically not attacked.

I trust that my remarks will have given a useful survey of the technical application of ionic exchange. In view of the wide range of possible applications, it has been necessary to mention only a few processes, which are of significance primarily in the pharmaceutical field. Nevertheless, these examples show that ion exchange has now achieved the importance of such classic techniques as adsorption, extraction, filtration and distillation. There can be no doubt that the opportunities for development, which it affords, will continue to be fruitfully exploited in the improvement of pharmaceutical working methods.

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ION EXCHANGERS IN ANALYTICAL CHEMISTRY: APPLICATIONS AND PROBLEMS

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At the International Congress for Applied Chemistry in London in 1909, Siedler advanced the idea that artificial zeolites could be applied to quantitative analysis. Siedler was right although permutites were at first only applied in a limited way to analytical methods. Thus a synthetic zeolite was used for the separation of ammonia from urine for colorimetric determination[1], and a permutite was used to exchange the alkaline earth metals for the alkali metals in a volumetric method for sulphuric acid in tap water[2,3]. The general applicability of permutites in analytical chemistry remained limited, however, owing to their instability towards acids and bases. It was not until the discovery and development of ion exchangers on a resin base that the way was opened for their many possible applications to analysis.

Individual Applications

Quantitative inorganic analysis

In qualitative analysis ion exchangers serve for the removal of interfering anions such as phosphate, oxalate, or tartrate[4,5,6]. After the precipitation of the sulphide group and the removal of hydrogen sulphide from the weak acid solution, this latter is slowly passed through a cation exchange column in the H-form. The cations are bound to the exchanger and the anions remain in solution and appear in the effluent. The cations are then eluted with 4N hydrochloric acid and estimated. In this procedure a green chromium (III) salt may pass through the column in small amounts since Cr^{+++} can form anionic or neutral complexes. Complex cyanides can be separated in a similar way[7].

Separations of metals can be carried out in various ways with ion exchangers. The amphoteric metals zinc, aluminium, molybdenum, tungsten and antimony can be separated from the non-amphoteric metals after exchange on a cation exchanger, by solution with alkali as complex anions; molybdenum and tungsten with a 2 per cent. caustic soda solution, zinc and aluminium with a 5 per cent. and antimony with a 10 per cent. solution[8–10].

The separation of molybdenum in qualitative analysis can be achieved by a method developed by Klement, wherein the molybdate is bound as a complex with organic acids[11,12]. A weak mineral acid solution containing the molybdate together with other cations is treated with excess citric acid and passed through a cation exchanger in the H-form. The molybdenum passes into the effluent as a complex and can be recovered quantitatively.

Bismuth can be eluted with a 1 per cent. potassium iodide solution from a cation exchange column laden with bismuth, copper and lead. Traces

of copper are also eluted, but not in sufficient quantity to interfere with the direct colorimetric determination of bismuth in the eluate.

From a solution of arsenic, antimony and tin, a cation exchanger of the sulphonic acid type holds back the antimony and tin, the arsenic appearing in the effluent[8].

Arsenic and antimony can be separated by means of a strong basic anion exchanger in the sulphate form which quantitatively holds back the arsenic but not the antimony[13].

QUANTITATIVE INORGANIC ANALYSIS

Determination of Cations

In quantitative analysis, many processes can be much simplified in that the cations can be exchanged for hydrogen ions on a cation exchanger in the H-form, the liberated acid determined with standard alkali and thus the equivalent amount of original cation can be calculated. This method can also be used for salts which are soluble only in acids, for example, calcium phosphate. In this case the increase in hydrogen ions is found and from this the content of cations can be calculated. Samuelson and colleagues and Klement and others have used this method in many cases with success. The determination of the anions in the solution is equally possible. Table I gives a series of possible separations.

For solutions containing several cations and anions, this method is however only applicable in rare cases. Here a simplification of the methods previously applied is possible in that the cations, exchanged in the usual way on a column, are dissolved with hydrochloric acid and determined in the acid solution.

TABLE I

THE USE OF EXCHANGERS IN THE DETERMINATION OF IONS

Cation	Anion	Exchanger	Reference
K	SO_4		(without separation
Alkalis	PO_4		of anions from
Al, Fe	PO_4		cations)
Ca	SO_3 (Sulphite-hydrochloric acid)		91
Na, K	$Fe(CN)_6'''$, $Fe(CN)_6''''$	H-exchanger	7
Na, K	$Co(CN)_6'''$, $Fe(CN)_5NO''$	H-exchanger	7
	$Cr(CN)_6$, $Mo(CN)_8''''$		
	$Cr(CN)_8''''$		
Na, K, Mg, NH_4, Cu, Ca	Cl	Wolfatit K	92
Sr, Ba	Cl	"	92
Cd	Acetate	"	92
Co	NO_3	"	92

In certain instances, owing to the instability of the anions in acid solution, an NH_4-exchanger must be employed instead of an H-exchanger. For example, the separation of the alkali metals from chromate, molybdate, tungstate, phosphomolybdate, phosphotungstate and silicotungstate and the determination of sodium and potassium in the presence of vanadate can be thus effected (Table II). Owing to the oxidising action of chromates in an acid medium and the instability of the other anions in acid solution, these cannot be treated with an exchanger in the H-form[7,10].

TABLE II

SEPARATION OF ALKALI METALS

Cations	Anions	Exchanger	Reference
Na, K	CrO_4''; MoO_4'', WO_4'', $P(Mo_2O_{10})_4'''$, $P(W_2O_{10})_4'''$	NH_4-Exchanger	7
Na, K	VO_3'	NH_4-Exchanger	10

A further possibility in quantitative determination of cations is to elute them with hydrochloric acid after they have been bound to a column as described above, and then to assay them in the hydrochloric acid solution. This procedure is especially useful when interfering anions must be removed. In the methods heretofore adopted an interfering anion had to be converted into an insoluble compound and removed by filtration. With exchangers this removal of interfering anions is accomplished very simply. Treatment of the solution with a cation exchanger binds the cations; and the anions which remain in solution, appear in the effluent. The cations are then eluted with hydrochloric acid and are determined without interference. Moreover the free acid in the effluent can be titrated and related to the cation content.

There are examples of this method; potassium in the presence of sulphuric acid, alkalis in the presence of phosphoric acid[5,6,14,15] and the estimation of aluminium and iron in the presence of phosphoric acid[16].

By complex formation of cations further possibilities may be envisaged, particularly the separation of cations from one another[7]. Thus the alkali metals can be separated from iron and cobalt. The mixture containing the alkali metals together with iron and cobalt is added to a solution of hydrocyanic acid which has been neutralised with ammonia. The heavy metals thus form complex cyanides, and, after passage through a column of exchange resin, appear in the effluent. The alkali metals are exchanged, eluted with hydrochloric acid and determined in the acid solution.

Finally, the different affinities of cations for the exchange resin can be used for their quantitative separation, for example, the separation of lithium, sodium and potassium and also cadmium and zinc by elution of a Wofatit KS column loaded with a mixture of the ions. Separation is brought about by the passage of the eluted cations through the unloaded part of the column which must therefore be sufficiently large. By means of a suitable apparatus for measuring conductivity, curves can be obtained showing when each cation appears in the effluent. By previous calibration with known solutions, the method can be made quantitative[17,18].

Determination of Anions

According to Samuelson, the anions which are principally determined are those which are bound as neutral salts, the solutions of which are passed through a cation exchanger in the H-form, and the equivalent amount of acid thus liberated is titrated with standard alkali. Table III gives possible separations and these proceed smoothly. Weak acids are adsorbed as non-polar molecules by the exchanger. For them, the

TABLE III

THE USE OF EXCHANGERS IN THE DETERMINATION OF IONS

Cations	Anions	Exchanger	Reference
Na, K	SO_4, NO_3	Wofatit KS	19
Li, Na, K, NH_4, Mg, Ca, Sr, Ba, Co, Ni, Zn, Mn, Al, Fe, Cr	NO_3, ClO_4, PO_4	Wofatit	7
Na, Cu, Fe^{++}, Cr Va	Cl, SO_4, NO_3, PO_4	Wofatit Sulphonic acid organolites	4, 7
K, Na, NH_4, Mg, Ca Al, Zn, Co, Fe, Cr (violet)	SO_4	Wolfatit K Wofatit KS	21, 22, 93, 94
Li, Na, K, NH_4, Ca, Sr, Ba, Co	Cl		95
K (black powder)	NO_3		7
Alkalimetal, NH_4, Cr, Fe, Al	PO_4	Wofatit K and KS	92, 96, 97
Li, Na, K, NH_4, Mg, Ca, Sr, Ba, Zn, Mn, Co, Ni, Al, Cr (green and violet)	Br, I, ClO_3		98
Fe	Br, ClO_3		98
Fe, Al, Co, Mn, Zn	SeO_3		98
Cd	Cl, Br, I, SO_4, NO_3, ClO_4, ClO_3, PO_4, Acetate, Oxalate		
Na	$H_4P_2O_7$	Wofatit K and KS	92
Alkalimetal	HPO_3		92
Ca, Fe	SiO_3		99
Na	Oxalate		92
Li, Na, K, NH_4, Mg, Ca, Sr, Ba, Zn, Mn, Co, Ni, Al, Fe, Cd, Cu, Pb	Acetate	Wofatit KS	6, 92, 24
Li, Na, K, NH_4, Mg, Ca, Sr, Ba, Zn, Mn, Co, Ni, Cd, Cu	Oxalate		6, 92, 24
Na	Tartaric acid	Wofatit K	92
K	$H_4Fe(CN)_6$	Wofatit K	92
K	HF	Wofatit K	92
K	CNS	Wofatit K	92
Mg	ClO_4	Wofatit KS	92
Na	$C_6H_5SO_3'$	Wofatit K	92
K	S_2O_8''	Wofatit KS	92

separation is quantitative only when large quantities of wash-water are used[19,20].

Bromate and iodate are reduced in contact with cation exchangers in the H-form and cannot therefore be separated.

For the determination of phosphoric acid, freshly precipitated alkaline earth metal phosphates are shaken with excess cation exchanger in the H-form. The precipitates dissolve completely in two minutes; the cations are bound on the exchanger and free phosphoric acid remains in the solution. Phosphoric acid can thus be separated from Li, Na, K, NH_4, Mg, Al, Fe^{+++}[7]. With Cr^{+++} separation can be effected in mixtures of chrome alum and chromium nitrate. On the contrary however separation is not possible with green Cr(III) salt solutions or solutions containing Cr^{+++} which have been boiled before treatment with the ion exchanger owing to the formation of complex cations[4].

Ion exchangers can also be used to advantage to remove ions which interfere with the usual quantitative procedures.

The precipitation of sulphate with barium chloride for gravimetric determination of sulphate suffers considerable interference in the presence of cations such as Al^{+++}, Cr^{+++}, Ca^{++}, whose removal with cation exchangers can be effected without difficulty. A pure sulphate solution

free from interfering ions is obtained in the effluent[21,22]. Any nitric acid present simultaneously can be removed by evaporation of the effluent. The presence of phosphoric acid requires the usual procedure when precipitating sulphate with barium chloride. In the presence of green Cr(III)-salt solutions this method fails since, owing to the formation of complex sulphates of chromium, a part of the chromium appears in the effluent and a part of the sulphuric acid is bound as a complex with the chromium and retained on the exchanger. With violet chromium solutions correct values are obtained by working at low temperatures[23]. Complications can likewise arise with neutral solutions of iron ammonium alum since sulphate can be bound as cationic complexes[7]. On the other hand quantitative ion exchange is not affected by the hydrolysis of beryllium sulphate[24]. Other possible ways of removing interfering ions are given in Table IV.

TABLE IV

REMOVAL OF INTERFERING IONS

Method	Interfering Ions	Removal
Gravimetric determination of sulphate	Fe^{+++}, Al^{+++}, Cr^{+++}, Ca^{++}	H-Cation exchanger: Recovery of pure sulphuric acid.
Polarographic determination of selenide	Fe^{+++}, Al^{+++}, Co^{++}, Mn^{++}, Zn^{++}	H-Cation exchanger: separation of interfering ions.

The different affinities of chloride and bromide for a strongly basic anion exchange resin can be used for their separation in mixtures. On elution with a sodium nitrate solution at a controlled efflux rate, the first fraction contains no halide, the second fraction chloride and the third bromide[25].

Separation of the Rare Earth Metals

The separation of the rare earth metals or their compounds, which is very difficult without the use of ion exchangers, can be performed elegantly with their aid. The method utilises the ability of the rare earth metals to form complexes with citric acid the stability of which depends on the pH, and which exhibit very different degrees of adsorbability by the exchanger.

Analysis of Complexes

With the aid of resin exchangers, metal complexes may be separated into cationic, anionic and neutral complexes. The solution containing the complexes is passed successively over a cation- and then over an anion exchanger when the cationic- and then the anionic complexes are respectively exchanged. The neutral complexes appear in the effluent in both cases. Quantitative determinations are carried out on the eluates and on the effluent and the results give the composition of the complex types.

MICROCHEMICAL DETERMINATIONS

Inorganic Determinations

Wiesenberger was the first to use resin exchangers for microchemical estimations. He carried out neutral salt splitting with Wofatit-K and

estimated the acid alkalimetrically. After dissolving the neutral salts in water and treatment with the H-cation exchanger, he titrated the free acid in the effluent with 0·01N sodium hydroxide solution[26].

In human serum the calcium fraction can be determined easily with cation exchangers[27]. The calcium is exchanged by contact of the serum with Na-Wofatit-F, eluted with hydrochloric acid and determined in the eluate in quantities of the order of 0·1 to 1·0 mg.

In the determination of rubidium- and caesium salts in quantities of 0·15 milliequivalents, small-scale columns have been used for the microanalysis with good results[28].

Acetyl-group Determination

This determination is one of the most difficult organic group analyses. The micromethod of Freudenberg and Weber[29–31], which, according to figures reported in the literature gives excellent results, is by no means easy to carry out, but has been simplified by Wiesenberger[32] by the use of ion exchangers. He saponifies the ethyl acetate, obtained with *p*-toluene-sulphonic acid in the presence of ethanol, with strong caustic soda (instead of the 0·02N sodium hydroxide previously used) and treats the sodium acetate solution containing excess alkali with an H-cation exchanger so that all the Na^+ is exchanged with H^+. The liberated acetic acid is then titrated with 0·01N sodium hydroxide solution.

Exchange of Aldehydes and Ketones

The exchange of aldehydes and ketones can be achieved on an anion exchange column laden with bisulphite due to the well-known formation of hydroxy sulphonic acids thus:[33–37]

$$RCHO + HSO_3' \rightleftharpoons RCH(OH)SO_3'$$
$$R_2CO + HSO_3' \rightleftharpoons R_2C(OH)SO_3'$$

The reaction is reversible; elution is therefore possible with either alkalis or acids.

USE OF ION EXCHANGERS IN PLANT ANALYSIS

Alkaloids

The problems and possibilities of the quantitative estimation of alkaloids has been examined in detail by Büchi and Furrer[38]. Alkaloidal salts form large cations in aqueous solution:

$$Alk.HCl \rightleftharpoons [Alk.H]^+ Cl'.$$

These undergo exchange with cation and anion exchangers.

Use of Cation Exchangers

$$R^-\cdot H^+ + [Alk.H]^+Cl' \rightarrow R^-\cdot[Alk.H]^+ + HCl$$

In these exchanges (R = exchanger) the cation forms a salt-like compound with the ion exchanger. The alkaloid may then be recovered with alkali or ammonia according to the following equation:

$$R^-\cdot[Alk.H]^+ + NH_4OH \rightarrow R^-\cdot NH_4^+ + Alk. + H_2O.$$

Sparingly soluble alkaloidal bases are precipitated on the column and are extracted with an organic solvent[39,40]. When ethanolic ammonia is used, the liberated free bases are simultaneously dissolved by the ethanol.

Use of Anion Exchangers

$$R^{+}\cdot OH' + [Alk.H]^{+}\cdot Cl' \rightarrow R^{+}\cdot Cl' + Alk. + H_2O.$$

During the exchange of alkaloidal salts with anion exchangers in the OH-form, the salt-forming ion is exchanged with OH′ and the liberated alkaloidal base precipitated in the column. It is then eluted with an organic solvent.

Anion exchangers for the exchange of alkaloids must possess groups of a certain basic strength, otherwise selective exchange is not possible. An anion exchanger of the quaternary ammonium type converts not only alkaloids but alkali, ammonium and amine salts quantitatively into their bases or carbonates. Anion exchangers of the weak basic type do not completely convert strongly basic alkaloids to the free bases, for example, ephedrine and cotarnine. In both cases incorrect results are obtained; in the first case values are too high and in the second case too low[41].

For the analyses only ion exchangers with a loose network can be employed[42–45]. The natural and synthetic silicate exchangers have an average pore diameter of 3 to 5 Å[46]. The greatest diameter of the quinine ion is 15 Å[38]. Such ion exchangers are thus ill-adapted for the ion exchange adsorption of the large alkaloid cations. The earliest investigations with organic ion exchangers showed that even with these the total exchange capacity was not nearly reached[47].

Of the many cation resin exchangers examined, Duolite C-10 proved best for exchange with cinchona alkaloids[38]. Even here the use of strong ethanolic solutions somewhat lowered the exchange performance, but with a 46 per cent. ethanolic solution complete exchange was effected.

The elution of alkaloids from cation exchange resin in quantitative estimations is possible principally in two ways.

The alkaloid bound on the resin in the H-form, is exchanged with H^{+}-ions from an acid. The initial amount of acid is known and the excess acid which is not exchanged with alkaloid is back-titrated; or, the alkaloid is exchanged with other cations and is then transformed with alkali into the free base which separates on the column. To determine the alkaloid titrimetrically, two conditions must be fulfilled. Firstly, an easily volatile base must be employed, which can be driven off before the titration, and secondly the alkaloidal base must be dissolved with an organic solvent.

For the first of these two processes a considerable excess of acid is essential[38] both for sulphonic acid and carboxylic acid resins for the quantitative recovery of the alkaloid. With the second process of alkaline regeneration, very rapid quantitative recovery is achieved with an ethanolic ammonia solution which acts both as alkali and solvent[35].

In the determination of the total alkaloid content of Extractum Cinchonæ Pharm. Helv. V and Cortex Cinchonæ Pharm. Helv. V by exchange

on Duolite C-10, both with the H^+– and NH_4^+ forms, coloured compounds and mineral cations can be separated from the alkaloids using an aqueous ammonia solution containing a small amount of ammonium chloride[38]. For the determination of the total alkaloids of cinchona bark, both the filtration method and the contact exchange method are suitable. In this latter the exchanger is mixed directly with the bark from which the resin is easily separated by decantation with distilled water. For both methods, a water-formic acid mixture serves as solvent[38].

Ion exchangers can also be used for the separation of substances which interfere with the quantitative estimation of alkaloids.

After making alkaline with aqueous ammonia solution, ipecacuanha root is extracted with ether, the ether solution separated, and after the addition of sulphuric acid, the ether is removed. The sulphuric acid—emetine solution is passed through a column of synthetic alumino-silicate exchanger in the ammonium form, which effects separation of accompanying coloured materials. The exchanged alkaloid is eluted with a 10 per cent. ethanolic ammonia solution, and the alkaloid estimated titrimetrically after evaporation of the solvent. The values obtained were very good. The organic exchangers Amberlite IR-100 and Zeo-Karb were both unsuited to this method[48].

In the colorimetric estimation of morphine in pathological urine co-extracted materials interfere (extraction method of Pierce and Plant). These can be separated using synthetic zeolite (Permutit) since the morphine is exchanged but not the interfering substances. The morphine is eluted with saturated sodium carbonate solution and estimated colorimetrically by the addition of Folin-Denis phenol reagent[49].

The differing basic properties of alkaloids can be used for their separation. Cation exchangers of the carboxylic type enable weakly basic alkaloids (e.g., strychnine and caffeine) to be separated from strongly basic ones (e.g., quinine, brucine and nicotine)[43].

Glycosides

In the glycoside sphere, ion exchangers have been little used up till now. Anionotropic aluminium oxide[50] was used in the isolation of digicornin. By chromatography of sugars and some glycosides on borate-buffered exchange columns, separation can be effected owing to the formation of different borate complexes conditioned by the spacial configuration of the OH-groups[51].

Glycosides of Mustard Oil. The anionic character of mustard oil glycosides, shown by their ability to migrate in electrophoresis experiments[52], enables the exchange of the glycoside anions on an anion exchanger to take place with ions originally present.

Strongly basic ion exchangers (Lewatit MI and Amberlite IR-400) in the OH-form exchange accompanying materials besides the glycoside, so that the eluate residue contains 60 to 70 per cent. of glycoside. The weakly basic ion exchangers Amberlite IR-45 and Amberlite IR-4B yield an eluate whose residue consists of up to 90 per cent. of glycoside.

Anionotropic aluminium oxide-Woelm can also be used in the same way as the weakly basic anion exchangers.

Anthraquinone Glycosides[53]. For the analysis of drug extracts containing anthraquinone glycosides strongly basic anion exchangers have been shown to be the best (Lewatit MI and Amberlite IR-400). On elution with glacial acetic acid, the materials retained on the column pass gradually into the eluate without separation. Flavone derivatives as well as anthraquinone glycosides and anthraquinone aglycones can be identified in the eluate.

Determination of Reducing Sugars. The estimation of reducing sugars in plant extracts is frequently inaccurate owing to the presence of other reducing substances. These interfering substances may be removed, however, with ion exchangers. After passage through a two-stage column or treatment of the solution with mixed resins, it is freed from interfering substances. The ability of monosaccharides to form sugar-borate complexes with borate ions, and to react with bisulphite enables an exchange to be effected. Fructose, glucose, mannose and galactose in dilute sodium borate solution are quantitatively retained by strongly basic ion exchangers in the OH-form[54]. Xylose and mannose are quantitatively held back from an aqueous solution by an ion exchanger in the bisulphite form[55]. Fructose does not react with such an exchanger and can thus be separated from xylose and mannose (Fig. 1). By stepwise elution with ethanol solutions of decreasing concentration a separation is effected since the stability of the addition compounds differs[55].

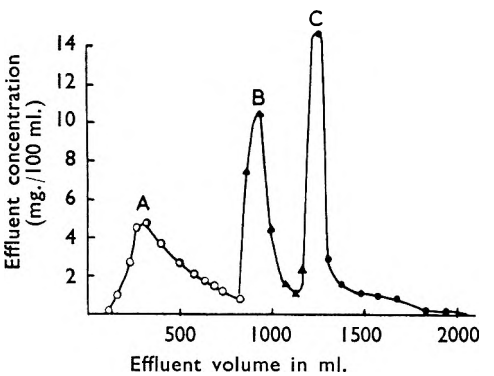

FIG. 1. Chromatographic separation of monosaccharides by stepwise elution.
Column: 9 × 150 mm.; Amberlite IRA-400 [HSO_3^-; <0·12 mm.].
Flow rate: 0·7 ml./min.
Elutriants: × 99·5 per cent. ethanol (by weight).
▲ 95·0 per cent. ethanol.
● water.
A. Fructose. B. Glucose. C. Mannose.

The carbonyl content (end-groups) of polysaccharides are estimated by transformation to carboxyl groups by the cyanhydrin method with $Na^{14}CN$. The radioactive carboxylic acid derivative is exchanged on an anion exchange resin and thus separated from other polysaccharides. The radioactivity after elution gives the carbonyl content (end-groups)[56].

APPLICATION OF ION EXCHANGERS IN THE ANALYSIS OF DRUGS

Local Anæsthetics

Of the local anæsthetics, larocaine, tutocaine, percaine, procaine, amylocaine, amethocaine, can be exchanged with Amberlite IRA-400 and

quantitatively determined[57]. Local anæsthetics in tablets and solutions for injection could not be determined since these preparations contained too great a quantity of electrolytes[58], but a procaine determination in an ointment was successful[57].

Sympathomimetic Drugs

Of sympathomimetics, methylamphetamine, ephetonal, ephedrine, naphazoline and amphetamine were estimated using without difficulty Amberlite IRA-400[59]. Compounds with one or more phenolic hydroxyl groups attached to the benzene ring were, however, not determinable, e.g., adrenaline, sympatol. They are bound only partly by the exchanger. The authors explain this as due to the weakening of the basic properties of these compounds by the phenolic hydroxyl groups which is in agreement with the work of Kunin and McGarvey[60], who found that phenols could be bound with a strongly basic exchanger[58].

Determination of Sympathomimetic Amines and Antihistamines in Tablets

The quantitative analysis of sympathomimetic amines[59] (ephedrine, methylamphetamine, ephetonal, naphazoline) and antihistamines[61] (antazoline, mepyramine, promethazine) was carried out in the following way[58]:

An extract with 10 ml. of 50 per cent. ethanol of tablets or dragees containing 20 to 100 mg. of active constituent is filtered through cotton wool. The diluents retained on the filter paper are extracted several times with hot 95 per cent. ethanol to make a total quantity of 50 ml. of extraction liquid. The combined filtrates are passed through a column containing 8 to 10 g. of Amberlite IRA-400 in the carbonate form at a rate of 7 ml. per minute. For washing through, 40 to 80 ml. of hot 95 per cent. ethanol is sufficient. After dilution with 40 ml. of hot distilled water, the solution is then titrated potentiometrically with 0·1N hydrochloric acid, or a suitable indicator (methyl red) may be used.

Regeneration of the column is effected with 10 ml. of 4 per cent. caustic soda solution, followed by washing with water until the effluent is no longer alkaline to phenolphthalein. The column cannot be used indefinitely since it is gradually rendered unusable by "filling" materials.

Determination of Spasmolytic and Cough-sedative Materials in Tablets

Substances such as acedicon, caramiphen, amprotropine and adiphenine can be assayed by the above method for the determination of the content in tablets of sympathomimetic amines and antihistamines[58].

Amino-acids

Ion exchange chromatography is of significance for the analytical and preparative separation of amino-acids. Different exchange potentials of different ions with similar properties enable separation to be effected using ion exchangers in principle exactly as in ordinary chromatographic analysis. Work on amino-acids and nucleotides as well as on the separation of the rare earth metals and isotopes in the inorganic realm have contributed much to the development of ion exchange chromatography.

Amino-acids are firstly separated into groups and then these are separated into their individual components.

In group-separations, exchangers with a functional group are selected, which selectively exchange only a few acids with isoelectric points in a definite pH-range from a series of amphoteric amino-acids with isoelectric points extending over a wide pH-interval. Thus the groups of aliphatic neutral, aliphatic basic and aliphatic amino-acids with two carboxyl groups can be separated (Table V).

TABLE V

SEPARATION OF AMINO-ACIDS

Cation exchanger	Exchange	References
Sulphonic acid type in H-form	Total amino-acids	62, 100, 104,
Sulphonic acid type in salt form	From neutral solution only basic amino-acids	105
	From acid solution also neutral amino-acids	
Carboxylic acid type: at pH 4·7	Total basic amino-acids	106
at pH 7·0	Lysine and arginine	106
Anion exchanger:		
Strong basic type in OH-form	Total amino-acids except arginine	
Strong basic type	Dibasic amino-acids	101, 107
Wofatit M, previously treated with 0·2N acetic acid	Tryptophane	63, 108

Aliphatic and aromatic amino-acids are separated from one another with activated charcoal. The aromatic amino-acids (phenylalanine, tyrosine, tryptophane) are selectively adsorbed by activated charcoal[62,63].

Kunin and Winters[64] combined anion- and cation exchangers and separated acid, neutral and basic groups, and the latter into arginine, lysine and histidine as shown in Figure 2.

The chromatographic separation[65–68] of the amino-acids depends upon the degree of ionisation of individual acids, the van der Waals forces between the ion exchanger and the acids and lastly the charge on the ions[69]. When there are little or no van der Waals forces, the distribution of the amino-acids must take place according to their dissociation constants. With methionine, van der Waals forces operate so that it does not appear in the series of the other amino-acids in accord with their dissociation constants during separation, but is more strongly adsorbed. When the van der Waals forces are reduced by raising the temperature, it then appears in approximately the position in the series of amino-acids, conditioned by its dissociation constant[67].

Experimentally a cation exchanger is used and the amino-acids taken up by the resin are displaced with bases which have a higher affinity for it (e.g., NH_3 or NaOH). The eluate is analysed by means of its conductivity or pH or better, collected with a fraction-collector and analysed by paper chromatography.

By such a procedure, however, many amino-acids are not separable, but groups are obtained. In order to effect a separation, further methods have been developed. After a further adsorption, the fraction can be treated with another eluant[70] or it can be eluted step-wise by successive

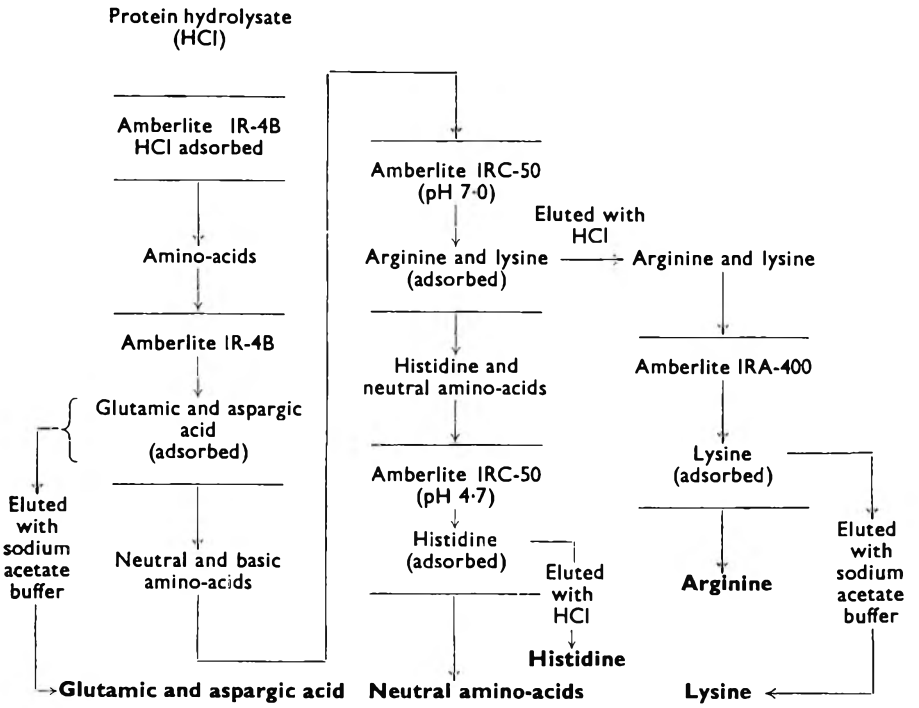

FIG. 2. Scheme for the separation of amino-acids into three groups by means of synthetic ion exchange resins.

solvents with increasing acid concentration. Also, using a resin in the Na-form buffer solutions of increasing pH may be employed[71,72].

In the separation of an amino-acid mixture containing 3 to 6 mg. the following acids were quantitatively obtained by using a buffer of pH 3·4, and were divided off according to their maxima: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine and valine; at a pH of 4·25 the following acids were obtained: methionine, *iso*leucine, leucine, tyrosine and phenylalanine; at pH 8·3, histidine; at pH 9·2, lysine and at pH 11, arginine[72].

Nucleotides are separated on the same principle as amino-acids[73].

Vitamins

Ion exchangers in determination of vitamins serve not only to remove interfering substances but also for the separation of the vitamins themselves.

Vitamin B_1. Vitamin B_1 can be quantitatively determined in ultra-violet light after oxidation to thiochrome in an alkaline medium. When vitamin B_1 is to be detected in biological material, substances may be co-extracted which interfere with the determination. Their removal can be achieved with ion exchangers, since they are retained. Synthetic zeolites[74,75] or Amberlite IR-100 in the free acid- or in the Na-form[76] or cation exchangers of the carboxylic acid type in the Na-form may be used[64].

For details of the method *cf.* ref. [77]. By this method aneurine was detected in urine[78–81], blood[82] and cereals[81–83]. The determination of aneurine after separation with an ion exchanger can be done colorimetrically[84].

Vitamin B_2. Vitamin B_2 can be separated from vitamin B_1 with ion exchangers and then quantitatively determined.

Vitamin B_6. Vitamin B_6 is exchanged on a cation exchanger of the carboxylic acid type[85] and thus freed from interfering substances which are co-extracted from yeast and which render accurate determination impossible.

Nicotinamide. For the removal of substances which interfere with the exact quantitative fluorimetric estimation of nicotinamide, the extract is adjusted to pH 5·0 and treated with a cation exchanger of the carboxylic acid type in the Na-form. At this pH no nicotinamide is exchanged. Then the anionic impurities are removed with a strong basic anion exchanger in the OH-form[86].

Panthenol can be separated by means of a column of Amberlite IRA-400 in the OH-form from ascorbic acid and vitamins of the B-complex-aneurine, riboflavine, pyridoxine, nicotinic acid and panthotenic acid, and can be quantitatively determined colorimetrically in the effluent[87].

Antibiotics

Penicillin. With penicillin it is evident that the degree of cross-linking of the resin has a decisive influence on the exchange capacity for such a large molecule as penicillin[88].

Streptomycin. A streptomycin broth can be purified to such an extent by the use of ion exchangers, that a sufficiently pure streptomycin solution is obtained which gives reliable and accurate results on determination.

The broth is diluted with a 0·2M disodium phosphate solution to a strength of 20 to 50 units per ml., adjusted to pH 8·5 to 9·0 with 0·2N sodium hydroxide solution and centrifuged. The prepared solution (5 ml.) is passed through a cation exchange column in the Na-form followed by 0·5 ml. of distilled water, and then followed by wash-water at an efflux rate of 0·3 ml./minute. The streptomycin cations are adsorbed quantitatively at this pH. Immediately after the wash-water has passed through the column, 25 ml. of 0·2N hydrochloric acid is passed at 0·5 ml./minute. The first 20 ml., which contains the eluated streptomycin free from interfering substances, is used for the determination.

Streptomycin is hydrolysed to maltol by the addition of 0·2 ml. of 4N sodium hydroxide solution to 4 ml. of the corresponding acid eluate; it is heated for 6 minutes in a boiling water bath and immediately cooled to room temperature in ice. Ultra-violet absorption at 322 mμ is measured before and after heating in a 1 cm. cell of a Beckman-Spectrophotometer[89].

Aureomycin. For the quantitative determination of aureomycin in blood and urine a synthetic zeolite (Decalso) may be used, which retains it, and from which it can be dissolved at an elevated temperature with 5 per cent. sodium carbonate solution. The quantitative determination is carried out fluorimetrically on the eluate[90].

Very recently an attempt has been made to combine paper chromatography and ion exchangers by impregnating the paper with ion exchangers.

This short review shows how varied are the possible applications in analytical chemistry opened up by the use of ion exchangers. Their further development too, will take a long time to explore fully.

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ION EXCHANGE RESINS IN CLINICAL MEDICINE

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To appreciate the use and the limitation of ion exchange resins it is necessary first to consider their mode of action[1]. The cation exchange resins are the most used in clinical medicine, and of these, there are two main types in general use—a sulphonated resin and a carboxylic resin. The latter is the more weakly acidic and the less effective on the acid side of neutrality but the more active weight for weight at optimal reaction. In practice, when used at the reaction of the alimentary tract, there is no material difference in their general action. Both resins have the same physico-chemical reactions. Their acidic nature enables them to bind loosely a series of cations. In weak solution—about M/100 cations with a higher valency are more firmly bound than those with lower valency and ions of equal valency are bound more firmly the higher their place in the periodic table.

In stronger solutions, the law of mass action applies however, and if the medium contains both sodium and potassium in amounts over M/10, then the resin will be found to bind sodium as well as potassium. With excess of sodium the resin will absorb more sodium than potassium. The rate at which these exchanges take place varies with the concentration of cations, size of resin particle and degree of mixing. With particles of size 50 to 100 μ, such as are used in pharmaceutical preparations, with constant mixing and cation concentration about that found in the gut, equilibrium can be established in about ten minutes in *in vitro* experiments.

In the human alimentary canal, the composition of the fluid surrounding the resin will vary with its progress through the gut. Initially, the composition will be largely determined by the food, but the dilution of the bolus in the upper alimentary tract with digestive juices containing a high proportion of sodium will cause the resin to take up sodium in excess of potassium. In the colon, the fluid excreted contains much more potassium and so the resin will relinquish its sodium in favour of potassium, and also of calcium and magnesium.

These effects have been well shown by Spencer, Ross and Lloyd-Thomas[2]. They also demonstrated the effect of aperients which decrease the time of stay of the resin in the large gut and showed that the amount of displacement of sodium by potassium increased with the delay in the colon, and that the result of giving aperients was therefore, to increase the amount of sodium and decrease the amount of potassium combining with the resin. However, their figures show some increased loss of potassium in the stool as a result of the aperient.

The amount of calcium and magnesium taken up by the resin will be greater as the relative amount of sodium and potassium in the fluid lessens with passage down the colon. As only ionic concentration is concerned in ion exchange, the bulk of the calcium and magnesium will

not enter into the exchange, owing to their being present in an insoluble form. Also the amount of calcium excreted in the digestive juices must be very small.

Resins can be used in several forms, combined with hydrogen, ammonium, potassium or sodium. The hydrogen form will readily exchange its H for sodium or potassium, thus freeing a H ion with which the body will have to deal and being acidic it may cause ulceration of the mouth.

TABLE I

DAILY EXCRETION OF CATIONS IN THE STOOLS

	Total (m.Eq)		Bound to Resin (m.Eq/g.)		
	Na	K	Na	K	Na/K
Resin only	21 (13–37)	69 (58–106)	0·32	1·2	0·26
Resin and purge	63 (39–114)	103 (73–127)	0·68	0·93	0·73

Taken from a paper by Spencer, Ross and Lloyd-Thomas, *British Medical Journal*, 1954, 1, 603.

The ammonium form is neutral but although causing no trouble when taken by mouth, ammonium is exchanged for sodium and potassium, the product when metabolised by the liver, also produces an acid ion. Both these resins are therefore, liable to produce an acidosis. The potassium form will exchange its potassium for sodium, but the amount of sodium ultimately bound to resin will not be great as the potassium ions of the resin will increase the proportion of potassium to sodium present in the surrounding fluid. In the same way, the sodium form will exchange with potassium. Neither the potassium or sodium forms will disturb the acid base balance but both will exchange with calcium and magnesium.

Resins are used mostly in conditions in which there is retention of sodium and water, such as cardiac failure, renal disease (nephrotic syndrome) toxæmia of pregnancy and cirrhosis of the liver. They are also used in hypertension.

The homeostatic control of the extracellular fluid space is a complex one[3,4], involving the maintenance at optimum level of the osmotic and oncotic pressures of the plasma, the correct adjustment of the glomerular filtration rate, the consumption of adequate salt and water, and a normal adrenocortical function. If this is carried out normally, the total body sodium will be kept at a constant level. The distribution of the extracellular fluid between plasma and interstitial fluid will depend on the plasma proteins and the capillary pressure, and the even distribution between various parts of the body depends on the normality of the circulation and muscle tone. Resins can thus be really effective only if the remaining mechanisms are capable of functioning at a reasonable degree of efficiency.

In general, œdema indicates the presence of excess sodium in the body. Dietary restriction of sodium to less than 0·5 g. per day is difficult, Carey[5] states that 2 g. of sodium chloride (0·8 g. of sodium) per day is the lowest the diet can be reduced to without being rendered completely unpalatable. As there is obligatory loss of sodium, both in urine and

sweat, restriction of sodium intake to this level will prevent an accumulation of more sodium in the body, and if steps are taken to increase the sodium loss by sweating and a high fluid intake, a slow loss of sodium to the body can be produced. It is not possible to cause a rapid loss of sodium at all easily by dietary restrictions alone.

Ion exchange resins will remove sodium from the body and reduce the excess sodium load, providing the remaining mechanisms are functioning adequately. This will in turn get rid of the œdema. The underlying pathological condition will not be affected, although removal of the mechanical effects of fluid retention will often allow embarrassed mechanisms to function better.

The fundamental action of resins as a sodium remover are similar in all the clinical conditions for which they are used, except in hypertension without obvious œdema. In this condition it is claimed that a low sodium diet is often effective in reducing the blood pressure and resins have been used as a reinforcement of a low sodium diet[6], or to enable some salt to be taken safely in the diet. In the absence of œdema it is unwise to remove sodium from the body but it may well be that by reducing the sodium to be excreted by the kidney, there will be some beneficial readjustment of the kidney's pressure needs for filtration. However, the clinical reports of the usefulness of resins in hypertension, are in general, not very optimistic[7].

Many authors have noted that resins can be used with advantage in conjunction with mercurial diuretics[8,9]. After a time, many patients become resistant to mercurial diuretics and Schwartz and Relman[10,11] explained this by showing that the prolonged effect of mercurial diuretics produced hypochloræmic alkalosis. If this became marked, the diuretic effect of the mercurials ceased. As the effect of resins is usually to cause an acidosis, it can be easily seen how the combination of resins and mercurials will mutually aid each other's action.

In cardiac failure, resin treatment has a definite place[12,13]. When prolonged treatment has to be undertaken intermittent use of resins is to be preferred, periods of about six months appear to be satisfactory, but after that, many people record less gratifying results[7] owing to the side effects, especially possible loss of calcium. In the same way, regular treatment with mercurial diuretics may be found to be unsatisfactory over prolonged periods. The intermittent treatment gives the opportunity to replenish depleted calcium stores and quite likely also other unrecognised depletions, such as iron.

If the patient is being treated with digitalis, the serum potassium level must be watched, since with lower potassium levels, the action of the digitalis becomes more marked[14]. It is wiser to reduce the digitalis in advance and only increase it when a state of electrolyte equilibrium has been established.

When ascites or hydrothorax complicate the œdema, resins are less effective, and removal of the fluids by puncture is recommended. If the fluid accumulated only because of the tissue œdema, it should not return if the resin is controlling the œdema.

The long term use of resins calls for careful attention. The amount of sodium removed by the resin, together with the loss in sweat and urine, will exceed the sodium in the diet if a low sodium diet is given. This will cause a reduction of the extracellular fluid below the optimum level and frequently instead of this happening the percentage of sodium in the fluid will fall to below the normal value and symptoms of sodium lack will occur, such as marked lassitude, loss of appetite, vomiting, and a rising blood urea and blood pressure, which may mimic uræmia.

This low salt syndrome[15] occurs in two forms. It can occur more or less spontaneously, in which case it has a very bad prognosis, and attempts at replacing the sodium by giving salt in the diet will merely result in increased œdema without improving the lot of the patient. If, on the other hand, it is the result of active therapeutic measures, such as mercurials or resins, then an adequate amount of hypertonic saline will rapidly remove the symptoms and quite often results in a loss of the œdema.

To be worth using, a treatment which needs constant watching should be more effective than other less troublesome methods. In nephrotic œdema in children this is by no means the case as often loss of renal function is sufficient to prevent the use of resins and even when these are initially successful, the œdema will return, but with a low serum sodium which prevents their further use[16]. In the adult variety of nephroic œdema, which often behaves differently to that of children, the recorded results of resin treatment are less gloomy[17].

In both hypertension and nephrotic œdema, the kidney function may be impaired and the prolonged use of resins in the ammonium cycle—the most effective form—will cause a progressive acidosis owing to the kidney being unable to bring into action the various base saving mechanisms. In nephrosis in particular, the damaged kidney may be unable to maintain the normal plasma osmotic pressure and when the excess sodium is removed by the resin (or even by prolonged low sodium diet), the water in which it was dissolved remains in the body and no reduction of œdema occurs. As a result the sodium may fall to a dangerously low level. A similar renal failure may also occur during the course of an apparently successful treatment, and this will be shown by an increase in œdema (or in weight) with no obvious cause. It is therefore essential in all long-term treatments for frequent blood electrolyte studies to be made.

Another complication of using the hydrogen or ammonium cycle resins alone, is the removal of potassium from the body which would cause hypokalæmic symptoms (cardiac failure, abdominal distension and œdema, etc.) to appear. This is avoided by using a mixture of the ammonium and potassium resins, but it is by no means certain that an optimum mixture is being used in every case. Close attention must be given to the plasma potassium level, not only to prevent hypokalæmia but also hyperkalæmia, since with damaged renal function this state has been found when the standard mixture of ammonium and potassium resins were employed.

A further complication sometimes recorded is tetany. Since so little calcium is removed that most balance studies fail to show a negative

balance[15], and as there is so much calcium available in the bones, it seems unlikely that resins alone could account for the low serum calcium. It is well recognised that the blood calcium is maintained at a very constant level irrespective of any dietetic measures, its level being set apparently by the activity of the parathyroid gland. There is very considerable variation in the literature on the effect of resins on the calcium level in the blood[5,6]. Many record constantly normal results and state that they have observed no clinical manifestations on prolonged use of resins. Others report occasional tetany[18], and many more record slight lowering of the calcium in the blood, but very rarely levels below 8 mg. per 100 ml. It is unlikely that symptoms of tetany will occur with a calcium no lower than this. On the other hand, for example in nephrosis, spontaneous tetany is known to occur quite apart from the use of resins.

In cirrhosis of the liver, treatment has a peculiar danger if resin in the ammonium form is used[9]. If there is much liver damage, the ammonium liberated will not be metabolised to urea and severe toxic symptoms may occur due to raised blood ammonia. If the liver function is good however, resin treatment can be successful[13,19].

Good results have been claimed in early toxæmia of pregnancy[5,20], and as there is no question of a chronic long-term treatment, it would seem a reasonable form of treatment.

Another use of resins is in acute renal failure. While it is possible by dietetic measures to minimise the rise of the blood urea, the development of acidosis and the occurrence of œdema, it is impossible to control the steady rise of serum potassium, which may indeed be the final cause of death. Resins in the sodium form can be used with marked success[21,22] in particular, as the amount of potassium to be removed is not very great; the loss of one gram in an average adult would reduce the blood level by about 7 mg., this would only need about 20 to 30 gm. of resin. A similar use may occur occasionally in the treatment of acute adrenocortical failure when the serum potassium may rise to very high levels.

While the cation binding resins are the most used, the anion binding resins formed by using amino-acid groups instead of the sulphonic or carboxyl groups have also some clinical application. In an endeavour to counteract the acidosis caused by the hydrogen or ammonium resin, anion binding resins have been added to the mixture. This will increase the bulk of the resins needed to bind a given amount of sodium and in practice has not been very successful[23].

Anion binding resins have also been tried as an antacid in gastric ulcer treatment. It shares with several other insoluble acid bonding substances the advantage that any unused excess will not be absorbed and so increase the alkalosis which inevitably results from the removal of large amounts of free hydrochloric acid from the body. Segal *et al*[21], in a paper reviewing the results of using resins in ulcer treatment, record a reasonably successful result in about 65 per cent. of the cases, using a dose of approximately 2 g. of resin every two hours. They found no side effects from this treatment, and in particular, had no trouble with constipation.

It is rather difficult to assess the real place of resins in therapy. They are of great value in hyperkalaemic states and a supply of the sodium form should be available in all hospitals to treat acute cases. For all other conditions, as good or better methods of treatment are available which do not call for so much clinical chemistry. For those who find a low salt diet both advantageous and intolerable, resins will give some slight lightening of their dietetic burden. In most cases however, the main usefulness of resins will be as an adjuvant or a temporary alternative to mercurial or other diuretics, or to low salt diets.

Resins can be of value as alternative methods of treatment over short periods and some patients will prefer to use resins rather than have periodic injections of mercurial diuretics. Others, however, find the taking of the large amount of resin so unpleasant, that they do not tolerate the treatment for prolonged periods.

The complication of severe constipation and even of semi-obstruction, adds one more difficulty in their use, but there is no doubt that there is a definite place in therapeutics for the ion exchange resins.

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RESEARCH PAPERS

THE *IN VIVO* POTENTIATION BY MAGNESIUM SALTS OF THE UTERINE RESPONSE TO POSTERIOR PITUITARY EXTRACTS IN THE BOVINE

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INTRODUCTION

It has been known for some years that the reactivity of isolated uterine muscle of posterior pituitary extracts varies with the magnesium ion concentration of the fluid environment. An augmenting effect of increased magnesium concentration was first demonstrated by Van Dyke and Hastings[1] using unfractionated posterior lobe extracts on the isolated guinea-pig uterus and this has since received confirmation by de Jalon[2] and Hsu[3]. Genell[4] showed that this *in vitro* phenomenon applies to species other than the guinea-pig.

Frazer[5] and Stewart[6] have studied this effect using fractionated vasopressor and oxytocic preparations. Frazer found that increase of magnesium chloride concentration can augment the response of the isolated guinea-pig uterus to either fraction but that a greater and more consistent potentiation is seen with vasopressin than with oxytocin. Stewart reinvestigated this problem over a wider range of concentrations of magnesium chloride and found potentiation of vasopressin to be greater than that of oxytocin with relatively low concentrations of magnesium chloride, but that at high concentrations oxytocin was potentiated to a greater extent than vasopressin.

The mode of action of magnesium ions in producing this augmentation is unknown. The augmentation phenomenon is known to affect uterine muscle more than other forms of smooth muscle[4]; to be inapplicable to other oxytocic drugs such as ergometrine and histamine[3,5]; and to affect vasopressin in a different way to oxytocin[5,6]. This implies a considerable degree of biochemical specificity. Genell[4] has suggested that the role of the magnesium ion as an enzyme catalyst is implicated.

Although the effect of magnesium salts is well established *in vitro* no comparable *in vivo* experiments have been traced. In the course of other work on the reactivity of the bovine uterus and cervix, to be published elsewhere, it became possible to investigate this relationship in the intact animal.

APPARATUS AND METHODS

Ten experiments were made using three cows, all of whom were non-pregnant throughout the period of observations.

Serum magnesium concentration was estimated by Allcroft's modification[7] of the method of Dennis[8]

The techniques used for recording uterine activity were essentially

similar to those used in pregnant women by Schild, Fitzpatrick and Nixon[9].

Intra uterine pressure was recorded using a hollow metal cannula carrying terminally a small latex rubber balloon (capacity 2 ml.),* the cavity of which communicated with the shaft of the cannula. This in turn was connected via pressure tubing to a mercury manometer, the movements of which were linearly magnified and recorded on a kymograph. The whole transmission system was water filled.

After sterilization, and with the balloon completely deflated, the instrument was inserted *per vaginam* until the tip lay within the body of the uterus, approximately two inches rostral to the internal cervical Os as judged by rectal examination. This position was maintained by clipping the shaft of the cannula to the vaginal protuberance of the cervix. The balloon was then partially inflated by injecting into the transmission system, a volume of water slightly less than the mould volume of the balloons.

For this procedure and throughout the subsequent experimental period the animals were maintained in the normal standing posture under extreme posterior epidural anæsthesia (segments S4 and S5) using 2·5 per cent. procaine hydrochloride.

Drugs

All drugs were given via an indwelling polythene cannula inserted into the external jugular vein.

Commercial preparations of Pitocin and Pitressin were used as sources of oxytocin and vasopressin respectively. The terms oxytocin and vasopressin when used below refer to these preparations. The manufacturers' statements, of potency and freedom from more than 5 per cent. cross contamination, were confirmed by assaying each product for both oxytocin and pressor activity.†

EXPERIMENTAL

In each experiment several doses of oxytocin, vasopressin or pituitrin were given intravenously to indicate the normal pretreatment response of the animal under prevailing conditions. Subsequently a dose of magnesium chloride or magnesium sulphate was given intravenously and the magnitude of response compared with that obtained before treatment. In some experiments the responses to both oxytocin and vasopressin were studied before and after magnesium treatment.

The observations fall into two groups. In the first, the same dose of active principle was given repeatedly throughout the experiment (Experiments 1, 4, 5, 6, 7 and 10). In the second type of experiment the dose of active principle was varied before administration of magnesium to indicate approximately the dose-response relationship over a restricted range (Experiments 2, 3, 8, 9, 10, and 11). An interval of 40 or 45

* The balloons were specially moulded by the London Rubber Co.

† These were very kindly done by G. A. Stewart of Burroughs, Wellcome and Co., Dartford.

minutes separated successive doses of hormone and this time factor precluded further elaboration of the pattern of the experiments.

RESULTS

Preliminary dosage experiments were made in which the concentration of magnesium in the blood serum was estimated before, and at intervals after, the intravenous administration of various doses of magnesium chloride. A typical experiment is illustrated in Figure 1 in which a function of the serum concentration of magnesium is plotted against time.

Treatment with magnesium chloride was found to augment uterine response of some posterior pituitary preparations but not to all. The results are summarised in Table I.

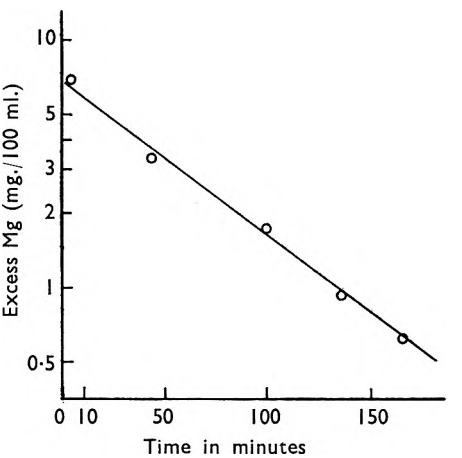

FIG. 1. Serum concentration of magnesium in a cow after the intravenous injection of 22·5 g. magnesium chloride. The logarithm of the increase in serum concentration of magnesium above the pre-injection value, is plotted against time. The slope is approximately linear.

Pituitrin. The unfractionated extract was used only in the first experiment of the series. Responses of reasonably consistent intensity were obtained when the same dose of pituitrin was given three times in succession before magnesium treatment. A fourth dose of the same magnitude, given 10 minutes after 6 g. of magnesium chloride intravenously, elicited an augmented response. This might be attributed to potentiation of either or both fractions, oxytocic and vasopressor. All subsequent experiments were made using the fractionated preparations.

Oxytocin. Uterine response to oxytocin was studied in eight experiments, in four of which vasopressin was also given. In no experiment was evidence obtained of an augmentation after magnesium even though the serum concentration was raised to 8 or 9 mg./100 ml. In most experiments (3, 5, 8, 9, 10 and 11) the treatment appeared to produce inhibition but this was not significant ($P = 0{\cdot}05$) for the group as a whole. This inhibition is shown graphically in Figure 2.

Vasopressin. Uterine responses to vasopressin were recorded in six experiments in each of which augmentation of response after magnesium was seen clearly (Fig. 2). This effect was highly significant for the group as a whole ($P = 0{\cdot}01$).

However the magnitude of the increase in response was not proportional to the corresponding serum magnesium concentration. This lack of relationship is possibly due to the discrepancy between the concentration

TABLE I

REACTIVITY OF BOVINE CORPUS UTERI BEFORE AND AFTER MAGNESIUM SALTS

Expt.	Dose	Serum concentration of magnesium in mg. per 100 ml.	Response in mm. mercury	Per cent. augmentation of response after magnesium
1	35 U. pituitrin	2·3	22	
	" " "	"	20	
	" " "	"	21	
	6 g. $MgCl_2$			
	35 U. pituitrin	3·2	29	+38
2	1 U. oxytocin	2·6	10	
	3 U. "	"	11·5	
	9 U. "	"	15	
	3 U. "	"	12	
	10 g. $MgCl_2$			
	3 U. oxytocin	5·0	12	nil
	15 g. $MgCl_2$			
	9 U. oxytocin	8·0	14	−7
3	1 U. oxytocin	2·7	5·0	
	3 U. "	"	9·5	
	9 U. "	"	15·0	
	18 g. $MgCl_2$			
	3 U. oxytocin	7·3	6·0	−37
4	3 U. oxytocin	2·7	13·5	
	3 U. "	"	14·0	
	15 g. $MgCl_2$			
	3 U. oxytocin	7·1	14·5	+3·5
5	10 U. oxytocin	2·3	15	
	10 U. "	"	13	
	4 g. $MgCl_2$			
	10 U. oxytocin	3·2	11	−15·5
6	9 U. vasopressin	2·0	10·5	
	9 U. "	"	9·0	
	5 g. $MgCl_2$			
	9 U. vasopressin	3·5	14·0	+55
7	30 U. vasopressin	3·0	34·0	
	30 U. "	"	30·0	
	30 U. "	"	39·0	
	30 U. "	"	37·5	
	20 g. $MgCl_2$			
	30 U. vasopressin	8·3	45·0	+20
8	1 U. oxytocin	3·0	3·0	
	5 U. vasopressin	"	2·0	
	2 U. oxytocin	"	4·0	
	15 U. vasopressin	"	10·0	
	6 U. oxytocin	"	15·0	
	40 U. vasopressin	"	20·0	
	23 g. $MgCl_2$			
	2 U. oxytocin	9·5	1·5	−62
	15 U. vasopressin	7·0	16·0	+60
	6 U. oxytocin	4·6	5·0	−67
9	1 U. oxytocin	3·2	5·0	
	3 U. "	"	13·0	
	18 U. vasopressin	"	20·0	
	6 U. oxytocin	"	18	
	20 g. $MgSO_4$			
	3 U. oxytocin	6·6	9·0	−18
	18 U. vasopressin	6·2	28·0	+40
	6 U. oxytocin	5·4	16·0	−11
10	9 U. vasopressin	3·0	13·0	
	2 U. oxytocin	"	15·0	
	6 U. vasopressin	"	7·0	
	2 U. oxytocin	"	12·0	
	18 g. $MgCl_2$			
	9 U. vasopressin	8·0	19·0	+46
	2 U. oxytocin	5·5	9·0	−25
	9 U. vasopressin	4·9	18·0	+40
11	3 U. oxytocin	2·4	14·0	
	6 U. "	"	32·0	
	15 U. vasopressin	"	13·0	
	30 U. vasopressin	"	24·0	
	17½ g. $MgSO_4$	"		
	3 U. oxytocin	7·8	8·0	−43
	15 U. vasopressin	4·9	18·0	+38
	6 U. oxytocin	4·6	24·0	−25
	30 U. vasopressin	3·9	30·0	+25

Doses of magnesium chloride and magnesium sulphate are calculated as anhydrous salts.

of magnesium in the blood and that in the immediate environment of the uterine cells or perhaps within the cells[10].

Comparison of responses to oxytocin and vasopressin

The differences seen between oxytocin and vasopressin in these experiments are well illustrated in the four experiments in which both oxytocin and vasopressin were given (Experiments 8, 9, 10 and 11). In all four experiments oxytocin responses showed no change or were depressed after administration of magnesium salt, whilst under the same experimental conditions responses to vasopressin were augmented. Figure 3 illustrates kymograph records from one such experiment (Experiment 8).

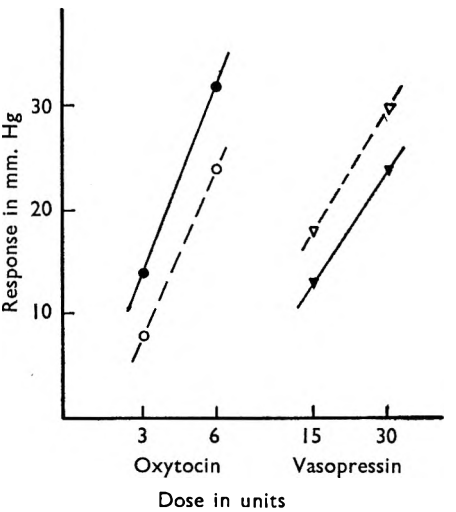

FIG. 2. Data from experiment 11. The uterine pressure response is plotted against the dose of oxytocin (circles) or vasopressin (triangles) on a logarithmic scale. Reactivity before magnesium is represented by solid symbols and continuous lines; that after magnesium by open symbols and interrupted lines. Reactivity to oxytocin is depressed after intravenous injection of 17·5 g. magnesium sulphate although at the same time reactivity to vasopressin is augmented.

DISCUSSION

Experiments of this nature suffer *in vivo* complications which make it impossible to compare the potency of posterior pituitary preparations before and after magnesium treatment, with the precision to be expected from *in vitro* experiments employing multi-dose assay procedures.

Within these limitations our results clearly indicate that an increase in the magnesium concentration of body fluids augments uterine reactivity to vasopressin, a finding in keeping with the relationship already established *in vitro* by previous workers. On the other hand our experiments showed no augmentation of oxytocin although such an effect has been detected *in vitro* by Hsu[3], Stewart[6] and others. However if the concentration of magnesium is considered, this difference is largely resolved. Frazer[5] was the first to study *in vitro* this effect over a wide range of magnesium concentrations. His results indicated progressive augmentation of vasopressin as the concentration of magnesium in the perfusion fluid increased from 2 mg. per cent. $MgCl_2$ to 5, 10, 20, 25 and 50 mg. per cent. No such augmentation was seen over this range with oxytocin and in fact depression was recorded with concentrations of 10 and 20 mg. per cent. This may be compared with the depression seen in our experiments. Stewart[6] in a somewhat similar investigation showed that at low concentrations of magnesium (2·5 and 5·0 mg per cent. $MgCl_2$)

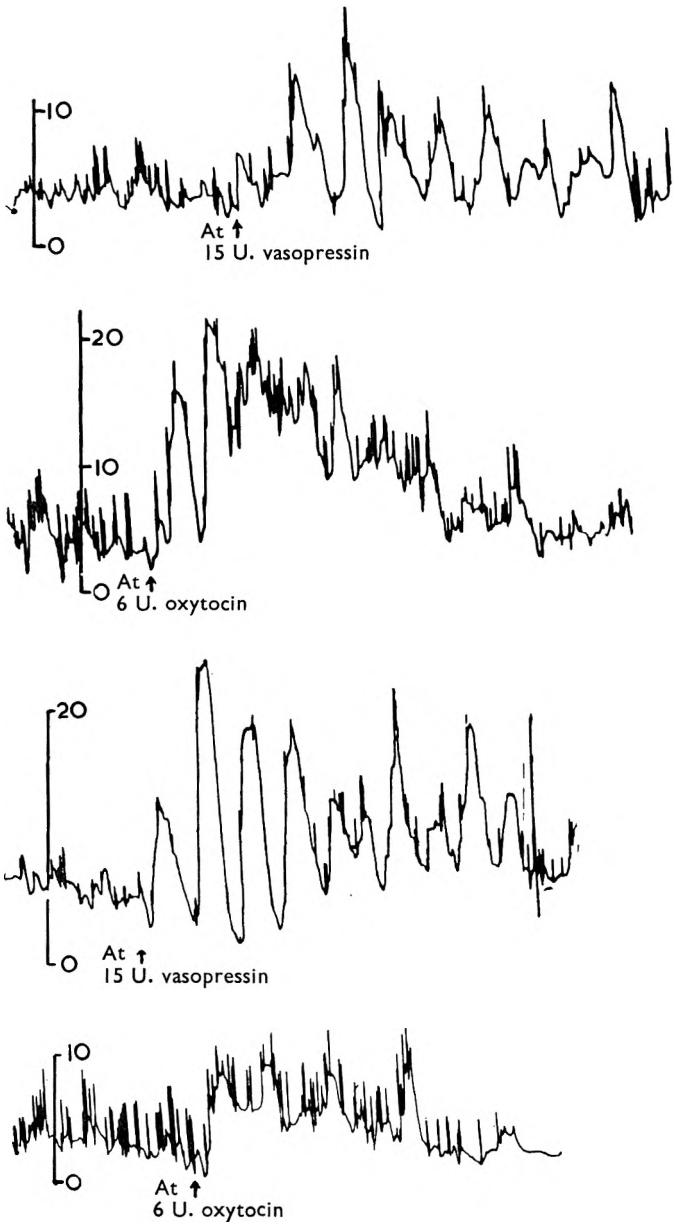

FIG. 3. Kymograph records obtained in experiment 8. Upper two tracings before, and lower two tracings after, the injection of 23 g. magnesium chloride intravenously. Before magnesium treatment the response to 6 units oxytocin is greater than that to 15 units vasopressin. Subsequent to magnesium treatment the response to the same dose of oxytocin is depressed whilst that to vasopressin is augmented, so that the vasopressin effect is the greater.

augmentation of vasopressin was predominant whereas only at high concentration (50 and 100 mg. per cent. $MgCl_2$) was oxytocin augmentation predominant.

Our *in vivo* observations were not extended to the higher concentrations used by these authors since such concentrations would certainly be toxic by virtue of central nervous system depression[11]. Similarly our lowest concentrations could not be less than those physiologically normal and it is significant that Frazer[5], Hsu[3] and Stewart[6] found evidence of magnesium augmentation of oxytocin when the concentrations studied were between zero and 2·5 mg. per cent. $MgCl_2$.

Thus the *in vitro* evidence of magnesium augmentation of oxytocin is restricted principally to concentrations of magnesium that are either too small or too large to be studied *in vivo* in experiments such as ours. Our *in vivo* evidence, of a preferential augmentation of uterine response to vasopressin consequent upon administration of magnesium salts, is in reasonable agreement with *in vitro* observations if comparison is limited to magnesium concentrations which are compatible with life.

SUMMARY

1. Intravenous injection of magnesium chloride and magnesium sulphate to intact non-pregnant bovines augments the response of the myometrium to vasopressin but not that to oxytocin. These results are discussed in relation to comparable observations *in vitro*.

The author is grateful to Dr. H. O. Schild for his advice throughout these experiments and to the Veterinary Laboratory, Weybridge for estimations of serum magnesium concentration.

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POLAROGRAPHY OF SOME PURINE DERIVATIVES

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Certain purines and purine derivatives are known to be antitumour agents because of their ability to act either as antagonists or antimetabolites in cell metabolic processes[1–3]. During the syntheses and study of some of these compounds a rapid and sensitive method for the detection of certain functional groups was needed. Because the ultra-violet absorption spectra of the purines lie close together in a region where they are often difficult to distinguish, and because the bands in the infrared have not yet been definitely assigned, we have been investigating the use of the polarograph as an analytical tool.

The comprehensive review on the subject of the nucleic acids and their chemistry[4], notes only two references to the application of polarography: Heath[5] has shown that of the naturally occurring purine and pyrimidine ribonucleotides, only adenylic acid and its derivatives are reduced in 0·1M perchloric acid at the dropping mercury electrode. Cavalieri and Lowy[6] have studied a series of variously substituted pyrimidines and give a list of twenty-six, ten of which yield polarographic waves. Heath suggests that in adenine, reduction takes place between the nitrogen and the carbon at the 1:6 positions in the pyrimidine portion of the molecule, whereas Cavalieri and Lowy conclude that the system —N=C—C=C— is responsible for the polarographic activity in the pyrimidines they tested.

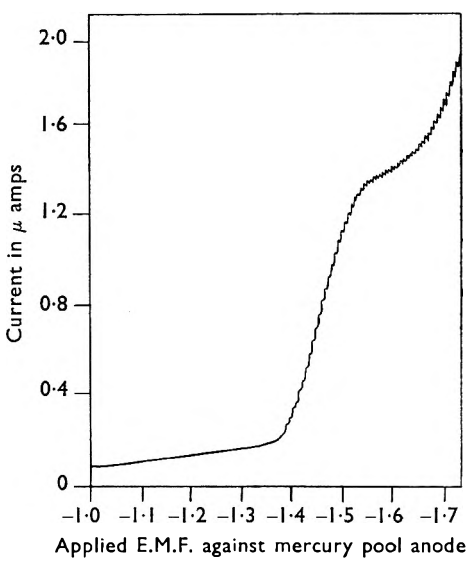


Fig. 1. Polarogram of adenine (*ca* 10^{-4} M) in perchloric acid (0·1 M).

An interesting correlation appears to exist between the presence of an amino group in the six-position of the purine ring and the appearance of a polarographic reduction wave. Besides 6-aminopurine (adenine) the following compounds reduce at the dropping mercury electrode: 2:6-diaminopurine, 6-acetamidopurine (acetyladenine), 2:6-diacetamidopurine. As distinguished from adenine which gives only one step, the acetylated

derivative shows two reduction steps (see Figs. 1 and 2). One of these must be due to the presence of the acetyl group for when that is hydrolysed, the wave at the more positive potential disappears and only the one due to the adenine moiety remains. Futhermore, 2:6-diacetamidopurine shows only two reduction waves although both amino groups have been acetylated

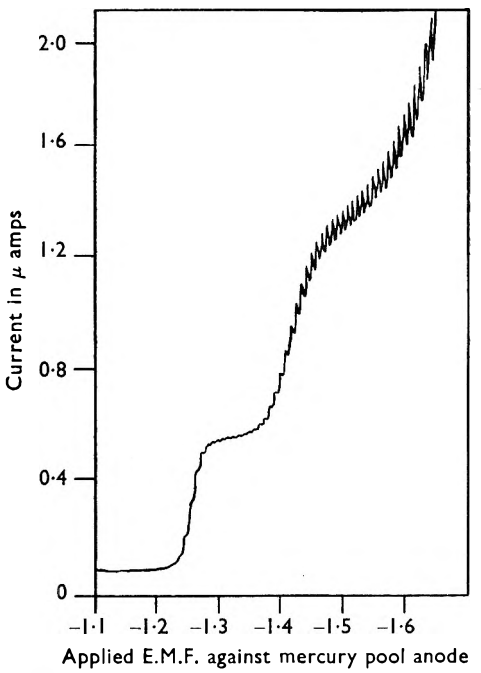


FIG. 2. Polarogram of acetyladenine (*ca* 10^{-4} M) in perchloric acid (0·1 M).

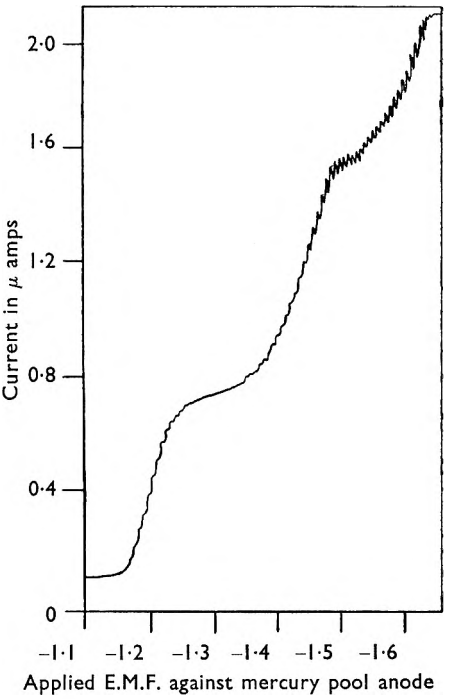
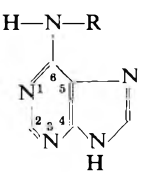


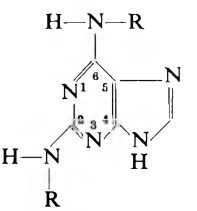
FIG. 3. Polarogram of 2:6-diacetamidopurine (*ca* 10^{-4} M) in perchloric acid (0·1 M).

(see Fig. 3). These steps are very similar in behaviour to acetyladenine and have approximately the same half-wave potentials. This also substantiates the assumption that reduction is associated with the amino group* (or its *N*-acetyl derivative) when it is in the 6-position of the purine ring (see I and II).



R = — H = Adenine
R = — $COCH_3$ = Acetyladenine

I



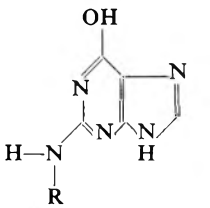
R = H = 2:6-Diaminopurine
R = — $COCH_3$ = 2:6-Diacetamido-purine

II

* The fact, however, that 6-mercaptopurine shows a polarographic wave in alkaline solution indicates that the character of the functional group in the C(6) position of the purine ring is also important.[7]

However, the presence of an acetyl group in *N*-acetylguanine (see III) does not promote reduction, and like the parent compound, guanylic acid and its derivatives, shows no polarographic activity.

A similar behaviour exists in the corresponding pyrimidines: the 6-amino and 2:6-diaminopyrimidine molecules reduce polarographically, whereas 2-amino-6-hydroxypyrimidine (*iso*cytosine) does not[6].



R = — H = Guanine
R = — $COCH_3$ = Acetylguanine

III

Experimental

All measurements were made on a Tinsley pen-recording polarograph Mark 15. Fresh stock solutions of our model compound acetyladenine (0·002M) were prepared in 50 per cent. aqueous ethanol made millimolar with perchloric acid. From this, appropriate dilutions for 2, 4, and 8×10^{-4} molar were made so that the final solution contained 0·001M perchloric acid and 0·01 per cent. starch all made up to volume with 50 per cent. (v/v) aqueous ethanol.

For the first step the instrument setting was 2 microamperes for full scale deflection in all concentrations examined. The work was carried out in the electrolysis stand supplied with the instrument and approximately 10 ml. of each solution was used. Nitrogen was bubbled through the cells to remove dissolved oxygen at which time no mercury was present. A saturated calomel half cell was used as the anode, and the temperature of the system was maintained at 20·8° C. ($\pm$ 0·2°).

All the polarograms were taken at two heights of the mercury reservoir, 36 cm., and 64 cm., drop times 6·3 and 3·6 seconds respectively. Both damped and undamped waves were obtained; the former were used to measure the diffusion current, while on the latter, the half-wave potentials were measured graphically. Derivative waves were also taken to aid the resolution of the two reduction steps produced, and to help in reading the $E_{\frac{1}{2}}$ values.

Results

A quantitative study was made on acetyladenine as a representative compound. Although previous polarographic determinations on adenine and the nucleotides have been made in 0·1M perchloric acid[5], it was found that acetyladenine hydrolysed rapidly in this supporting electrolyte giving non-reproducible wave heights. Additional proof that the changing values were due to the hydrolysis of the acetyl group was obtained from the ultra-violet absorption data which shifted from the known value of acetyladenine ($\lambda_{max.}$ 287 mμ to that of adenine ($\lambda_{max.}$ 262 mμ). Table I.

Of the two polarographic waves produced by acetyladenine in 0·1M

perchloric acid, the first became almost negligible and the second increased after one hour on the steam bath. This indicated that the first wave came from the acetyl group and the second from the adenine portion of the molecule. When the acetyladenine was completely hydrolysed, both the remaining single polarographic wave and the ultra-violet absorption data were indistinguishable from the parent compound, adenine.

TABLE I

CHANGE WITH TIME IN ULTRA-VIOLET ABSORPTION OF ACETYLADENINE IN 0·1M PERCHLORIC ACID

mμ	Fresh solution*	1 day	2 days	Plus 2 days at 37° C.
λ Maximum ..	287†	274	270	262
λ Minimum ..	235	232	232	228‡

* Concentration: 10 mg./100 ml. † Known value for acetyladenine. ‡ Known value for adenine.

However, acetyladenine could be stabilised in 50 per cent. aqueous ethanol and when adjusted to a concentration of 0·001M in perchloric acid gave two polarographic waves which were reproducible even after the solution had stood for 24 hours at about 20° C. That these reduction waves occurred only in acidic solutions, and in addition were not simply dependent on the perchlorate ion, was shown by the fact that neither in 50 per cent. aqueous ethanol, nor in this medium containing potassium perchlorate did any polarographic steps appear. However, acidification again produced the two reduction waves which improved in appearance upon the gradual addition of perchloric acid to a concentration of 1 millimolar at which they were stabilised.

TABLE II

POLAROGRAPHIC BEHAVIOUR OF ACETYLADENINE IN 0·001M PERCHLORIC ACID

(Reduction Wave at $E_{\frac{1}{2}} = -1{\cdot}03$ V. against the Saturated Calomel Electrode)

Concentration* $M \times 10^{-4}$	Diffusion Current i_d at 36 cm. (μa.)	Diffusion Current i_d at 64 cm. (μa.)	$\frac{i_d \text{ 36 cm.}}{i_d \text{ 64 cm.}}$
2	0·29	0·39	0·75
4	0·56	0·76	0·74
8	1·11	1·47	0·75

* $E_{\frac{1}{2}}$ remained constant in the range of concentration studied. Temperature was maintained at 20·8° C. (± 0.2°)

Although both waves of acetyladenine could be used for identification and quantitative estimation we chose the first, with a half-wave potential of −1·03 volts (against saturated calomel electrode) for investigation, because it was more clearly defined over a wide range of pH. For this reduction step, the wave height was proportional to the concentration (see Table II) and the plots of the diffusion current i_d against concentration for two values of the height of the mercury reservoir were found to be straight lines. In addition, a comparison was made with the reduction of the cadmium ion whose polarographic wave is known to be diffusion controlled[8]. The current readings of a standard solution of cadmium in 0·1M potassium nitrate were found at two heights of the mercury reservoir,

36 cm. and 64 cm. respectively. The ratio of these values was 0·76 which is in good agreement with the calculated figure of 0·75, i.e., the ratio of the square roots of the two heights chosen. From Table II it can be seen that the acetyladenine steps examined gave similar ratios.

Comparison was made with the polarographic behaviour of yeast nucleic acid. A freshly-prepared solution of yeast nucleic acid in 0·2M potassium chloride showed a reduction wave which was for the most part diffusion controlled and whose half-wave potential was — 1·28 volts against the saturated calomel electrode. The step height was nearly linear with concentration in the range between 4 and 16 mg. per cent. during which interval the $E_{\frac{1}{2}}$ value remained constant. Since of the isolated nucleotides only adenylic acid gave a reduction wave, it may be inferred that the adenine portion was probably involved in the polarographic activity of the nucleic acid.

Discussion

The work of Heath[5] was repeated and confirmed.

The second of the two polarographic steps shown by acetyladenine—the one with the half-wave potential at the more negative value—was the more pH sensitive. As distinct from the first step which is relatively unaffected by changes in acidity of the supporting electrolyte, the second step was more clearly defined in 0·1M than in 0·001M perchloric acid. In the absence of ethanol, large maxima occurred which could be suppressed by the addition of starch solution.

For exact measurement of the second wave carefully controlled conditions were required to prevent the hydrolysis of the acetyl group, a process which caused the observed fluctuation in step heights, and the $E_{\frac{1}{2}}$ values. However, if a water solution was acidified immediately before polarographing, both waves could be determined quantitatively.

The half-wave potential for each wave remained constant with concentration. However, the $E_{\frac{1}{2}}$ values varied with pH and were found to be more negative with decreasing acidity: in 0·001M perchloric acid the value was — 1·03 volts for the first step and — 1·26 volts for the second step, but in 0·1M perchloric acid the corresponding potentials were — 0·85 and — 1·0 volts against the saturated calomel electrode respectively. It was also observed that in less acid solutions, better resolution between the two waves was obtained when the concentration of the acetyladenine was ca. 1×10^{-4}M, a fact which might be of some importance in biological investigations. Table III gives a list of purine derivatives which show polarographic waves in 0·1M perchloric acid.

The polarograph was also used to decide quickly between various methods of acetylation, since the acetylated compounds show the characteristic step with a half-wave potential in the region of —1·2 volts against the anode pool in addition to the purine reduction step in the region of — 1·5 volts. Yet a further application of this method was its use in

identifying adenine as an impurity in a commercial sample of a non-reducible nucleotide. The presence of adenine was later confirmed by R_F values obtained from paper chromatography of the sample.

TABLE III

POLAROGRAPHIC BEHAVIOUR OF SOME PURINE DERIVATIVES IN 0·1M PERCHLORIC ACID

Compound*	Analyses		$E_{\frac{1}{2}}$† volts
	Found	Calc'd.	
Adenylic acid			− 1·34
Adenosine			− 1·42
Adenine	C, 44·35 H, 3·85	C, 44·44 H, 3·73	− 1·46
Acetyladenine‡	C, 47·1 H, 4·0	C, 47·45 H, 3·95	− 1·22, − 1·43
2:6 Diaminopurine	N, 56·1	N, 55·97	− 1·4
2:6-Diacetamidopurine	N, 35·95	N, 35·9	− 1·27, − 1·5
Nucleic acid§			− 1·32

* All compounds were determined at a concentration of ca. 1×10^{-4} molar, capillary drop time 3·4 sec.
† Against the mercury pool anode, ± 0·02 volts.
‡ $E_{\frac{1}{2}}$ against the saturated calomel electrode = − 0·85, − 1·0 volts.
§ $E_{\frac{1}{2}}$ against the saturated calomel electrode = − 1·28 volts.

SUMMARY

1. The acetylation of 6-aminopurine derivatives were followed polarographically since two reduction waves were produced in distinction to the parent compound which gave but one. That the first of the two waves is due to the presence of the acetyl group was shown by its disappearance when this group was hydrolysed. Confirmation was also obtained by following the changes in ultra-violet absorption.

2. A quantitative study was made on acetyladenine and the reduction step with the more positive half-wave potential was determined in 50 per cent. aqueous ethanol made millimolar with perchloric acid, in which medium it was stabilised.

3. The position and nature of a functional group on the purine ring was found to be a critical factor for polarographic activity. Examples are given which show that the reduction waves obtained were associated with the amino group of its *N*-acetyl derivative in the 6-position of the purine ring.

4. Nucleic acid gave a well-defined polarographic step in 0·2M potassium chloride. Since of the isolated nucleotides only adenylic acid (or its derivatives) were reducible at the dropping mercury electrode, it was inferred that the adenine moiety was involved, a fact which may prove useful in biological investigations.

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CHRONIC TOXICITY STUDIES ON FOOD COLOURS

PART II. OBSERVATIONS ON THE TOXICITY OF FD&C GREEN NO. 2 (LIGHT GREEN SF YELLOWISH), FD&C ORANGE NO. 2 (ORANGE SS) AND FD&C RED NO. 32 (OIL RED XO) IN RATS

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THIS paper describes further studies on the chronic toxicity of food colours. In Part I of this series some chronic effects of Oil Yellow AB and OB in rats were reported[1]. It has previously been reported by other workers that some colours belonging to the azo and triphenylmethane classes caused tumours to develop in animals after subcutaneous injections[2,3]. As some of these colours are being used in food it was thought worthwhile to examine them for chronic oral effects in rats. The effects of the oral administration of FD&C Green No. 2, FD&C Orange No. 2 and FD&C Red No. 32 on growth, food consumption, food efficiency, blood hæmoglobin, and on the pathology of a number of organs are presented in this paper.

METHODS

The methods employed were similar to those reported for the two yellow colours[1]. The food colours were incorporated in the laboratory diet in the following concentrations: FD&C Green No. 2, 0·03, 1·5 and 3·0 per cent; FD&C Orange No. 2 and FD&C Red No. 32, 0·03, 0·75 and 1·5 per cent. The rats were approximately five to six weeks of age at the start of the experiment. The animals were kept in groups of two to a cage and were given free access to their respective diets and water. Their body weight and food consumption were recorded weekly. For more accurate evaluation of food consumption it would have been preferable to place only one rat in a cage, but this was not possible. Post-mortem examinations were made where possible on rats which died on test. All surviving rats were killed at the end of the experiments and post-mortem examinations were made. Many of the organs were weighed and prepared for histological examination. As a result of an unfortunate accident at the end of the fourth week to the male rats on a dietary concentration of 1·5 per cent. FD&C Green No. 2, data on these rats are not included.

RESULTS AND DISCUSSION

The Effect on Growth Rate, Food Consumption and Food Efficiency

Growth, food consumption and food efficiency curves for the groups receiving the various dietary concentrations of the three colours are shown in Figures 1 and 2. Growth rate, food consumption and food

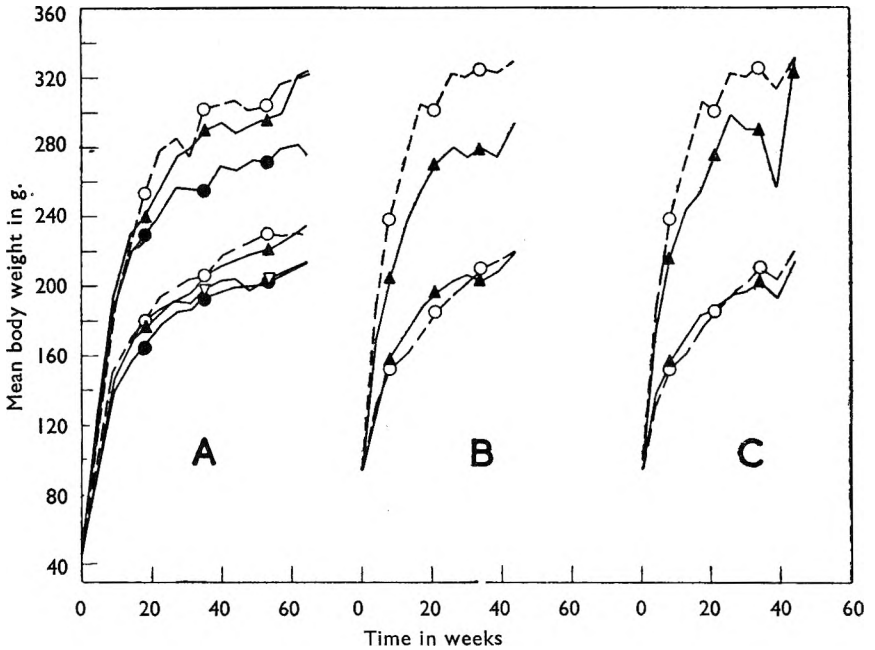

FIG. 1. Growth rate curves for control rats and those receiving the colours. Lower curves are for female rats and upper curves for male rats.

A. FD&C Green No. 2: B. FD&C Orange No. 2: FD&C Red No. 32

○ control; ▲ 0·03 per cent.; ▽ 1·5 per cent.; ● 3·0 per cent.

efficiency were not significantly affected by any of the three colours at the 0·03 per cent dietary level. However, for FD&C Red No. 32 and FD&C Orange No. 2 at the higher concentrations of 0·75 and 1·5 per cent. of the diet, growth rate, food consumption and food efficiency were noticeably affected and all rats in these two groups died before completion of the experiment. For FD&C Green No. 2, two dietary levels, 0·03 and 1·5 per cent. had no effect on growth rate, food consumption or food efficiency, but the 3·0 per cent. level noticeably affected growth rate. This may have been partly due to the amount of food consumed, which was somewhat reduced on this dietary amount. Daily doses of 200 mg./kg. and 400 mg./kg. of FD&C Red No. 32 and FD&C Orange No. 2 for 20 weeks to rats affected growth, food consumption and food efficiency, as shown in Tables I and IV.

The Effect of Mortality

At the end of the test period (65 weeks) the mortality of rats on FD&C Green No. 2 ranged from 52 to 68 per cent. for the respective groups, as shown in Table II. The control group mortality was 68 per cent. The mortality for the respective groups on dietary concentrations of FD&C Green No. 2 was not significantly different from the control. For the other two colours there was 100 per cent. mortality at the 0·75 and

TABLE I

SUMMARY OF DATA ON MORTALITY, FOOD CONSUMPTION AND FOOD EFFICIENCY WHEN FOOD COLOURS WERE GIVEN BY STOMACH TUBE FOR 20 WEEKS

Dose	Sex	No. rats on test	Mortality	Food consumption g./rat/day	Food efficiency g. gain/g. food consumed × 100
Control	M	10	0	13·2	5·2
	F	10	0	11·1	4·7
200 mg./kg./orally/daily ..	M	10	6	12·5	2·9
FD&C Red No. 2 ..	F	10	0	12·7	3·1
200 mg./kg./orally/daily ..	M	10	6	13·9	3·3
FD&C Orange No. 2 ..	F	10	2	10·3	4·5
400 mg./kg./orally/daily ..	M	10	5	12·1	1·7
FD&C Red No. 32 ..	F	10	3	10·1	1·8
400 mg./kg./orally/daily ..	M	10	6	10·3	1·9
FD&C Orange No. 2 ..	F	10	6	10·0	0·6

1·5 per cent. levels by the time the experiment was ended as shown in Table III. By the end of 20 weeks all the rats on the 1·5 per cent. level of FD&C Red No. 32 had died, and at the end of 40 weeks all the rats on the 0·75 per cent. level had died. It was not possible to make autopsies on all the animals which died during the experiment, but the tissues and organs of many of those dying on the 0·75 and 1·5 per cent. dosage were stained with the colours. The kidneys were soft, dark and swollen. The spleen was enlarged and dark in colour. The picture was one of acute toxæmia.

TABLE II

CUMULATIVE NUMBER OF DEATHS

Concentration of colour in diet	Sex	No. Rats on test	Time in weeks on test														
			1	3	5	10	15	20	25	30	35	40	45	50	55	60	65
FD&C Green No. 2																	
Control	M	25	1	6	7	8	10	11	12	13	14	14	14	14	14	16	17
	F	25	0	1	1	2	3	3	3	6	6	7	8	8	8	8	10
0·03 per cent.	M	25	0	0	0	1	1	2	4	8	8	8	8	9	11	13	14
	F	25	0	0	0	0	1	4	4	5	6	7	9	11	12	13	13
1·5 per cent.	F	25	0	0	0	0	1	1	2	2	2	3	5	7	13	13	14
3·0 per cent.	M	25	0	0	2	3	3	3	3	4	10	10	11	11	12	13	16
	F	25	0	0	0	2	2	3	4	6	8	8	9	10	10	14	14

The Effect on Organ Weights

Organs of surviving rats were weighed at the termination of the test. The mean weights (in mg./g. of body weight) are shown in Table IV. The mean weights of a number of organs deviated significantly from those of corresponding controls. Heart, liver, spleen, kidneys and testes were the organs chiefly affected. In very few instances where increases or decreases in organ weights occurred was it possible to demonstrate pathological changes; the changes demonstrated in the testes are an exception. In a number of cases the organ weights were about the

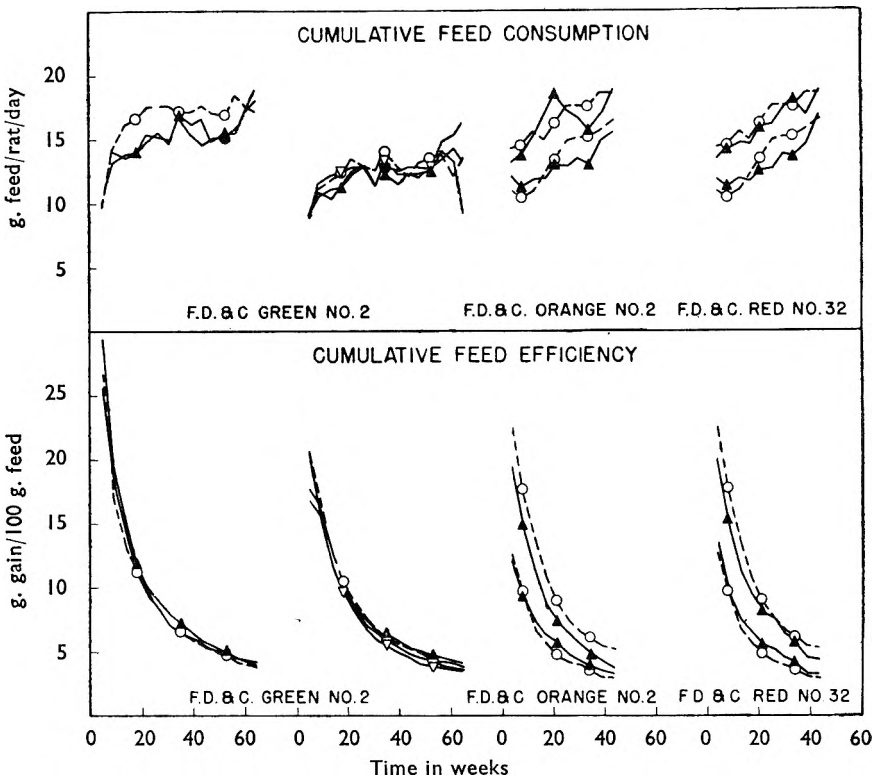

FIG. 2. Food consumption and food efficiency curves for control and test rats, male and female. For FD&C Green No. 2, values for males are shown on the left. For the other two colours, the upper curves represent male rats and the lower curves female rats.

○ Control; ▲ 0·03 per cent.; ▽ 1·5 per cent.; ● 3·0 per cent.

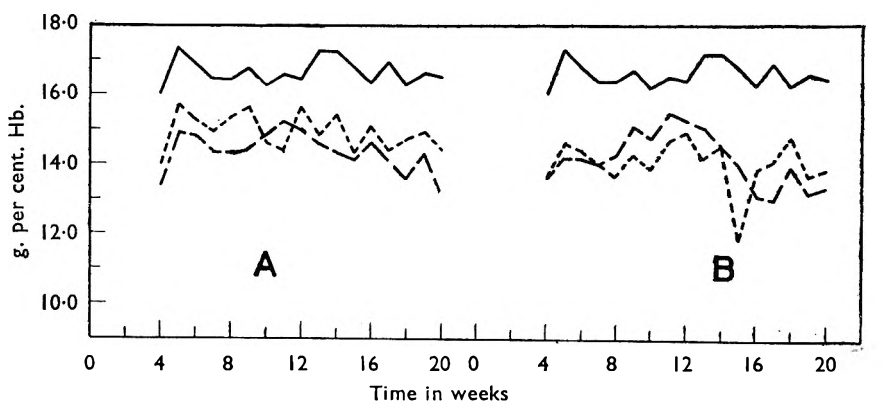

FIG. 3. Combined results of hæmoglobin determinations on both sexes of control rats and those given the two colours at different doses. A. F.D.&C. Red No. 32. B. F.D.&C. Orange No. 2.

— Control: - - - 200 mg./kg./rat, oral: — — 400 mg./kg./rat oral.

same as the controls but the body weights of the test animals were less than the controls, suggesting the possible utilization of muscle protein. In other cases the body weights of test and control animals were about the same but the organ weights differed significantly. The changes observed in the organs in these cases appeared like compensatory changes. These results do not demonstrate a correlation of organ weight and the histopathological changes except with the testes.

TABLE III

CUMULATIVE NUMBER OF DEATHS

Concentration of colour in diet	Sex	No. Rats on test	Time in weeks on test											
			2	4	8	12	16	20	24	28	32	36	40	44
FD&C Red No. 32														
Control	M	20	0	2	3	4	5	7	10	11	11	12	13	13
	F	20	0	0	2	2	3	6	7	7	7	7	7	7
0·03 per cent.	M	20	0	0	2	3	3	7	9	9	9	9	16	18
	F	20	0	0	1	1	5	5	6	6	6	6	8	9
0·75 per cent.	M	20	2	3	4	11	15	15	15	16	17	19	20	
	F	20	0	0	0	9	11	13	15	15	15	16	20	
1·5 per cent.	M	20	2	8	11	17	19	20						
	F	20	0	2	10	18	19	20						
FD&C Orange No. 2														
Control	M	20	0	2	3	4	5	7	10	11	11	12	13	13
	F	20	0	0	2	2	3	6	7	7	7	7	7	7
0·03 per cent.	M	20	0	0	1	2	4	6	6	7	8	9	13	13
	F	20	0	0	0	0	1	2	3	4	5	6	7	7
0·75 per cent.	M	20	2	5	10	17	20							
	F	20	3	6	10	17	19	20						
1·5 per cent.	M	20	3	12	17	20								
	F	20	4	10	16	20								

Hæmatology

Hæmoglobin determinations were made weekly for 20 weeks on groups of male and female rats, 10 rats to a group, given daily oral doses of 200 mg./kg. and 400 mg./kg. respectively of FD&C Red No. 32 and FD&C Orange No. 2. A slight modification of the pyridine-hæmochromogen method of Rimington was used[4]. The combined results of these determinations on both sexes are shown in Figure 3, and the mean values of the final determinations are shown in Table IV. The combined blood hæmoglobin values for both sexes show a significant decline in all groups on both colours. This trend was also evident from an examination of the data obtained on each sex.

Blood hæmoglobin values were also determined on the surviving rats from the other experiments. These values, also shown in Table IV, were about the same as those for the controls.

Histopathology

A detailed examination was made of the hæmatoxylin-eosin stained paraffin sections of a number of organs including lung, heart, liver,

TABLE IV

COMPREHENSIVE SUMMARY OF OBSERVATIONS ON RATS FED FD&C RED NO. 32, FD&C ORANGE NO. 2 AND FD&C GREEN NO. 2

Product	Dosage	No. weeks on test	No. rats surviving / No. rats on test	Mean body weight g. ± s.e.		Mean Hg (g. per cent.) ± s.e.†	Mean organ weight, mg./g. rat ± s.e.					
				Initial	Final		Heart	Liver	Kidneys	Adrenals	Spleen	Testicles
Males												
Control		44	7/20	97·7±5·1	329·1±26·3	15·8±0·29	3·3±0·08	28·6±1·57	6·4±0·07	0·08±0·007	2·4±0·13	8·8±0·35
FD&C Red No. 32	0·03 per cent. of diet	44	2/20	98·4±6·3	330·0± 6·0	16·1±0·75	3·6±0·05	37·0±4·45	7·0±1·20	0·09±0·007	2·6±0·15	8·9±0·73
FD&C Orange No. 2	0·03 per cent. of diet	44	7/20	94·1±5·8	269·4±24·9	15·3±0·50	3·4±0·03	32·5±3·31	7·2±0·13	0·11±0·013	2·4±0·22	8·4±0·63
Control		20	4/10	118·7±5·7	251·0±11·0	17·3±0·40	3·6±0·05	34·9±1·55	7·6±0·25	0·09±0·005	2·5±0·28	9·7±0·38
FD&C Red No. 32	200 mg./kg./day	20	4/10	113·1±4·8	155·0±18·9*	14·1±0·81*	4·6±0·35*	38·2±0·95	9·5±1·55	0·15±0·038*	2·9±0·60	9·6±1·32
FD&C Red No. 32	400 mg./kg./day	20	5/10	115·7±6·4	160·2±14·0*	12·8±1·50*	4·7±0·38*	53·1±2·96*	9·6±0·71*	0·13±0·014*	4·5±0·44*	9·6±1·65
FD&C Orange No. 2	200 mg./kg./day	20	4/10	115·9±4·0	200·0±18·6*	14·1±0·40*	4·1±0·32	44·4±4·46	8·8±0·73	0·12±0·014	4·5±0·52*	11·0±0·70
FD&C Orange No. 2	400 mg./kg./day	20	4/10	110·7±4·0	152·8± 8·6*	13·8±0·64*	4·2±0·18*	44·8±1·59*	8·9±0·49	0·12±0·006	5·0±0·14*	12·9±0·47*
Control		65	8/25	45·6±2·7	323·4±20·0	17·2±0·35	3·2±0·11	30·3±1·01	6·2±0·19	0·08±0·008	2·7±0·26	8·6±0·40
FD&C Orange No. 2	0·03 per cent. of diet	65	11/25	45·5±2·7	323·8±10·9	16·6±0·19	3·4±0·07	32·6±0·82	6·1±0·12	0·08±0·004	2·6±0·11	6·5±0·38
FD&C Green No. 2	3·0 per cent. of diet	65	9/25	45·4±2·7	274·0±12·5*	16·0±0·25*	3·4±0·10	34·9±2·14	6·9±0·38	0·09±0·005	2·3±0·15	5·0±0·69*
Females												
Control		44	12/20	91·7±3·5	219·8± 4·9	15·9±0·22	4·1±0·06	35·7±0·80	7·4±0·21	0·21±0·007	3·4±0·36	
FD&C No. 32	0·03 per cent. of diet	44	11/20	91·8±2·7	214·1± 2·9	14·9±0·66	4·2±0·15	39·2±1·38*	8·1±0·27	0·23±0·010	3·9±0·64	
FD&C Orange No. 2	0·03 per cent. of diet	44	11/20	95·1±3·4	219·4± 5·8	15·6±0·22	4·2±0·13	37·8±1·36	7·8±0·18	0·24±0·011	3·6±0·15	
Control		20	9/10	93·0±3·9	166·9± 6·5	15·7±0·65	4·5±0·41	37·5±1·38	8·2±0·45	0·26±0·031	3·1±0·33	
FD&C Red No. 32	200 mg./kg./day	20	10/10	92·3±4·0	147·6± 5·8*	14·7±0·23	4·5±0·18	43·0±1·73	8·7±0·34	0·20±0·004	4·3±0·15*	
FD&C Red No. 32	400 mg./kg./day	20	7/10	91·6±3·5	124·9± 4·6*	13·4±0·45*	5·0±0·18	51·8±1·92*	9·6±0·14	0·16±0·007*	6·5±2·05	
FD&C Orange No. 2	200 mg./kg./day	20	8/10	88·8±3·3	153·6± 3·1	13·7±0·19*	4·3±0·07	40·2±1·34	7·8±0·30	0·17±0·011	5·6±0·27*	
FD&C Orange No. 2	400 mg./kg./day	20	4/10	89·9±4·0	102·8±11·2*	12·9±1·37*	5·0±0·31	55·1±5·14*	9·9±0·43	0·20±0·022	5·3±0·73*	
Control		65	15/25	39·6±2·4	227·9± 3·9	16·0±0·19	4·1±0·07	35·4±0·84	7·0±0·18	0·22±0·008	3·6±0·13	
FD&C Green No. 2	0·03 per cent. of diet	65	11/25	39·4±2·4	236·7± 6·9	16·4±0·30	3·9±0·09	31·9±1·05*	6·6±0·21	0·21±0·011	3·2±0·06*	
FD&C Green No. 2	1·5 per cent. of diet	65	11/25	39·6±2·3	213·6± 8·7	16·1±0·33	3·6±0·06*	24·4±0·39*	6·1±0·13*	0·16±0·017*	2·7±0·20*	
FD&C Green No. 2	3·0 per cent. of diet	65	11/25	39·7±2·4	210·6± 7·3*	16·0±0·21	4·3±0·12	34·9±1·16	7·3±0·17	0·19±0·005*	3·6±0·22	

* significant at P = 0·05 † determination on 5 rats

TABLE V

Summary of Histopathological Findings

	Control	Control	FD&C Green No. 2	FD&C Orange No. 2	FD&C Red No. 32	Control	Control	FD&C Green No. 2	FD&C Orange No. 2	FD&C Red No. 32	Control	FD&C Green No. 2	Control	FD&C Green No. 2	Control	FD&C Green No. 2	Control	FD&C Orange No. 2	FD&C Red No. 32	Control	FD&C Orange No. 2	FD&C Red No. 32	Control	FD&C Orange No. 2	FD&C Red No. 32	Control	FD&C Orange No. 2	FD&C Red No. 32
Sex	Male					Female					Female		Male		Female		Male			Female			Male			Female		
Dose	0·03 per cent. in diet					0·03 per cent. in diet					1·5 per cent. in diet		3·0 per cent. in diet		3·0 per cent. in diet		200 mg./kg./day			200 mg./kg./day			400 mg./kg./day			400 mg./kg./day		
Number of rats on test	25	20	25	20	20	25	20	25	20	20	25	25	25	25	25	25	10	10	10	10	10	10	10	10	10	10	10	10
Number of survivors	8	7	11	7	2	15	7	12	13	11	15	11	8	9	15	11	4	4	5	9	8	10	4	4	5	9	4	7
Number of rats examined	5	5	6	7	2	4	7	6	11	10	4	6	5	8	4	6	4	4	4	6	8	10	4	4	5	6	4	6
Duration of test-weeks	65	44	65	44	44	65	44	65	44	44	65	65	65	65	65	65	20	20	20	20	20	20	20	20	20	20	20	20
Bladder parasites			3	4	1	1		2	2	3		2		3		1												
Hydronephrosis						2		1	1	1						2					1							
Chronic glomerulonephritis						1															1							
Pyelonephritis																										1		
Interstitial nephritis																										1		
Altered spermatogenesis				1										7										1	3			1
Necrotic casts in testes tubules														6														
Ovary fibroma									1																			
Ovary cystadenoma									1																			
Perioophoritis																1												
Liver necrosis														1														
Lung papilloma																										1		
Purulent pericarditis																										1		1
Laryngeal abscess																1												
Acute tracheitis							1																					

spleen, thyroid, pancreas, stomach, small intestine, kidney, urinary bladder, adrenal, testes, ovaries and thymus. A summary of the findings is given in Table V. There were no consistent histopathological changes observed in the tissues or organs studied that could be attributed to the toxic effects of the colours with the possible exception of testicular change which was observed in animals on the higher dietary concentrations. These findings were particularly noticeable in rats on dietary levels of 3·0 per cent. FD&C Green No. 2 and in those receiving daily oral dosage of 400 mg./kg. of FD&C Red No. 32. On the 3·0 per cent. level of the green colour testicular change was a constant finding. In seven out of eight animals there was tubular atrophy and incomplete spermatogenesis. The change, although constant, was variable in degree. In some testes a few tubules showed complete absence of spermatogenic cells and in others the spermatogenic cells were greatly reduced. Several tubules in each testis contained deep-blue-staining caseous necrotic casts. Remnants of sperm were present in the necrotic debris. The spermatogenic cells and the supporting network of the tubules were undergoing varying degrees of degenerative changes. In some tubules it was apparent that the necrotic casts were being formed from the degenerating cells.

SUMMARY

1. FD&C Orange No. 2, FD&C Red No. 32 and FD&C Green No. 2 in concentrations of 0·03 per cent. in the diet did not affect growth, food consumption or food efficiency in either male or female rats.

2. In groups receiving FD&C Orange No. 2 and FD&C Red No. 32 in concentrations of 0·75 and 1·5 per cent. in the diet, there was 100 per cent. mortality before the completion of the experiment.

3. FD&C Green No. 2 in a concentration of 3·0 per cent. in the diet adversely affected the growth rate which may have been due in part to the amount of food consumed. At the 1·5 per cent. concentration in the diet of female rats no effect on growth rate, food consumption or food efficiency was observed.

4. Hæmoglobin production was not affected by 0·03 per cent. in the diet of either FD&C Orange No. 2 or FD&C Red No. 32, but oral doses of 200 and 400 mg./kg. of these two colours caused a decline in hæmoglobin values which was significant in both sexes at 20 weeks. Hæmoglobin values were not affected by any of the dosage levels of FD&C Green No. 2.

5. The only significant pathology was found in the testes of those rats receiving a dietary level of 3·0 per cent. of FD&C Green No. 2, or an oral dosage of 400 mg./kg. of FD&C Red No. 32.

The authors wish to acknowledge the technical assistance of Miss Elizabeth Carmichael, Miss Elaine Connell and Miss Rita Carioto.

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BACTERICIDAL ACTIVITIES OF SOAP-PHENOL MIXTURES*

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The modification of bactericidal activities of phenols when dissolved in varying concentrations of soaps has been the subject of several communications during the past few years. These reports differ in the postulated mode of action of the soap, and the experimental data are conflicting. The present investigations were undertaken in an attempt to relate the previous findings.

Agar and Alexander[1] and Alexander and Tomlinson[2] measured the extinction times of *Bacterium coli* when exposed to phenol and chlorinated phenols dissolved in aqueous solutions of anionic and cationic surface-active agents. In all cases, the extinction times were minimal at the critical micellar concentration of the surface-active agent. When the phenol concentration was kept constant and the concentration of surface-active agent varied, extinction times rapidly increased at concentrations either below or in excess of the critical micellar concentration of the surface-active agent. The concentration exponent of the phenol-soap mixture was found to remain virtually constant and Alexander and Tomlinson were led to postulate that, as the soap concentration was increased beyond the critical concentration, the extinction times of the mixtures would increase until a toxic concentration of the surface-active agent was reached, the only activity remaining being due to the surface-active agent alone.

Enhanced activity when the soap concentration was increased to the critical concentration was ascribed to the formation of an interfacial "complex" at the bacterium-water interface, the effect being similar to that observed by Alexander and Trim[3] in the anthelmintic activity of hexyl-resorcinol-soap mixtures. At soap concentrations in excess of the critical, they considered, the phenol passed into the micelles. Reduction in bactericidal activity was therefore due to a phenol depletion of the aqueous phase. At very high concentrations of soap, nearly all of the phenol would be dissolved in the micellar phase and any activity of the mixture would then reside only in the activity of the soap itself.

The studies undertaken by Bean and Berry[4,5] employed two phenols of lower water solubility than those investigated by Alexander and his colleagues. They used only one soap, potassium laurate, and they used mixtures which contained not a constant phenol concentration, but a constant phenol/soap ratio.

In agreement with the findings of Alexander and Tomlinson, Bean and Berry observed that increases in soap concentration up to the critical

* This account formed part of a thesis submitted by one of us (B.A.W.) for the degree of Ph.D. in the University of London.

micellar concentration resulted in increased bactericidal activity. But as soap concentrations increased in excess of the critical, the bactericidal activity at first decreased sharply and then increased. The soap concentration of minimal activity was also that at which the relative amount of solubilisation of the phenol began to decrease. The activities of mixtures containing micellar concentrations of soap were explained in terms of saturation of the micelles with the phenol, in contrast to Alexander's explanation in terms of "free" phenol concentration in the aqueous phase.

It is difficult to correlate the findings of both groups. The phenol concentrations used were different and the soaps employed were of differing bactericidal activity: the potassium laurate used by Bean and Berry was much more toxic than the aerosol MA and aerosol OT used by Alexander and his colleagues. In the present communication one soap, potassium laurate, and three phenols of widely differing solubility were used. Mixtures containing a constant phenol concentration and mixtures containing a constant phenol/soap molar ratio were separately studied.

Experimental Details

Materials. The soap solutions were prepared from a lauric acid of high purity (m.pt. 42·5–43·5° C.; acid value 279; iodine value, nil). 0·5M solutions of potassium laurate were adjusted to pH 9·6–9·8 and stored under nitrogen until required for use. The phenols used were 4-benzylphenol (m.pt. 83·5–84·0° C.; soluble in 6400 parts of water at 20° C.); 2-hydroxydiphenyl (m.pt. 56–57° C.; soluble in 1400 parts of water at 20° C.); and phenol (analytical reagent quality). All soap-phenol mixtures were prepared with water free from carbon dioxide and were stored under nitrogen.

Test Organism. Bacterium coli (*Escherichia coli*), laboratory strain, type I, 44° C.-positive, formerly N.C.T.C. No. 5933.

Media. The medium used in the determination of extinction times was of the same composition as that employed by Berry and Bean[6]. The solid medium used for cultivation of the test organisms contained 1 per cent. "Oxoid" peptone and 0·5 per cent. sodium chloride, solidified with 2 per cent. Davis bacteriological agar, and was adjusted to pH 7·2.

Experimental Technique. This closely followed the extinction method described by Berry and Bean[6]. The test suspension was adjusted to a density of 2×10^9 organisms per ml., and the inoculum consisted of 10 drops of this suspension, delivered with the improved dropping pipette described by Cook and Yousef[7] and Cook[8]. An experiment consisted of between 15 and 20 replicate determinations, each at 8 or 9 different contact times. The results were analysed by the loglog analysis of Mather[9] as described by Cook and Wills[10]. In a trial series of experiments, the extinction times to varying concentrations of potassium laurate were determined. The first and second calculated approximations to the loglog regression gave estimates with limits of error within which the visual estimate of the mean single survivor time easily lay. Hence, all

subsequent estimations of extinction times of soap-phenol mixtures could be made visually from the regression line best fitted by inspection.

Determinations of extinction times to mixtures containing 2-hydroxydiphenyl or 4-benzylphenol were made at 20° C. Mixtures of phenol with potassium laurate developed so heavy a turbidity due to hydrolysis of the soap that determinations could be undertaken only at a higher temperature.

The Bactericidal Activities of Solutions of Potassium Laurate

In order to assess the contribution of the soap alone to the bactericidal activities of soap-phenol mixtures, determinations of extinction times of *Bact. coli* on exposure to solutions of potassium laurate were made over a wide range of concentration. The effect of varying the pH of the soap solutions was also studied. Figure 1 shows the relation between logarithms of extinction times and logarithms of potassium laurate concentrations. The four relations, A, B, C, and D, were obtained from the use of stock 0·5M soap solutions of pH 10·4, 9·9, 9·7 and 9·7 respectively. Batches C and D are represented by a common regression. Curve E of Figure 1 was obtained from determinations with batch D of soap solution at 25° C.

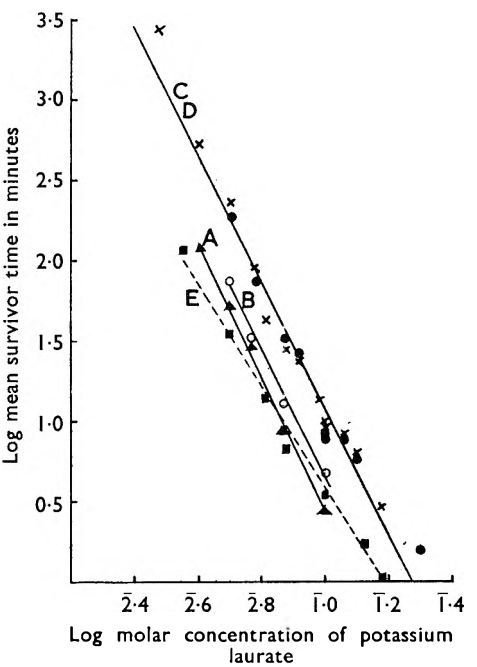

Fig. 1. The relationship between extinction times of *Bacterium coli* and concentration of potassium laurate. Curves A, (▲——▲), B (○——○), C (×——×) and D (●——●) were obtained from determinations at 20° C., using batches of stock 0·5M soap solution of pH 10·4, 9·9, 9·7 and 9·7 respectively. Curve E (■——■) was obtained from determinations undertaken at 25° C., using batch D of stock soap solution.

It is clear that batches C and D had almost equal bactericidal activity; batch B, which was only 0·2 pH unit more alkaline in reaction, was much more toxic; and batch A was the most highly bactericidal. A further batch of stock solution adjusted to pH 9·4 could not be used for reliable bactericidal determinations because of the very pronounced hydrolysis on dilution. The precipitated hydrolysis products occluded dropping pipettes to such an extent that neither the rate of delivery nor the volumes delivered could be controlled.

Solubilities of Phenols in Aqueous Solutions of Potassium Laurate

The concentrations of the phenols present in phenol-saturated solutions of varying potassium laurate concentration were determined spectrophotometrically. All determinations were performed in duplicate. 100 ml. of each concentration of potassium laurate solution was prepared with water free from carbon dioxide. Half of the solution was placed in each of two 60 ml. glass-stoppered bottles. An excess of the phenol was added to each bottle, which was heated in a boiling water bath with frequent vigorous shaking. The bottles were removed after 15 minutes, and placed in a water bath maintained at 20° C. for at least 48 hours.

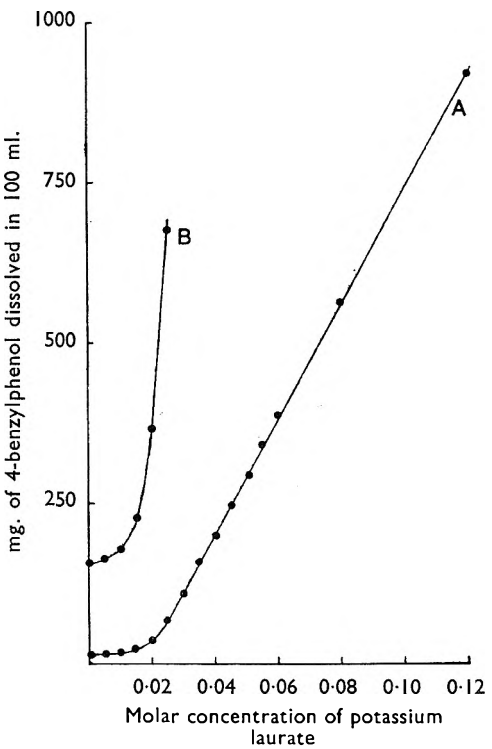

FIG. 2. The solubility of 4-benzylphenol in aqueous solutions of potassium laurate at 20° C. Curve B, for which the values of ordinates have been divided by 10, is an enlargement of curve A.

The contents of each bottle were separately filtered and an aliquot suitably diluted so that the final dilution for estimation contained between 50 and 100 mg. per litre of the phenol, together with 50 per cent. ethanol and 0·05N hydrochloric acid. The E (1 per cent. 1 cm.) of 4-benzylphenol at 277 mμ was determined from eight separately prepared solutions over a tenfold range of known concentration as $89{\cdot}65 \pm 0{\cdot}84$ ($P = 0{\cdot}99$) with coefficient of variation of 0·756. Similarly 2-hydroxydiphenyl gave a value for E (1 per cent. 1 cm.) of $612{\cdot}5 \pm 4{\cdot}8$ ($P = 0{\cdot}99$) with a coefficient of variation of 0·629 at 245 mμ.

The saturation weights of 4-benzylphenol are shown plotted against potassium laurate concentrations in Figure 2. It is seen that up to a soap concentration of 0·015M, very little more of the phenol was dissolved than in water alone. Over a concentration range exceeding 0·02M, however, the weight of the phenol dissolved increased linearly with increasing soap concentration. A change in solubilising properties occurred between 0·015M and 0·020M, the increased solubility being due to the presence of micelles at that concentration. A small increase in solubility occurred over the premicellar range of concentration, similar

behaviour to which was reported by Heller and Klevens[11] for the solubility of ethylbenzene in potassium laurate solutions.

Figure 3 shows the saturation phenol/soap molar ratios, i.e., saturation molar concentration of 4-benzylphenol/molar concentration of potassium laurate, plotted against potassium laurate concentrations. The shape of the curve, at concentrations in excess of the critical, is in general agreement with those of other workers who used a variety of solutes and soap: Hartley[12], McBain and Johnson[13], Bean and Berry[14]. At concentrations below 0·015M, however, the saturation molar ratio again increased, since the weight of phenol solubilised decreased only slightly with decreasing soap concentration over the premicellar range. All previous illustrations of solubility in soap solutions, save that of Heller and Klevens[11], have failed to show this initial fall in the curve, either because the solute employed had an extremely low water solubility or because determinations at soap concentrations below the critical were not undertaken. The ethylbenzene employed by Heller and Klevens was of comparable solubility in water to 4-benzylphenol. Figure 3 also shows saturation 2-hydroxydiphenyl/potassium laurate molar ratios plotted against potassium laurate concentrations. The relationship is generally similar to that obtained for 4-benzylphenol.

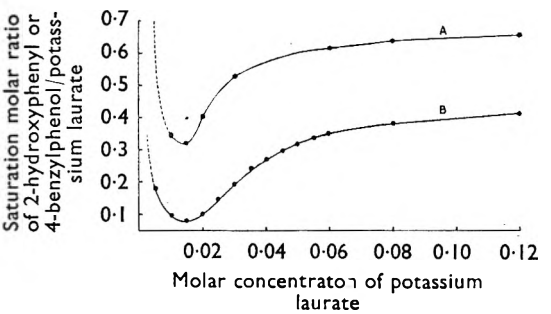

FIG. 3. The solubilities of 2-hydroxydiphenyl (A) and 4-benzylphenol (B) in aqueous solutions of potassium laurate at 20° C. Solubilities are expressed as saturation 4-benzylphenol or 2-hydroxydiphenyl/potassium laurate molar ratios.

THE BACTERICIDAL ACTIVITY OF 2-HYDROXYDIPHENYL IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE

Extinction times were determined when (*a*) a constant concentration of the phenol was maintained with variation of soap concentration, after the scheme of Alexander and Tomlinson[2]; (*b*) with a constant phenol to soap molar ratio with varying soap concentrations; (*c*) with varying phenol concentrations but constant soap concentration, as was adopted by Bean and Berry[4,5]. In addition, it was necessary to determine the bactericidal activity of the phenol in aqueous solutions without soap.

Regression line A of Figure 4 was obtained by plotting logarithms of mean single survivor times against logarithms of 2-hydroxydiphenyl concentrations in solutions containing no soap. Curves B, C, D, E and F in the same graph were obtained from the use of test solutions of varied phenol content and of potassium laurate molar concentrations 0·010, 0·015, 0·020, 0·030 and 0·040 respectively. It can be seen that the presence of soap greatly enhanced the bactericidal activity of the phenol with all

the concentrations of phenol and of soap which were studied. The batch of soap solution used in these determinations (batch B), gave an extinction time of 76 minutes for a 0·05M solution, so that here the soap alone did not exert any appreciable bactericidal effect.

Curve A of Figure 5 represents the relation between logarithms of extinction times and concentrations of potassium laurate for soap-phenol

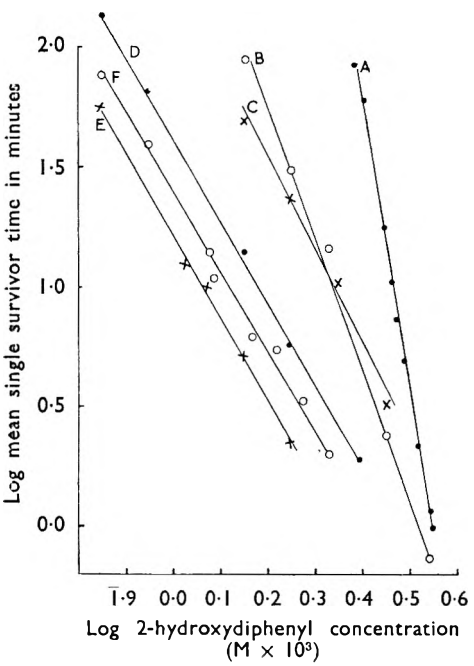

FIG. 4. The bactericidal activity of 2-hydroxydiphenyl against *Bacterium coli* at 20° C. in aqueous solution and in aqueous solutions of potassium laurate. Logarithmic relationships between concentrations of 2-hydroxydiphenyl and extinction times of *Bact. coli*, using five concentrations of potassium laurate: 0·01M (curve B ○——○), 0·015M (curve C ×——×), 0·02M (curve D ●——●), 0·03M (curve E ×——×) and 0·04M (curve F ○——○). Curve A (●——●) represents the activities of solutions of 2-hydroxydiphenyl containing no added soap.

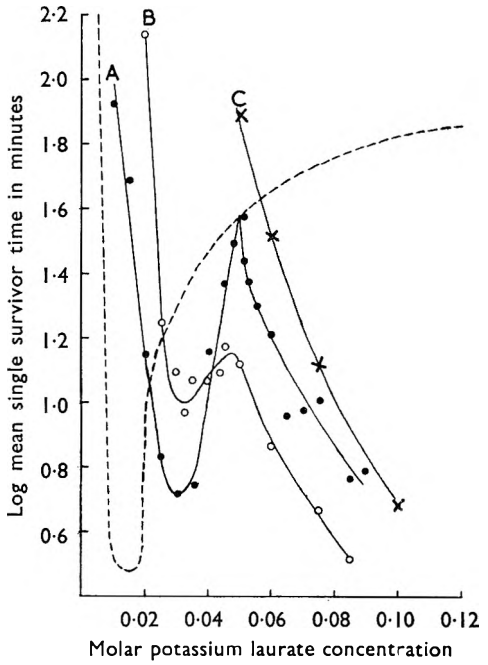

FIG. 5. The bactericidal activities of 2-hydroxydiphenyl-potassium laurate mixtures against *Bacterium coli* at 20° C. Curve A (●——●): mixtures containing a constant phenol concentration of 0·00141M. Curve B (○——○): mixtures containing a constant 2-hydroxydiphenyl/potassium laurate molar ratio of 0·0353. Curve C (×——×): solutions of potassium laurate (batch B) containing no phenol. The broken curve represents the solubility of 2-hydroxydiphenyl in potassium laurate solutions (see Fig. 3).

mixtures containing a constant 2-hydroxydiphenyl concentration of 0·00141M. Extinction times of mixtures containing a constant 2-hydroxydiphenyl/potassium laurate molar ratio of 0·0353 are represented by curve B of Figure 5, and extinction times of the soap alone are plotted logarithmically against soap concentration to give curve C. Curves A and B intersect at a potassium laurate concentration of 0·040M, the solutions represented by each curve possessing the same phenol concentration at this soap concentration.

The Bactericidal Activity of 4-Benzylphenol in Aqueous Solutions of Potassium Laurate

Saturated aqueous solutions of 4-benzylphenol at 20° C. were found to be devoid of measurable bactericidal activity. The scheme for the investigations undertaken with this phenol closely followed that employed with 2-hydroxydiphenyl. However, difficulties were encountered when a new batch of stock soap solution was introduced midway through the investigations.

Using the same batch of soap solution as that used in the investigations of 2-hydroxydiphenyl, two series of determinations were carried out in which the test bactericide contained constant 4-benzylphenol/potassium laurate molar ratios of 0·0326 and 0·0652. The results are shown as logarithms of extinction times plotted against potassium laurate concentrations (curves 1 and 2 respectively of Figure 6). A third series of determinations, using a phenol/soap molar ratio of 0·0489, yielded curve 3 of Figure 6. A new batch of stock 0·5M potassium laurate had to be used for this series, also for preparing test solutions containing a constant 4-benzylphenol concentration of 0·00163M, but varying concentrations of potassium laurate (curve 1, Fig. 7). The bactericidal activity of this second batch of soap solution (batch C) is represented by curve 5 in Figure 6 and curve 2 in Figure 7, and that of the first batch (batch B)—the values having been obtained shortly after preparation—is represented by curve 4 of Figure 6.

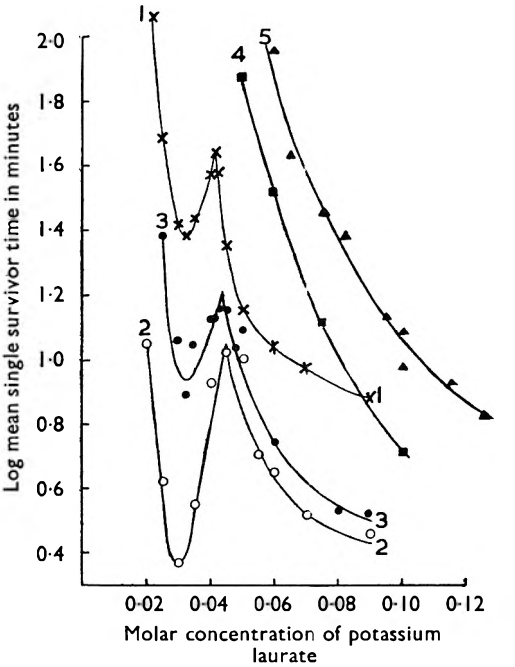

Fig. 6. The bactericidal activities of 4-benzylphenol-potassium laurate mixtures at 20° C. Curves 1 (×——×), 2 (○——○) and 3 (●——●) relate to mixtures containing constant 4-benzylphenol/potassium laurate molar ratios of 0·0326, 0·0652 and 0·0489 respectively. Curves 4 (■——■) and 5 (▲——▲) represent the bactericidal activities of batches B and C of potassium laurate solution in the absence of the phenol.

From the data represented in curves 1–3 of Figure 6 and curve 1 of Figure 7, eight potassium laurate concentrations could be found at each of which had been made four determinations of extinction times in the

presence of varying concentrations of 4-benzylphenol. Logarithms of extinction times are plotted against logarithms of 4-benzylphenol concentrations in Figures 8 and 9, the relations being shown on two graphs in order to avoid confusion in relating the plotted values to the appropriate regression lines. The correlations are poor because of the variations between the two batches of soap solution.

After completion of the work described above, when little of batch B of stock soap solution remained, its bactericidal activity was found to have declined: a concentration of 0·10M, which initially gave extinction after exposure for 4·8 minutes now required 26·3 minutes. No appreciable loss of activity on storage of further batches of potassium laurate solutions under a variety of conditions could be demonstrated.

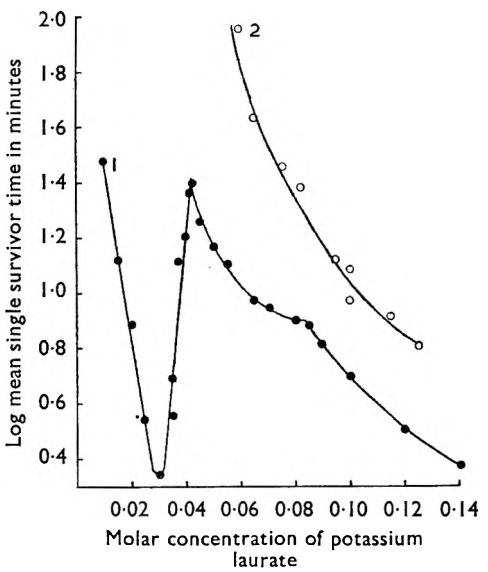

FIG. 7. The bactericidal activities of 4-benzylphenol-potassium laurate mixtures at 20° C. Curve 1 (●——●) relates to mixtures containing a constant 4-benzylphenol concentration of 0·00163M. Curve 2 (○——○) represents the bactericidal activity of batch C of potassium laurate solution in the absence of the phenol.

The influence of the variations in toxicity of soap solutions upon the activities of their mixtures with the phenol was investigated as follows. The bactericidal activities of solutions of the phenol prepared from four different batches of potassium laurate solution known to possess differing bactericidal activity were measured. Two soap concentrations were examined: 0·025M and 0·030 M. Two batches, B and C, had already been tested; the others had initial reactions of pH 10·4 and 9·4. The data were analysed by testing the adequacy of common slope and coincident regression line from a knowledge of the residual sums of squares of regressions separately fitted to each batch soap data and computing the residual sum of squares of a common regression. The analysis is described by Tippett[15]. It was found that the four relations could be regarded as being represented by a common coincident regression. Thus alteration in batch of 0·5M stock soap solution had so little effect on extinction times of 4-benzylphenol-soap mixtures, relative to variations between estimates of extinction times with individual batches of stock solution, that the differences between the stock solutions at the concentrations examined could be neglected.

The Bactericidal Activity of Phenol in Aqueous Solutions of Potassium Laurate

Logarithms of extinction times of *Bact. coli* when exposed to soap-phenol mixtures containing a constant phenol concentration of 0·0159M are shown plotted against potassium laurate concentrations in curve A of Figure 10. Curve C relates logarithms of extinction times to molar concentrations of potassium laurate in solutions containing no added phenol. Additional estimations were made with solutions of corresponding soap concentrations with and without phenol; a precaution which was adopted in the hope that day-to-day variations in extinction time estimates would not lead to the postulation of fallacious relationships between the soap-phenol and soap systems. Curve B of Figure 10 relates to mixtures containing a constant phenol/potassium laurate molar ratio of 0·399.

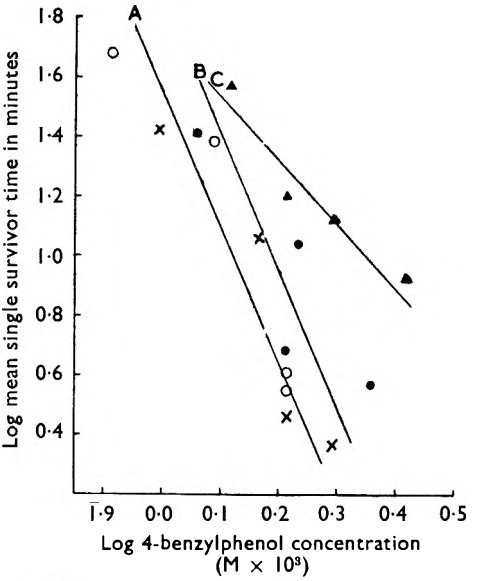

FIG. 8. The bactericidal activity of 4-benzylphenol in aqueous solutions of potassium laurate at 20° C. Curves A (○——○: ×——×), B (●——●) and C (▲——▲) represent phenol-soap mixtures containing 0·025, 0·030, 0·035 and 0·040M potassium laurate respectively.

Also, the phenol contents of the test solutions were varied at the following molar potassium laurate concentrations: 0·028, 0·0325, 0·040, 0·050 and 0·080. The observed extinction times are plotted logarithmically against phenol concentrations to give curves B, C, D, E, and F in Figure 11. Curve A in the same graph relates to solutions of phenol containing no added soap. As explained earlier, all determinations with phenol were made at a temperature of 25° C.

Discussion

Estimates of bactericidal activities of potassium laurate-phenol mixtures containing a *constant phenol concentration*, using three phenols of widely differing solubility, lead to the principal inference that, over the range of concentration studied, activity is governed by the existence of three soap concentrations of limiting activity.

The First Concentration of Limiting Activity

At a potassium laurate concentration of about 0·03M, the phenols exerted maximal bactericidal activity in soap solutions over a range 0 to 0·1M. This characteristic effect was observed with all phenols at all

concentrations and was probably independent of the initial pH of the stock soap solution from which the mixtures were prepared. This concentration of limiting activity must be identified with the critical concentration for the formation of micelles, and throughout the remainder of this discussion the critical concentration of potassium laurate will be taken to be 0·03M. As the soap concentration is increased to this point, the formation of an interfacial "complex" of soap and phenol appears to be responsible for the logarithmic increase in bactericidal activity (curve A, Fig. 5; curve 1, Fig. 7; curve A, Fig. 10). The adsorbed film may promote penetration of the phenol by supplying a high phenol concentration at the surface of the organism—considerably higher than would be found in the bulk solution. Adsorption of the soap may also cause a breakdown of the lipoid cell surface, so that phenol may more readily penetrate the cell, a view which has been put forward by Alexander[16]. Gale and Taylor[17] showed that a variety of surface-active agents and phenol separately bring about damage of the bacterial cell wall and it has been argued that soaps and phenols possess a synergistic action, the surface activity of the complex being greater than that of either soap or phenol alone.

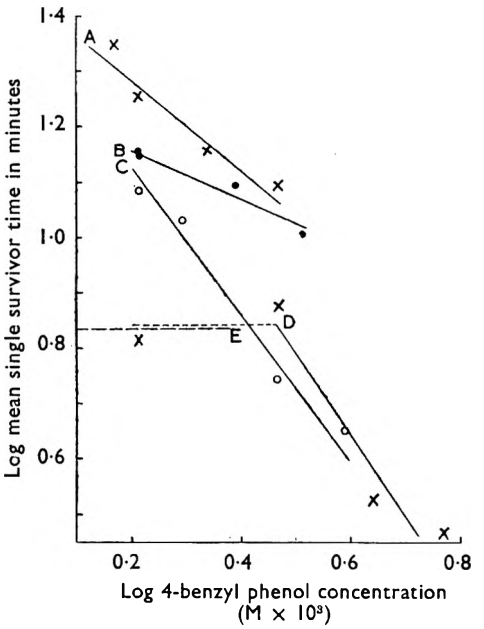

FIG. 9. The bactericidal activity of 4-benzylphenol in aqueous solutions of potassium laurate at 20° C.—continued. Curves A (×——×), B (●——●), C (○——○) and D (×——×) represent phenol-soap mixtures containing 0·045, 0·05, 0·06 and 0·09 M potassium laurate respectively. E represents the extinction time of 0·09M potassium laurate (batch B stock solution) in the absence of the phenol.

The Second Concentration of Limiting Activity

At a potassium laurate concentration of about 0·045M, all of the phenols exhibited a minimal bactericidal activity. The decrease in bactericidal activity at soap concentrations immediately in excess of the critical has been differently explained by Alexander and Tomlinson[2] and by Bean and Berry[4,5]. The presence of a second concentration of limiting activity, although found by Bean and Berry with their constant ratio mixtures, was not reported for mixtures containing a constant phenol concentration by Alexander and Tomlinson.

Extinction time determinations and counts of survivors were undertaken

by Alexander and Tomlinson at five concentrations of soap in excess of the critical concentration (1 per cent. Aerosol MA). The first three determinations showed no change from that at the critical, and the fourth determination, at 2 per cent. Aerosol MA, gave an increase in extinction time from 15 to 40 minutes. The last determination, at Aerosol concentration 4·5 per cent., gave an extinction time of over 480 minutes. The corresponding survivor counts gave an almost constant percentage of survivors (about 25 per cent.) after exposures of from 10 to 60 minutes, after which no further counts were made. Thus no extinction time or true index of activity was in fact determined at this soap concentration. Their soap, without addition of phenol, was almost equally toxic at the same concentration, judging from their recorded counts of survivors, so that one might expect that the extinction times of the mixtures would be falling with increase in soap concentration in this region.

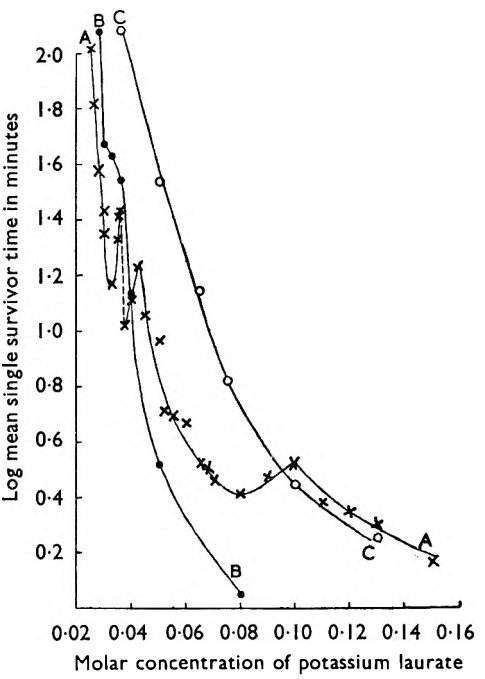

FIG. 10. The bactericidal activities of phenol-potassium laurate mixtures at 25° C. Curve A (×——×) relates to mixtures containing a constant phenol concentration of 0·0159M. Curve B (●——●) relates to mixtures containing a constant phenol/potassium laurate molar ratio of 0·399. The activity of the potassium laurate in absence of phenol at 25° C. is represented by curve C (○——○).

Our results (Figs. 5, 7, 10) cannot be fully explained by the interpretation of Bean and Berry without modification, for we have demonstrated a similar behaviour in solutions containing a constant concentration of the phenol, where the presence of more micelles with increasing soap concentration can result only in a phenol depletion of the aqueous phase and a reduced phenol concentration in the micelles. The second concentration of limiting activity, beyond which activity sharply increases, must be explained either as intervention of the bactericidal activity of the soap itself or as a sudden change in the mode of action of the soap-phenol mixture. Since soap solutions were used which differed considerably in bactericidal activity at 20° C., and determinations at 25° C. giving very much shorter extinction times were undertaken, while the concentration of minimal activity was not substantially changed, it is considered that the soap itself cannot be sufficiently toxic to account for the break in the curve.

That limiting activity was still observed at the same concentration when mixtures of varying toxicity were used, so that the extinction time of the mixture was either only a little shorter or very much shorter than that of the soap alone, may also be taken as an indication of independence of soap toxicity.

A sudden change in the mode of action of the mixtures might be explicable in terms of multilayer adsorption of the soap. The limiting concentration could represent the lowest concentration at which micelles begin to be adsorbed on to the bacterial surface to produce a multilayer. Another possible explanation is that at this concentration, the soap produces more extensive damage to the cell surface, this having the effect either of allowing much easier access of the phenol or of causing such extensive leakage of cellular contents that the time required for disinfection is much reduced at higher soap concentrations. It may be that the adsorbed complex exists in a different state at this concentration of soap—that a new, more highly adsorbable complex is formed between the phenol and the soap or its hydrolysis products, as described by Allawala and Riegelman[18]. It may be impossible to draw either correct or complete conclusions until more is known of the structure of micelles, the structure of bacterial surfaces and the ways in which surface-active agents modify these surfaces.

The Third Concentration of Limiting Activity

At concentrations of potassium laurate exceeding the second concentration of limiting activity, the extinction times of the mixtures decrease, at first rapidly. But when the soap concentration lies in the range 0·065–0·080M there is little decrease in extinction times, which may, in fact, increase. After a concentration of about 0·08M has been reached, the extinction times decrease with increasing soap concentration to the same extent as do those with the soap alone, the activity of the mixtures being only a little greater. With phenol (Fig. 10) the extinction times of the mixtures were a little greater than those for the soap alone, and this concentration of the phenol is sufficiently high to promote hydrolysis of the soap. There were not enough estimations with 2-hydroxydiphenyl to draw conclusions (Fig. 5).

The reduction in the rate of increase in activity with increase in soap concentration over the range 0·065–0·080M seems to be due to the effect of the factor responsible for the increase in extinction times at concentrations of soap just above the critical micellar concentration. The rapid increase in activity caused either by adsorption of a more toxic interfacial complex or by increased damage to the cell surface is not maintained because the "free" phenol concentration in the aqueous phase is further reduced by presentation of a much enlarged micellar phase. The activity of the phenol is also reduced by increased dissociation occurring in the more alkaline solutions at these soap concentrations. Only at soap concentrations exceeding this third limiting soap concentration—0·08M—is activity due solely to the soap, the phenol now being largely in solution in the micelles.

Hence the typical extinction time curves for the constant phenol concentration mixtures can be regarded as being made up of two parts, the second part being a repetition of the first part and explicable in similar terms. The first half begins at very high dilutions of the soap and the second half at about 0·045M, ending at that concentration at which the mixture and the soap alone have nearly equal activities. The mechanism is capable of and worthy of further investigation.

Mixtures of Constant Phenol/Soap Molar Ratio

The behaviour of soap-phenol mixtures in which the phenol concentration is increased proportionately to increase in soap concentration varies according to the solubility of the phenol. The least soluble phenol examined, 4-benzylphenol, showed steep increases in extinction times at concentrations in excess of the critical (curves 1–3, Fig. 6), the effect being comparable to that described by Bean and Berry[4] for their chlorinated benzylphenol which was also of comparable solubility in water. The phenol of intermediate solubility, 2-hydroxydiphenyl, exhibited only a slight increase in extinction time over the same soap concentration range (curve B, Fig. 5), whereas the most soluble phenol exhibited only a barely perceptible halt at soap concentrations just above the critical micellar concentration.

The difference in behaviour must be attributed to the differing partitions of the phenol between the micellar and aqueous phases of the solutions over the concentration range. It follows that a phenol of high solubility in water will attain a higher "free" phenol concentration in the aqueous phase in the presence of micelles than will a phenol of low solubility, where the aqueous phase will be much more depleted and the extinction times increased to a much greater extent. Thus constant molar ratio mixtures will exhibit the second limiting concentration effect only when the phenol possesses a partition coefficient permitting low partition in the aqueous phase as compared with the micellar phase.

Concentration Exponents of Soap-phenol Mixtures

The relations between phenol concentrations and extinction times at varying soap concentrations for each of the three phenols (Figs. 4, 8, 9, 11) show certain characteristics which are common to the three systems:

(*a*) Where the bactericidal activity could be determined in aqueous solutions containing no soap, the relationships between extinction time and phenol concentration (curve A, Fig. 4; curve A, Fig. 11) were steeper, i.e., the concentration exponents higher, than in solutions containing soap at any concentration.

(*b*) The more soluble the phenol, the more was its activity increased by addition of all proportions of soap.

(*c*) In the presence of soap, the relationships between phenol concentration and extinction time gradually changed at high dilution of the soap, the slope changing with increase in soap concentration to yield a constant slope over concentrations immediately above and below the critical concentration. Only when the soap concentration was increased beyond

0·04M did the slope become less steep. At that concentration at which activity of the constant concentration mixtures began to increase (about 0·05M) the slope was less than at any other soap concentration. Further increases in soap concentration resulted in a steepening of the curves, the slope probably remaining unchanged over the range 0·06–0·09M.

Thus the concentration exponent of the phenol changes with increasing soap concentration as follows: it is gradually reduced over a concentration range ending at 0·015M, remains constant until that concentration is reached at which extinction times rise towards the maximal value (0·04M), decreases markedly over the range 0·04–0·05M, and then increases to values which, however, are lower than those found around the critical micellar concentration but which probably remain constant from 0·06–0·09M. Hence the second soap concentration of limiting activity is associated with minimal concentration exponents of soap-phenol mixtures.

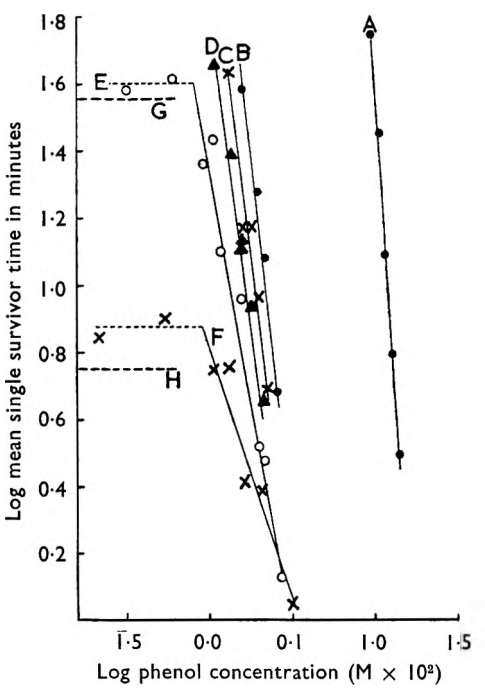

FIG. 11. The bacterical activity of phenol in aqueous solutions of potassium laurate at 25° C. Logarithmic relationships between concentration of phenol and extinction times of *Bact. coli* using five concentrations of potassium laurate: 0·028M (curve B ●——●), 0·0325M (curve C ×——×), 0·040M (curve D ▲——▲), 0·050M (curve E ○——○), and 0·80M (curve F ×——×). Curve A (●——●) represents the activities of solutions of phenol containing no soap, and G and H represent bactericidal activities of potassium laurate alone in concentrations of 0·050 and 0·080M respectively.

It is suggested that each range of changing concentration exponent represents an initiation of a new mode of bactericidal action. At high dilutions (below 0·015M), it is the transitional stage between uptake of phenol by the unmodified bacterial surface and uptake through an interfacial soap-phenol complex at the bacterium-water interface. The second change corresponds to the multilayer adsorption of the mixed film or further modification of the bacterial surface with concomitant increase in bactericidal activity of the mixtures.

That the concentration exponent remains constant over limits of about ± 50 per cent. of the critical concentration supports the findings of Alexander and Tomlinson[2] who demonstrated a roughly constant value over rather wider limits of concentration. Bean and Berry[4] were led to consider that relations between phenol concentration and extinction time

were parallel for different soap concentrations as a result of observations at two concentrations: 0·039 and 0·065M. The results shown in Figures 8 and 9, obtained with a phenol of similar solubility, indicate that, although the slopes at these concentrations may not be greatly dissimilar, a large difference will be found at intermediate concentrations.

Comparison of Activities of the Phenols

Examination of the plotted values in Figures 4–11 at once reveals that phenol is by far the least active under all conditions—in solutions containing no soap and in solutions containing all concentrations of soap—whereas 4-benzylphenol and 2-hydroxydiphenyl have much more nearly equal activity, giving nearly equal extinction times at any given phenol concentration.

Molar concentrations of the two phenols required to give the same extinction time in the presence of varying amounts of soap are recorded in Table I, which also expresses the concentrations as percentages saturation of the solutions, i.e.

$$\frac{\text{phenol/soap molar ratio} \times 100}{\text{saturation phenol/soap molar ratio}}.$$

It is seen that the most saturated systems do not give the highest bactericidal activity irrespective of the nature of the phenol, a 22·8 per cent. saturated solution of 4-benzylphenol having the same activity as a 7·24 per cent. saturated solution of 2-hydroxydiphenyl at soap concentration 0·03M.

TABLE I

MOLAR CONCENTRATIONS AND PERCENTAGES SATURATION OF 2-HYDROXYDIPHENYL AND 4-BENZYLPHENOL REQUIRED TO GIVE IDENTICAL EXTINCTION TIMES (20° C.) IN SOLUTIONS OF POTASSIUM LAURATE

Molar concentration of potassium laurate	Mean single survivor time (minutes)	2-Hydroxydiphenyl		4-Benzylphenol	
		Molar concentration ($\times 10^3$)	Percentage saturation	Molar concentration ($\times 10^3$)	Percentage saturation
0·03	10·0	1·15	7·24	1·32	22·8
0·04	10·0	1·31	5·81	2·29	21·4
0·05	12·6	1·76	5·97	2·11	12·6
0·06	7·4	2·11	5·73	2·63	12·5

At the four different soap concentrations the ratios of molar concentrations of 4-benzylphenol/2-hydroxydiphenyl required to produce extinction of *Bact. coli* in the same time were 1·15, 1·75, 1·20 and 1·25. The second value is much higher than the others, which give an estimate of the ratio of activity of 1 : 1·2.

THE CRITICAL MICELLAR CONCENTRATION

Estimation of the critical concentration for micelle formation of potassium laurate by the two methods described previously gave a wide discrepancy. Solubility determinations, in which the soap solutions were saturated with 4-benzylphenol or 2-hydroxydiphenyl, gave estimates of

0·015M with both phenols. The estimates provided by the concentration of maximal bactericidal activity of mixtures with phenols were always between 0·030 and 0·033M. A possible explanation for the lower values obtained from solubility measurements was depression of the critical concentration on saturation of the solutions with phenols. McBain, Merrill and Vinograd[19] reported that solubility measurements generally gave estimates of critical concentration lower than those obtained by other methods, and McBain and Merill[20] maintained that this was found because increase in solubilising power provided a much more sensitive method than measurement of several other properties. On the other hand, Klevens[21] has shown that his estimates from solubility agree well with estimates by most other methods.

Summary

1. Solubilities in solutions of potassium laurate of two phenols, 2-hydroxydiphenyl and 4-benzylphenol, which exhibited a nearly five-fold difference in water solubility, were estimated spectrophotometrically. The solubility curves are similar to those described by Bean and Berry[4,5] for a chlorinated benzylphenol and for a chloroxylenol.

2. Soap-phenol mixtures containing constant concentrations of phenol, 2-hydroxydiphenyl and 4-benzylphenol, with varying concentrations of potassium laurate, have been examined for bactericidal activity against *Bacterium coli*. The characteristic extinction time-soap concentration curve shows three soap concentrations of limiting activity, the significance of which has been discussed.

3. Mixtures containing a constant phenol/soap molar ratio and varying potassium laurate concentrations give changes in bactericidal activity which depend on the aqueous solubility of the phenol.

4. Increased water solubility of the phenol is associated with greater enhancement of bactericidal action in the presence of soap at all concentrations.

5. The concentration exponents of aqueous solutions of phenols containing no soap are greater than those of solutions containing soap. The concentration exponents of solutions of phenols containing potassium laurate are roughly constant over a soap concentration range extending from 0·015 to at least 0·040M. Possible interpretations of deviations at either extreme of this range have been put forward.

6. 4-Benzylphenol and 2-hydroxydiphenyl have been shown to be nearly equally active as bactericides despite their difference in solubility in water and in solutions of potassium laurate. Equally saturated solutions of different phenols are therefore not necessarily equally active. Comparable bactericidal activity depends upon the concentration of the compounds in total solution and, over a range of soap concentrations, 2-hydroxydiphenyl was found to be about 1·2 times more active than 4-benzylphenol.

The authors wish to express their thanks to Mr. A. Edwards and Mr. D. S. May for technical assistance.

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AN ASSAY FOR PRIMARY ARYLALKYLAMINES

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Baker, Harbourne and Ollis[1] showed that salicyloylacetone reacted with primary aliphatic amines, but not amino-acids, to give yellow fluorescent *o*-β-alkylaminocrotonylphenols. Attempts were made to apply this reagent for estimation of 1-(*p*-alkyloxyphenyl)ethylamines without success. Salicylaldehyde was a satisfactory alternative. Formation of aldimines to discriminate between primary and alkylated amines has attracted little attention for assay purposes. Loeper[2] made reference, without details, to the use of salicylaldehyde for estimation of tryptamine, though later *m*-nitrobenzaldehyde was specified as the reagent[3].

Experimental

Salicyloylacetone was made by the method of Badcock, Dean, Robertson and Whalley[4]. Salicylaldehyde was purified through its bisulphite compound and twice distilled, b.pt. 40°/0·5 mm. Derivatives of these carbonyl compounds with primary amines were prepared by setting aside equimolecular proportions of the reactants in benzene. After 12 hours the solvent was removed under reduced pressure and the residue crystallised from light petroleum. New derivatives are listed in Table I.

TABLE I

Derivatives of primary amines with salicyloylacetone and salicylaldehyde

Amine	Salicyloylacetone derivative		Salicylaldimine	
	m.pt.°C.	Analysis	m.pt.°C.	Analysis
Mescaline	119	Found: N, 3·7·$C_{21}H_{25}O_6N$ requires N, 3·8 per cent.	112	Found: N, 4·4· $C_{18}H_{21}O_4N$ requires N, 4·4 per cent.
1-(*p-cyclo*Hexyloxyphenyl)-ethylamine	95	Found: N, 3·7· $C_{24}H_{29}O_3N$ requires N, 3·7 per cent.	108	Found: N, 4·5· $C_{21}H_{25}O_2N$ requires N, 4·3 per cent.
1-(*p-iso*Propyloxyphenyl)-ethylamine	—	—	73	Found: N, 5·0· $C_{13}H_{21}O_2N$ requires N, 4·9 per cent.

The yellow fluorescence of several *o*-β-alkylaminocrotonylphenols in ultra-violet light was quenched by common laboratory solvents. Solutions in methanolic sulphuric acid developed a blue fluorescence, probably due to 2-methylchromone arising from hydrolysis and cyclisation of the salicyloylacetone produced[5]. The intensity of fluorescence corresponded to equivalent amounts of salicyloylacetone under similar conditions. It was dependent on the final concentration of acid, showing a sharp maximum at 47 per cent. v/v. Sensitivity was adequate, the fluorescence developed by pure ketimines being detectable at 0·01 μg./ml. but the yields of derivatives at high dilution were too variable and low for practical use.

Alkaline solutions of salicylaldehyde had a bright yellow colour and a

greenish yellow fluorescence in ultra-violet light. The salicylaldimines of Table I and that of amphetamine had the same characteristics, the intensity of colour corresponding to the equivalent salicylaldehyde content (Table II). There was a linear relation between concentration of aldimine, and colour or fluorescence intensity, up to 100 μg./ml. and 1 μg./ml. respectively.

TABLE II

EQUIVALENCE OF COLOUR INTENSITY DEVELOPED BY SALICYLALDEHYDE AND SALICYLALDIMINES IN ALKALI

239 parts amphetamine salicylaldimine ≡ 122 parts salicylaldehyde. Other amines mentioned in the text gave a similar result

Amphetamine aldimine		Salicylaldehyde	
Concentration (μg./ml.)	Reading	Concentration (μg./ml.)	Reading
23·9	0·279	12·2	0 285
47·8	0·554	24·4	0 561
81·7	0·810	36·6	0 817
95·6	1·05	48·8	1 08
119·5	1·29	61·0	1·29

Method of Assay

The solution of amine was acidified with a few drops of hydrochloric acid, warmed to 40° C. and carbon dioxide swept out by a current of nitrogen. The solution was adjusted to pH 9 and the base extracted by shaking five times with 5 ml. portions of volatile solvent, preferably benzene. The extract was lightly centrifuged to separate alkaline globules and the solution transferred to a flask containing about 10 molecular proportions of salicylaldehyde in benzene. By concentration to small bulk in a current of air on a sand bath the solution was dried (more benzene being added if necessary) and the aldimine formed. The volume was adjusted to 20 ml. and 5 ml. portions transferred to ground joint tubes containing 0·5 ml. 1 per cent. acetic acid in benzene. Solvent was removed completely in a current of air on a sand bath and the excess aldehyde distilled under reduced pressure at 0·5 mm. for 30 minutes with the tubes completely immersed in water at 70° C. A blank and a suitable amount of pure alkimine as standard were submitted to the same process as the extracted amine. The residues were dissolved in 5 ml. of warm absolute ethanol and 1 ml. of N sodium hydroxide added followed by 4 ml. of water. The yellow colour, developed after a brief lag, was measured at 415 mμ.

Modifications. The procedure required slight modification according to the amine being assayed. Mescaline was preferably extracted continuously with benzene for 12 hours. Amphetamine salicylaldimine, a yellow liquid b.pt. 140–150° C./0·4 mm. (bath temp.) was appreciably volatile at 70° C./0·5 mm. It was assayed satisfactorily by evacuating at 40° C./0·5 mm. The principle source of error was contamination of the initial solvent extract with alkali. This trapped salicylaldehyde and prevented its distillation. For *p-cyclo*hexyloxy-1-phenylethylamine this contamination could be removed by washing the ether extract with water,

but even a single wash would remove up to 30 per cent. of mescaline or the *iso*propyl ether (Table III). Centrifugation and the acetic acid additive were found to be adequate safeguards.

Sensitivity. Colour measurement was applicable to solutions containing not less than 10 μg./ml. salicylaldehyde equivalent. Below this concentration fluorescence measurement was applicable, with a practical lower limit of 0·1 μg./ml. The fluorimetric method was not developed fully due to difficulty in stabilising the mains supply to the ultra-violet source.

TABLE III

RECOVERY OF AMINES FROM 0·10 PER CENT. SOLUTIONS OF THEIR SALTS

Solutions of the salts at pH 9 were extracted three times with solvent and estimated colorimetrically as described in the text

Amine	No. of expts.	Solvent	Solution	Total mg.	Recovered mg.
1-(*p-cyclo*Hexyloxyphenyl)ethylamine	4	ether	water	10	9·9–10·0
	4	ether	water	1	0·85–0·92
	4	ether	human urine	1	0·87–0·89
	2	ether	rat urine	1	0·80–0·82
1-(*p-iso*Propyloxyphenyl)ethylamine	9	ether	water	10	4·82±0·92*
	3	ether	water	10	8·9–9·4
	2	ether	human urine	10	8·8; 9·2
Mescaline	4	chloroform	water	10	7·7–8·4*
	4	chloroform	water	10	9·9–10·3
	2	benzene†	water	10	10·0; 10·0
Amphetamine	3	benzene	water	10	7·9–8·2
	1	benzene	human urine‡	49	52

* Low recovery due to washing the solvent extract.
† Continuous extraction overnight.
‡ Supplied by Dr. Connell who found 49 mg. by the method of Brodie and Udenfriend[6].

RESULTS

Table III gives the recovery of amines added to water and to urines using the colorimetric process. Ammonia, acetone, acetoacetic ester and glycine did not interfere but bicarbonate and carbon dioxide could markedly reduce the proportion of amine recovered (Table IV). Secondary

TABLE IV

RECOVERY OF 1-(*p-cyclo*HEXYLOXYPHENYL)ETHYLAMINE FROM SALINE SOLUTIONS

10 mg. of the hydrochloride in 10 ml. saline solution with or without passage of 5 per cent. carbon dioxide, basified with 5-N sodium hydroxide. Ether extract estimated. Similar results were found with 1-(*p-iso*propyloxyphenyl)ethylamine and mescaline

Salt	Passage of carbon dioxide	Recovery per cent.
Sodium bicarbonate, 0·5 g.	+	27±3·4
Sodium bicarbonate, 0·5 g.	−	48; 50
Potassium bicarbonate, 0·5 g. ..	−	48
Potassium bicarbonate, 0·5 g. ..	+	28*
Sodium carbonate (hydrated) 1·0 g.	−	94
Sodium dihydrogen phosphate, 0·5 g.	+	94

* The aqueous residue acidified and freed of carbon dioxide, then basified and re-extracted raised recovery to 100 per cent.

amines e.g., methedrine, and tertiary amines, e.g., methadone, gave deep orange colours with salicylaldehyde but these disappeared during the vacuum distillation and low readings (0 to 3 per cent. apparent recovery) were obtained. Amines of low molecular weight, e.g., *iso*amylamine gave aldimines that were volatile at 70°/0·5 mm. and they were lost. Aromatic amines, e.g., *p*-aminosalicylic acid, gave yellow colours on the final addition of alkali but these faded within a few minutes.

Human urine (50 ml.) gave small positive readings. Of sixteen specimens from normal or mentally ill subjects, eight gave zero values, six gave values equivalent to the excretion of 115–264 μg. salicylaldehyde equivalent/24 hours. Two specimens from patients gave relatively high values equivalent to 1·23 and 1·53 mg. salicylaldehyde equivalent/24 hours. Adult female albino rat urine gave ether extracts corresponding to the excretion of 145 ± 35 μg. salicylaldehyde equivalent/24 hours. Similar animals rapidly metabolised *p-cyclo*hexyloxy- and *p-iso*propyloxy-1-phenylethylamine. After giving the 1-^{14}C labelled amines there was good recovery of ^{14}C, the major fraction being excreted in 24 hours, but only low recovery of primary amine was obtained (Table V). ^{14}C-metabolites not assayable as primary amine, were difficult to extract from urine even after acid hydrolysis, whether the solution was finally made acid, alkaline or neutral. Continuous extraction for three days with ether at pH 9 took out only half the labelled material present. No anticipated metabolite was traced.

TABLE V

RECOVERY OF AMINES FROM URINE OF RATS

The dose specified was divided between two rats and given i.p. Two similar rats receive saline. Radioactivity estimated on the evaporated urine at infinite thickness. Standards were prepared by addition of amines to rat normal urine

Amine	Dose (mg.)	Collection period (days)	Per cent. recovery of primary amine		Per cent. recovery of ^{14}C
			by assay	by ^{14}C	
1-(*p-cyclo*Hexyloxyphenyl)ethylamine	10	4	7·8; 10	—	—
	10	1	2·9	4·3	55
	20	2	19	16	69
	20	1	15*	—	—
1-(*p-iso*Propyloxyphenyl)ethylamine ..	10	1	14	5·8	81
Mescaline	20	1	12	—	—
	20	2	16	—	—

* Acid hydrolysis increased this to 28·5 per cent. but a benzene soluble pink substance interfered.

SUMMARY

1. A colorimetric method for the estimation of primary arylalkylamines is described.

Financial support from the Medical Research Council is gratefully acknowledged. Thanks are due to Miss W. J. Lynch, B.Sc. for technical assistance, and to Dr. H. Weil-Malherbe for advice on fluorimeter construction, to Drs. Connell and Rey for providing urine specimens from patients and to Professor H. McIlwain for his interest.

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Addendum to the footnote to the paper:

ORTHO SUBSTITUTED BENZOIC ACID ESTER OF DIALKYL AMINOALKANOL IN EXPERIMENTAL CARDIAC ARRHYTHMIAS

BY R. B. ARORA, V. N. SHARMA AND B. R. MADAN

This Journal, 1956, **8**, 323.

The full chemical name for the compound McN-A-29-11 is β-Diethylaminoethyl 2:3:5:6-tetramethyl benzoate.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Raunescine and *iso*Raunescine from *Rauwolfia canescens* L. N. Hosansky and E. Smith. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 639.) Two new alkaloids were isolated from a weakly basic alkaloidal fraction from which reserpine and canescine had been removed. The material was extracted with ether in the presence of acid and alkali to remove colour and other impurities. Raunescine and *iso*raunescine were then extracted with chloroform and separated on a silica column, by developing first with chloroform and then with chloroform containing 2 per cent. of methanol to obtain raunescine. *iso*Raunescine was obtained by continuing the development with chloroform containing 5 per cent. of methanol. The two alkaloids appeared to be isomeric, and gave ultraviolet absorption spectra almost identical with that of canescine. The infrared spectra were similar, but raunescine showed a doublet at 5·68 and 5·82 μ, in place of the single band at 5·79 μ in the spectrum of *iso*raunescine. As in the case of canescine, there was no absorption band at 6·18 μ, associated with the 11-methoxyl group. It is suggested that these alkaloids possess the same structure as canescine, with a hydroxyl group in place of methoxyl at position 17. G. B.

δ-Yohimbine from the Bark of *Rauwolfia verticillata*. H. R. Arthur. (*Chem. Ind.*, 1956, 85.) A hot methanolic extract from the bark of *R. verticillata* (prepared after initial extraction of the bark with light petroleum) was evaporated to dryness and the green tar which remained was extracted with hot benzene. The residue was triturated with water, the mixture filtered and the filtrate extracted with chloroform. The chloroform was evaporated to dryness and as much as possible of the residue dissolved in benzene and chromatographed on alumina, benzene being used as eluant. The alkaloidal fractions appearing in the eluate were combined and recrystallised from methanol to yield stout needles of δ-yohimbine m.pt. 252 to 253·5° C. A. H. B.

ANALYTICAL

Atropine in Mixtures, Polarographic Determination of. B. Novotný (*Československ. Farm.*, 1955, **4**, 448.) Atropine, which itself is inactive, can be determined polarographically in the form of its nitro derivative. For the determination of atropine in injections containing 0·01 g. of morphine hydrochloride and 0·0005 g. of atropine sulphate per ml., a 2-ml. sample is diluted with 30 ml. of water, 1 ml. of a 10 per cent. potassium hydroxide solution is added, and the mixture is extracted with chloroform. An aliquot of the extract, containing about 250 μg. of atropine, is transferred to a 50-ml. flask and the chloroform is removed in a stream of air. The residue is heated for 30 minutes on a water bath with 1 ml. of concentrated nitric acid and 0·1 ml. of 15 per cent. sulphuric acid. The solution is allowed to cool and 10 ml. of a 20 per cent. potassium hydroxide solution is added; it is then polarographed at 0·0 V., a stream of nitrogen being passed through the cell. A second aliquot of the chloroform extract to which 250 μg. of atropine sulphate has been added is treated in the same way. The atropine content of the sample is calculated with

the aid of a calibration graph constructed from the results obtained by polarographing solutions prepared from 200, 400, 600 and 800 μg. of atropine sulphate. The nitro derivative of atropine gives two waves and the height of the first wave is proportional to the concentration of atropine. E. H.

Bacitracin and Neomycin in Admixture, Determination of. J. Lingnau and G. Machek. (*Sci. Pharm.*, 1955, **23**, 234.) Since bacitracin is used therapeutically as such, while neomycin is employed in the form of sulphate, there is a possibility of separation of these two antibiotics by making use of their differing solubilities in alcohols. It was found that bacitracin is easily soluble, while neomycin sulphate is practically insoluble in ethanol or methanol (about 0·01 to 0·02 per cent. at 37° C.). Alcohols do not destroy the activity of these compounds. For the assay of mixed preparations, the material is extracted with cold ethanol (96 per cent.) and the undissolved neomycin sulphate is assayed microbiologically using *Micrococcus flavus*. The ethanolic solution is diluted with 0·1M phosphate buffer (pH 6) and assayed similarly, using *B. subtilis*. In the case of powders containing phenylmercuric acetate it is possible to determine the neomycin sulphate only. In the case of ointments a preliminary removal of fat by light petroleum is desirable. The results published show satisfactory agreement. G. M.

Benzene Hexachloride, Separation of Isomers of. R. G. Bridges, A. Harrison and F. P. W. Winteringham. (*Nature, Lond.*, 1956, **177**, 86.) Whatman No. 1 filter paper strips were washed with water to remove an inorganic halide contaminant present in the paper, dried, dipped in 5 per cent. w/v solution of white soft paraffin in ether, drained and dried. The mixture of the isomers was applied in acetone solution and the mobile phase consisted of 70 per cent. methanol and 30 per cent. water by volume. Descending chromatography was used at laboratory temperature. After 18 hours the strip was dried and the positions of the isomers was detected by dipping the paper in redistilled mono-ethanolamine, heating at 100° C. for $\frac{1}{2}$ hour and then dipping in 0·1N solution of silver nitrate acidified with concentrated nitric acid (10 vol. silver nitrate solution : 1 vol. acid). On exposure to ultra-violet light, brown spots appeared corresponding to the positions of the isomers. The alpha- beta-, gamma- and delta-isomers moved with mean R_F values of 0·33, 0·00, 0·40 and 0·58 respectively. The method of detection was sensitive to less than 5 μg. of all but the beta-isomer which was only just detectable in 5 μg. quantities. A. H. B.

Chloramphenicol, Periodate Oxidation in the Analysis of. A. Valseth and A. Wickstrom. (*Medd. Norsk. Farm. Sels.*, 1955, **17**, 345.) Hydrolysis of the amide linkage in chloramphenicol yields dichloroacetic acid and an aminodiol (+)-threo-1-(*p*-nitrophenyl)-2-amino-propanediol-1 : 3, which may be oxidised with periodate to *p*-nitrobenzaldehyde, formaldehyde, ammonia, and probably formic acid. The optimum pH region of the periodate oxidation of the aminodiol was found to range from 7·0 to 7·5. In the presence of a phosphate buffer of optimum pH the periodate uptake of the aminodiol mounted rapidly to the theoretical value of 2·0 molecules and did not exceed this value after one hour. The unused periodate was reduced with sodium arsenite, the excess being titrated against iodine. A method is also described which makes it possible to determine quantitatively 0·2–2 per cent. free aminodiol in chloramphenicol samples (125 mg.): the sample is oxidised with periodate, ammonia originating from any free aminodiol is separated from the reaction mixture by microdiffusion in Conway standard cells and then determined spectrophotometrically by Lubochinsky and

Zalta's modificaion of the colour reaction with phenate-hypochlorite. The amount of free aminodiol liberated from chloramphenicol eye drops by heating to 100° C., was evaluated by titration of the periodate uptake and by micro-determination of the ammonia evolved by oxidation with periodate. After heating at 100° C., for 30 minutes the chloramphenicol was hydrolysed to an extent of 3·8 per cent., the hydrolysis increasing to 15·4 per cent. after 2 hours. R. E. S.

Disulfiram, Colorimetric Determination of. B. Salvesen and L. Domange. (*Ann. pharm. franç.*, 1955, **13**, 499.) The method depends upon the formation of a yellow colour when a solution of disulfiram is treated with copper. This reaction appears to be specific for the group =N·CS·S·S·CS·N=. To 10-ml. quantities of solutions in acetone of the sample under examination and of known quantities of pure disulfiram (200–1000 μg.) is added 0·45 g. of copper turnings, previously treated with nitric acid and washed with water, ethanol and ether. The colour is developed by allowing the samples to stand for 7 hours with occasional shaking. The colour intensity is measured at intervals and readings are recorded when the maximum intensity is reached. Measurements are made with a photoelectric colorimeter and a screen having a maximum transmission at about 434 mμ. The quantity of disulfiram in the sample under test is read from the standard curve drawn from the figures obtained with the pure samples. Methanol may be used in place of acetone, but 0·2 g. of copper turnings should be used and the maximum colour intensity is obtained after 5 to 6 hours. About 25 hours is required when *cyclo*hexane is used as solvent. G. B.

Erythromycin, Colorimetric Determination of, using Methyl Sulphate. M. Pesez. (*Ann. pharm. franç.*, 1955, **13**, 513.) The following method is recommended. To 2 ml. of solution of erythromycin or erythromycin ethylcarbonate in methylethyl ketone add 8 ml. of methyl sulphate, shake and allow to stand for one hour. Measure the absorption at 480 mμ, by means of a spectrophotometer, and prepare a standard curve for quantities of 50 to 300 μg. of erythromycin. For the assay of tablets of erythromycin, shake a quantity of the powdered tablets equivalent to about 0·1 g. of erythromycin with 25 ml. of methyl ethyl ketone for 15 minutes. Filter, dilute the solution with the same solvent and complete the determination as above. The quantity of erythromycin is read from the standard curve, which is linear for quantities of 50 to 300 μg. G. B.

Glycyrrhizic Acid in Succus Liquiritæ, Determination of. H. Onrust, A. P. Jansen and B. S. J. Wöstmann. (*Rec. Trav. chim. Pays-Bas.*, 1955, **74**, 1515.) Glycyrrhizic acid is a glycoside composed of glycyrrhetic acid linked to two molecules of hexuronic acid. The method of determination involved dissolving the succ. liq. in a 50 per cent. dioxane-water mixture and then hydrolysis by refluxing with dilute sulphuric acid. Upon cooling, a precipitate of glycyrrhetic acid forms and most of it is dissolved by refluxing with chloroform. Subsequently the water-chloroform mixture is transferred to an extractor and extraction with chloroform continued for 1½ hours. After cooling, the chloroform layer is made up to a definite volume, an aliquot portion taken and evaporated to dryness and the residue dissolved in ethanol. This solution is then examined polarographically, the determination being made on 40 per cent. ethanol containing 0·1M acetate buffer [voltage range used was −1·2 to −1·7 V. (vs. S.C.E.)]. A. H. B.

Phenobarbitone and Diphenylhydantoin, Chromatography of. A. S. Curry. (*Analyst*, 1955, **80**, 902.) By the use of paper chromatography both phenobarbitone and diphenylhydantoin can be separated and a visual comparison made. The solvent system used was *n*-butanol, water, ammonia, sp.gr. 0·880 (100:66:33) with Whatman No. 1 paper; phenobarbitone had an R_F value of 0·50 and diphenylhydantoin R_F 0·65. The top layer of the solvent is used, the bottom layer being discarded. Two methods were used for the detection of the spots, the first being to contact print the dried chromatogram on Ilford Reflex Paper No. 50, using as the source, unfiltered light from a mercury-vapour lamp; after exposing to ammonia 10 to 20 μg. was visible as a white spot on a black background. In the second method the paper was dipped in a solution of 5 per cent. of mercuric oxide in 20 per cent. sulphuric acid followed by washing with water, acetone and ethanol; after dipping in 0·05 per cent. diphenylthiocarbazone in ethanol, reddish purple spots appeared after 1 to 2 minutes. Barbiturates with an allyl group in the molecule develop distinctly more blue than the fully saturated compounds, allobarbitone giving a blue spot and quinalbarbitone blue-purple. With this treatment 20 μg. phenobarbitone or 40 μg. diphenylhydantoin can be easily detected. R. E. S.

Reserpine, Fluorimetric Assay of. E. B. Dechene. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 657.) Solutions of reserpine in 5N acetic acid were treated with 1 ml. of solution of hydrogen peroxide (3 per cent.) to increase the intensity of the fluorescence due to reserpine, and diluted to 10 ml. with 5N acetic acid. The solutions were heated for 45 minutes in a boiling water bath, cooled and the fluorescence measured by means of a photoelectric fluorimeter. The fluorescence was compared with that of a solution of reserpine of known strength, similarly treated, and the reserpine content of the test solution calculated. The intensity of fluorescence was proportional to concentration of reserpine in the range of 0·4–1·8 μg. This method was applied to the determination of reserpine in tablets, and was also used in the assay of a powdered extract of *Rauwolfia serpentina*. In the latter case, preliminary treatment involving extraction with methanol, evaporation, maceration of the residue with N sulphuric acid, extraction with chloroform, washing with sodium bicarbonate solution, evaporation and solution in acetic acid was necessary. G. B.

Vitamins D, Colour Reaction for. W. I. Lyness and F. W. Quackenbush. (*Analyt. Chem.*, 1955, **27**, 1978.) Calciferol and vitamin D_3 were found to react with an iodine-ethylene dichloride reagent to produce a strong yellow colour which showed an absorption maximum at 450 mμ. The intensity of the colour was enhanced by mercuric *p*-chlorobenzoate and certain other compounds. Six sterols, lumisterol, ergosterol, 7-dehydrocholesterol, cholesterol, stigmasterol, and sitosterol, when tested at 0·05 and 0·5 mM concentrations showed no apparent reaction with the reagent. Vitamin A (0·20 mM solution) produced a medium blue colour which changed after approximately 1 minute to medium violet, the solution showing a broad absorption band with a maximum at 555 mμ and some absorption at 450 mμ; vitamin A interfered with the determination of vitamin D to give low values. When the mercuric *p*-chlorobenzoate was omitted from the reagent, colour development was qualitatively the same but quantitatively about 10 to 15 per cent. of the intensity. Temperature differences between 20° and 35° C., were shown to have no effect on the reaction. A precision within 2 per cent. was obtained under strictly controlled conditions. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

The Antivitamin B_{12} Activity of Some Compounds Related to Cobalamin. W. F. J. Cuthbertson, J. Gregory, P. O'Sullivan and H. F. Pegler. (*Biochem. J.*, 1956, **62**, 15P.) A cup-plate method has been devised for detecting compounds that antagonise the utilisation of cyanocobalamin by vitamin B_{12}-dependent organisms. Solutions of cyanocobalamin (0·1 μg./ml.) and the test substance (1–1000 μg./ml.) were placed in cups cut 8 mm. apart in vitamin B_{12} assay plates seeded with either *Escherichia coli* or *Lactobacillus leichmannii*, and the plates incubated overnight. Inert substances did not modify the circular zones of growth; non-specific inhibitors, such as phenol, produced circular zones of inhibition, cutting arcs from the growth produced by the vitamin in the adjacent cup. Competitive antagonists showed inhibition which decreased rapidly towards the centre of the growth zone and extended further round the edges than with non-specific growth inhibitors. Quantitative assessment was carried out in fluid media using graded levels of vitamin B_{12} and antagonist. The methylamide of the mixed monobasic acids derived from cobalamin was the most active of the substances tested. Vitamin B_{12}-deficient rats were fed mixtures of the vitamin and antagonist at varying dose levels and the antagonists depressed growth at doses of 0·1–3 μg./day. J. B. S.

Vitamin B_{12}, Antimetabolites from. E. Lester Smith, L. F. J. Parker and D. E. Gant. (*Biochem. J.*, 1956, **62**, 14P.) The mono-, di- and tri-carboxylic acids resulting from the mild hydrolysis of vitamin B_{12} have been reacted in anhydrous dimethylformamide solution, first with ethyl chloroformate and triethylamine, and then with amines (instead of ammonia as used in the regeneration of the vitamin) to yield amides, which have been examined for anti-vitamin activity. The products were purified by electrophoresis on paper, and by repeated paper chromatography. Antivitamin activity was demonstrated by plate assay with the B_{12}-requiring *Escherichia coli* mutant for the mono-amides of methylamine, ethylamine, monoethanolamine, ethylenediamine, dimethylamine, diethylamine, piperidine, phenylethylamine, *cyclo*hexylamine, aniline and sulphanilic acid. The di-anilides of the dibasic acids were inactive, whilst the tribasic acid gave a weekly active triethylamide.

J. B. S.

BIOCHEMICAL ANALYSIS

5-Hydroxyindolcacetic Acid in Urine, Determination of. A. Hanson and F. Serin. (*Lancet*, 1955, **269**, 1359.) A qualitative and a quantitative method are described for the determination of 5-hydroxyindoleacetic acid, the main excretory metabolite of 5-hydroxytryptamine, in urine. As a screen test, make 100 ml. of the 24 hour urine specimen alkaline to pH 8·5 with ammonia, filter and extract twice with ether to remove impurities. Acidify to pH 4 with dilute hydrochloric acid, filter and extract three times with 100 ml. of ether. Dehydrate the pooled ether extracts with anhydrous sodium sulphate, evaporate to dryness *in vacuo* and dissolve the residue in ethanol. Paper chromatography is used for identification, using a solvent mixture of *n*-butanol acetic acid water (4:1:5) and the spots are developed by spraying with an ethanolic solution of *p*-dimethylaminobenzaldehyde or a solution of 2-nitrobenzenediazonium-naphthalenesulphonate in dilute hydrochloric acid. For quantitative determina-

tion acidify 2 ml. of filtered urine with one or two drops of 10 per cent. hydrochloric acid and extract twice with 25 ml. of ether. Filter the pooled ether extracts, dehydrate and evaporate to dryness at 50° C. Dissolve the residue in 0·1N hydrochloric acid and to 2 ml. of this add 5 ml. of Ehrlich's aldehyde reagent. Heat the solution and a blank for 2 hours at 45–50° C., when the solution becomes blue. Dilute with cold 50 per cent. ethanol to 10 ml. and measure the colour in a spectrophotometer at 590 mμ. The urinary excretion has been studied in two patients with malignant carcinoid. G. F. S.

Urea in Blood and Urine, Determination of. H. L. Rosenthal. (*Analyt. Chem.*, 1955, **27**, 1980.) The Fearon condensation of urea with acidified diacetyl monoxime followed by oxidation with arsenic acid has been extensively studied in an effort to improve reproducibility and the linearity of response of the reaction. The concentration of mineral acid and oxidising arsenic was found to be critical; in 3·8N hydrochloric acid and 0·08N arsenic acid maximum colour is produced which conforms to Beer's law at urea concentrations up to 60 μg. per 10-ml. reaction volume. Dilution of the reaction mixture results in deviation from Beer's law, and the urea response curve no longer passes through the origin. The colour formation increased rapidly on heating in a boiling water bath, being 90 per cent. complete in 25 minutes; a 30 minute heating period was found to give the most reproducible results. Recovery experiments, with known amounts of urea added to blood and urine, gave individual recoveries ranging from 94 to 103 per cent. in blood and 92 to 110 per cent. in urine. Although the reaction is not specific for urea, only this substance gives a yellow colour with absorption maximum at 480 to 485 mμ.; for example, citrulline and other carbamyl amino-acids give maximum absorption at 550 mμ.

R. E. S.

CHEMOTHERAPY

Phenoxymethylpenicillin. W. J. Martin, D. R. Nichols and F. R. Heilman. (*Proc. Mayo Clin.*, 1955, **30**, 467.) Phenoxymethylpenicillin (penicillin V) is produced biosynthetically by *Penicillium chrysogenum* Q 176 in a culture medium containing a special type of nutrient substrate. It is an acid and therefore passes through the stomach unchanged, in contrast to benzylpenicillin which is partly inactivated in an acid medium. In the alkaline medium of the small intestine it dissolves and is absorbed. It is stable as a free acid and does not require to be prepared as a metallic or organic salt. Phenoxymethylpenicillin is readily absorbed into the serum when administered by the oral route. Administration of 200,000 units every 4 hours produces serum levels effective in combating minor infections due to susceptible organisms; for infections of moderate severity a dose of 400,000 units 4-hourly appears adequate, and 800,000 units has been given for fairly severe infections. The range of antibacterial activity appears to be similar to that of benzylpenicillin. Encouraging results were obtained from the use of phenoxymethylpenicillin in the treatment of 30 patients suffering from a wide variety of infections. The most frequently encountered side effect was a mild gastro-intestinal irritation manifested by slight abdominal cramping and diarrhœa. One patient experienced rather severe aphthous stomatitis. It appears that many of the infections that have been treated parenterally with penicillin in the past may now be treated with phenoxymethylpenicillin given orally. S. L. W.

Streptonivicin (Albamycin). W. J. Martin, F. R. Heilman, D. R. Nichols, W. E. Wellman and J. E. Geraci. (*Proc. Mayo Clin.*, 1955, **30**, 540.) Streptonivicin is an antibiotic produced by an actinomycete, *Streptomyces niveus*. It is relatively stable and is active against a variety of organisms; it is especially active, both *in vitro* and *in vivo*, against *Micrococcus pyogenes*. There is apparently no cross-resistance between streptonivicin and penicillin, streptomycin, chloramphenicol, the tetracycline group of compounds, neomycin, bacitracin, and erythromycin, and organisms resistant to them may be fully susceptible to streptonivicin. Laboratory evidence indicates that *M. pyogenes* can become resistant to streptonivicin. Administration of multiple doses of streptonivicin, 0·25 to 0·5 g., at intervals of 6 hours to a number of patients, showed that it is absorbed into the general circulation when given by this route; detectable levels persist in the serum for more than 12 hours after administration. The drug is distributed in blood, pleural and ascitic fluids, and thyroid tissue, and is excreted in the bile, urine and fæces. The kidneys are one of the main routes of excretion. There are indications that the antibiotic in the urine is changed but that it is biologically active. The drug was not detected in the cerebrospinal fluid. Streptonivicin appears to be relatively non-toxic in the doses used in this study, and none of the patients receiving it suffered damage to the kidneys, liver or hæmopoietic system. Some of the patients complained of nausea. If these experimental data are substantiated clinically, streptonivicin may prove effective in the treatment of infections caused by strains of *M. pyogenes* which are resistant to other antibiotics. S. L. W.

PHARMACY

NOTES AND FORMULÆ

Antacids, Comparative Evaluation by Various Methods. R. E. Booth and J. K. Dale. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 694.) Three antacid preparations were tested by 9 methods described in the literature and the results compared. Varying amounts of acid solution, sometimes containing pepsin, were used, the pH being determined at intervals. In some of the methods a quantity of solution was discarded at intervals and more acid added. In each case curves of pH against time were drawn, and considerable differences were observed when the same antacid was tested by different methods. In general, the pH increased more rapidly when the rate of stirring was increased. Increasing the working temperature had very little effect when testing rapid-acting products, such as a mixture of magnesium carbonate, magnesium trisilicate and calcium carbonate or a mixture of magnesium carbonate, magnesium oxide and calcium carbonate, but a much lower pH was reached at the higher temperatures with a preparation containing dried aluminium hydroxide gel and magnesium trisilicate. It was necessary to repeat the experiments several times in order to produce reliable curves. G. B.

Aneurine Hydrochloride, Effect of, on the Stability of Solutions of Crystalline Vitamin B_{12}. B. A. Feller and T. J. Macek. (*J. Amer. pharm. Ass. Sci., Ed.*, 1955, **44**, 662.) Solutions of reaction pH 4 containing vitamin B_{12} with 10 mg./ml. of aneurine hydrochloride were found to be stable at room temperature, but decomposition occurred rapidly on autoclaving, 62 per cent. of the vitamin B_{12} being decomposed in 60 minutes at 120° C. Experiments with solutions containing aneurine hydrochloride and its decomposition products, 2-methyl-4-amino-5-hydroxymethylpyrimidine and 4-methyl-5-β-hydroxyethylthiazole

showed that the decomposition was caused by the thiazole part of the aneurine molecule. Solutions of vitamin B_{12} alone were not decomposed by autoclaving at 120° C. G. B.

Turbidity, Limit Test for. K. Ilver, A. Jackerott and F. Reimers. (*Dansk. Tidsskr. Farm.*, 1955, **29**, 153.) Solutions of commercial chemical products may give turbid solutions owing to the presence of impurities and a limit test is desirable. Kaolin suspensions are not suitable for use as turbidity standards because of the variation between different samples of kaolin and because the particles are coarser than those usually encountered as impurities in commercial chemicals. Barium sulphate suspensions having a suitable particle size may be prepared as follows. Place at the bottom of a test-tube 1 ml. of solution of barium chloride containing 0·5 mg. of barium per ml. in ethanol (85 per cent. w/w) and add 1 ml. of M sulphuric acid, blowing it out from the pipette and shaking all the time. After 5 minutes add 10 ml. of water and mix. The suspension should be used as a standard of comparison within 20 minutes of its preparation. The presence of ethanol improves the reproducibility of the standard. In carrying out the limit test for turbidity, a solution of the sample under test is compared with the standard turbidity in daylight in clear colourless test-tubes observed horizontally in direct daylight against a black background. G. B.

PHARMACOLOGY AND THERAPEUTICS

Acetazoleamide, a Carbonic Anhydrase Inhibitor, Mechanism of the Anticonvulsant Action of. J. G. Millichap, D. M. Woodbury and L. S. Goodman. (*J. Pharmacol.*, 1955, **115**, 251.) The relationship between the anticonvulsant action of acetazoleamide and sulphanilamide and their inhibition of carbonic anhydrase was investigated in mice after oral administration. The time of maximum effect and the relative anticonvulsant potencies of the drugs were determined by the maximal electroshock seizure test. Brain inhibitor concentrations were measured both indirectly, from the degree of inhibition of brain carbonic anhydrase, and directly, by chemical and bioassay techniques. The anticonvulsant ED50 of sulphanilamide was 140 mg./kg. and of acetazoleamide 74 mg./kg., both doses inhibiting the brain enzyme by about 98 per cent. The anticonvulsant activity of the drugs was independent of the secondary metabolic acidosis caused by inhibition of kidney carbonic anhydrase. The time of peak anticonvulsant activity of acetazoleamide (3 hours) and of sulphanilamide ($1\frac{1}{2}$ hours) corresponded with their times of maximal inhibition of brain carbonic anhydrase. *In vitro* at 0° C., acetazoleamide was 100 times more active in inhibiting mouse erythrocyte carbonic anhydrase than was sulphanilamide. Anticonvulsant and brain-enzyme inhibiting potencies of the two drugs are reconcilable with the *in vitro* results, for at the same dose level, the ratio of brain localisation of acetazoleamide compared with sulphanilamide was 1 : 50. Phenobarbitone, trimethadione and diphenylhydantoin, in anticonvulsant doses, did not inhibit brain carbonic anhydrase. In the sulphonamides, a free $-SO_2NH_2$ group was found to be necessary for both anticonvulsant activity and inhibition of carbonic anhydrase; sulphathiazole had neither activity. CO_2 accumulation may be of significance in prevention of seizures by acetazoleamide; also, preliminary experiments show a decrease in total brain sodium and an increased brain intracellular/extracellular potassium ratio. G. P.

Analgesics and Nalorphine, Action of, on the Cough Reflex. A. F. Green and N. B. Ward. (*Brit. J. Pharmacol.*, 1955, **10**, 418.) Some morphine-like

analgesics were compared for antitussive activity on the cough reflex induced in lightly anæsthetised cats by electrical stimulation of the superior laryngeal nerve. This method did not induce the reflex in dogs, so with these animals and with guinea-pigs, coughing was caused by simple mechanical irritation of the tracheal mucosa or by a chemical method (introduction of SO_2 into the tracheal cannula). The suppression of the cough reflex did not appear to depend on the type of stimulus, nor was there any great species difference in the actions of the drugs. The order of antitussive activity of the analgesics, taking methadone, the most potent, as unity, were:—piperidyl amidone 1/2; thiambutene 1/4; morphine 1/8; pethidine 1/20; pholcodine 1/40; and codeine 1/80. The activity of narcotine was negligible. These values for potency are of the same relative order of magnitude as those already known to cause other morphine-like effects. In relation to its antitussive effect morphine depressed respiration most, and pethidine least, in the cat. Nalorphine readily antagonised the antitussive action of the analgesics, as did *N*-propylnormorphine with codeine, but nalorphine had no effect on the cough depressant actions of pholcodine or narcotine. G. P.

Antrycide, Action upon Trypanosomes *In Vitro*. F. Hawking and J. P. Thurston. (*Brit. J. Pharmacol.*, 1955, **10**, 454.) The minimal *in vitro* trypanocidal concentration of antrycide methylsulphate against *Trypanosoma equiperdum*, was 10^{-6} to $2{\cdot}5 \times 10^{-7}$. The trypanosomes were incubated at 35° C. for 20 hours with a horse serum—Tyrode mixture containing the drug. For an antrycide-resistant strain the minimal effective concentration under the same conditions was only four times greater; this resistant strain was unaffected by maximum tolerated doses *in vivo*. The power of normal trypanosomes to infect mice was destroyed by *in vitro* exposure to antrycide 10^{-6} for 5 hours at 35° C., whereas antrycide-resistant trypanosomes still retained high infective power after similar exposure to a concentration of 4×10^{-6}. This property of antrycide to abolish the infectivity is probably more important for its therapeutic action *in vivo* than is its direct trypanocidal action *in vitro*. Similar loss of infectivity is seen with suramin and phenanthridinium compounds. A possible explanation is that these compounds suppress the multiplication of the trypanosomes. G. P.

Benactyzine, General Pharmacology of. V. Larsen. (*Acta pharm. tox., Kbh.*, 1955, **11**, 405.) Benactyzine (benzilic acid diethylaminoethylester hydrochloride, Suavitil) was first synthesised during a systematic search for compounds with atropine-like activity. It has a low, both acute and chronic, toxicity when given to miec, rats, guinea-pigs, rabbits and cats. It reduces the barium-induced spasm and it also has an antiacetylcholine effect when tested on the isolated intestine of the guinea-pig and on the heart and the blood pressure of the rabbit. An anticholinergic effect on salivary secretion and on the movements of the stomach *in situ* after vagal stimulation can be demonstrated. It has strong local anæsthetic properties. It does not lower the body temperature in rabbits and it has neither adrenergic blocking nor ganglion blocking actions. In man benactyzine has a peculiar and rather specific effect on a series of higher functions of the brain, leading to some blocking of the thoughts, a certain insusceptibility to unpleasant mental impressions and decreased power of decision. It has no hypnotic effect *per se*, although it produces some drowsiness and dizziness and a feeling of considerable relaxation in the limb muscles. M. M.

Cardiac Glycosides, and Other Compounds, Effects of, on Cation Transfer in Human Erythrocytes. J. B. Kahn and G. H. Acheson. (*J. Pharmacol.* 1955, **115**, 305.) The influence of drugs on the cation exchange of human erythrocytes was studied employing standard techniques for separation of cells and plasma. Cation flux in fresh cells under steady state conditions was studied using radioactive potassium, ^{42}K. Concentration of cations was measured photometrically. Most values are recorded as plasma potassium (K_p) concentration and cellular sodium (Na_c) concentration, representing a shift of compartment of Na or K, with whole-blood values remaining constant. On cold storage, K_p concentration increased whilst Na_c concentration increased to a greater extent, giving a net gain of intracellular cation. Incubation at 37° C. reversed these changes, the cells gaining K and losing Na. In incubated cold-stored cells, the net influx of K was greater than the total influx of ^{42}K in fresh blood. Ouabain, strophanthidin, 3-acetyl strophanthidin, digitoxin, and desacetyl lanatoside-C, in suitable concentrations, completely inhibited the active phase of cation transport, and the flux of cations was slightly reversed. These changes were attributed to a block of the metabolic phase of cation transport. There were up to 30-fold differences of potency among the glycosides and genins tested, which did not result from differences in plasma binding as tested with ouabain and digitoxin. In glycoside-treated fresh blood, the rate of net efflux of K_c corresponded closely with the total efflux of K in equilibrium conditions (as deduced from the influx of ^{42}K) and remained roughly constant at one, two and four hours of incubation, despite a rise of K_p and a fall of K_c. Net efflux of K did not correlate with either K_p concentration, or the concentration gradient (K_c–K_p), but there was close correspondence with K_c concentration. Angelicalactone, butyrolactone and propiolactone, in a concentration one million times greater than ouabain, also inhibited cation transport. Other drugs tried, but which were without detectable effect in the concentration used (10^{-4} to 10^{-5}) included several pure veratrum alkaloids, andromedotoxin, adrenaline, tetraethylammonium, tubocurarine, 2 : 4-dinitrophenol and acetazoleamide. The presence of a lactone ring in a molecule does not necessarily confer activity in blocking cation transport. In active lactones, potency is enhanced by a *cyclo*pentanophenanthrene ring, the degree of increase possibly being related to the number and position of –OH groups on the steroid moiety, and the position of the double bond in the unsaturated lactones. G. P.

Chlorpromazine Hydrochloride, Side Effects of. E. M. Glaser and P. S. B. Newling. (*Brit. J. Pharmacol.*, 1955, **10**, 429.) The incidence of side effects of chlorpromazine, in single doses of 25 and 50 mg., was compared in normal healthy subjects with those of 0·75 mg. hyoscine hydrobromide and a lactose placebo. Each drug was given orally in tablet form, neither subjects nor experimenters knowing the identity of the tablets until completion of the tests. The 25 mg. dose of chlorpromazine had little effect, but with 50 mg. the effects produced were similar to those of the hyoscine, particularly sleepiness, tiredness and dryness of the mouth. However, 50 mg. chlorpromazine increased the heart rate, while the hyoscine reduced it. When subjects, previously given the placebo, hyoscine or the 25 mg. dose of chlorpromazine, received further similar doses of the same drugs, the frequency of symptoms reported after the fifth successive daily dose was significantly different from that obtained with the first dose (i.e., habituation to the experimental procedure had taken place). This did not occur with the 50 mg. dose of chlorpromazine, there being no significant difference between first and fifth doses; this suggests that this dose of chlorpromazine was inhibiting the induction of habituation, probably by depression of the cerebral cortex. G. P.

α-Cocaine. R. Foster, H. R. Ing and V. Varagić. (*Brit. J. Pharmacol.*, 1955, **10**, 436.) Contrary to the accepted statement by Willstatter that α-cocaine was without local anæsthetic activity, tests by the guinea-pig intradermal weal method and by application to the lumbar nerve plexus of frogs show it to be one-fifth as active as cocaine by the first method and three-fifths as active by the second. α-Cocaine inhibited, to the same degree, amine oxidase preparations from homogenates of cat and rabbit liver, of the nictitating membrane of the cat and of rabbit's uterus. However, on the isolated uterus, duodenum and ear preparations of the rabbit, α-cocaine either reduced the effects of adrenaline or had no effect. Cocaine, on the other hand, always potentiated the actions of adrenaline and noradrenaline on the uterus and perfused ear. There was occasional potentiation, by α-cocaine, of the action of adrenaline on the isolated auricles of the rabbit, but this was always less than that produced by cocaine. In the spinal cat, α-cocaine had no effect on the pressor action or contraction of the nictitating membrane caused by adrenaline and noradrenaline, although the doses of α-cocaine used were five times larger than doses of cocaine having a marked potentiating effect on the actions of the catechol amines. These results indicate that the suggestion by Burn and Robinson (*Brit. J. Pharmacol.*, 1952, **7**, 304), that cocaine potentiates adrenaline and noradrenaline through inhibition of amine oxidase, is incorrect. G. P.

Compound 48/80, Effect of, on Mammalian Skeletal Muscle. G. Sömjén and I. E. Uyldert. (*Brit. J. Pharmacol.*, 1955, **10**, 413.) The histamine liberator, compound 48/80, blocked neuromuscular transmission *in vivo* in the rat and rabbit, and in the isolated rat diaphragm—phrenic nerve preparation. The response of the muscles to direct stimulation was not affected. In some ways the block resembled that of (+)-tubocurarine in that a tetanus was not maintained during partial block and there was some post-tetanic reversal of the block; also, a dose of (+)-tubocurarine given between two 48/80 injections potentiated the effects of the second injection. In addition, 48/80 caused no contraction or contracture of the chronically denervated gastrocnemius muscle of the rat. However, neuromuscular paralysis, complete or partial, was not antagonised by anticholinesterases. Paralysis was apparently due to an action of the drug itself and not through histamine release, since there was no tachyphylaxis; nor did histamine reproduce the paralysis, or mepyramine antagonise it. G. P.

Cycloserine in Urinary Infections. R. D. Herrold, A. V. Boand and M. Kamp. (*Antibiotic Med.*, 1956, **1**, 665.) This report is based on clinical and bacteriological observations of 124 patients given cycloserine during a period of 8 months. All the patients were refractory cases who had failed to respond to intensive treatment with other antibiotics. Three groups of patients were studied, those with bladder or bladder and upper urinary tract infections, those with lower urinary tract infections (except gonococcic), and those with gonococcic infections. Among the 49 patients in the first group the predominant organisms were *Escherichia coli*, 17; *Proteus*, 14; followed by *Aerobacter aerogenes*, *Pseudomonas*, *Paracolon* and 2 instances each of staphylococci and streptococci; many were mixed infections. Of the 49 patients, there were 24 cures, 20 failures and 5 with insufficient follow-up. The group of 19 patients with lower urinary tract infections included cases of prostatitis with or without symptomatic urethritis; 12 had gram-positive flora, while 7 had some type of gram-negative bacilli usually mixed with gram-positive organisms. In this group there were 10 cures, in 3 of which cycloserine was

given concurrently with streptomycin, 5 failures, and 4 with insufficient follow-up. The third group consisted of 56 gonococcic infections, all in male patients. There was almost no improvement, clinically or bacteriologically, in any of these patients. The side reactions with cycloserine were few and consisted chiefly of vertigo, drowsiness, light headedness and ocular disturbance; there were occasional complaints of nausea, but no other gastro-intestinal disturbances. Children tolerated the drug well, the poorest tolerance being in elderly patients. The optimum dose is 1 g. daily by mouth in divided doses. The action appears to be bacteriostatic and often, in the more chronic infections, a favourable response is not evident in less than 10 to 14 days of medication, which would appear to be the minimum period of administration. In general, resistance to cycloserine does not seem to develop quickly. In several instances there seemed to be an advantage in combining cycloserine with streptomycin. S. L. W.

Dextran-Iron Complex in Hypochromic Anæmia. A. Grunberg and J. L. Blair. (*Arch. intern. Med.*, 1955, **96**, 731.) A dextran-iron preparation (containing 50 mg./ml. of iron) suitable for intramuscular use was given to 30 patients suffering from hypochromic anæmia. The response was satisfactory and was indistinguishable from that obtained with intravenous iron therapy. The total amount of iron administered was given in divided doses commencing with two injections of 100 mg. each and continuing with 250 mg. on subsequent occasions; injections were given on alternate days. Injections were given into the upper third of the outer side of the thigh. Apart from slight transient brownish discolouration at the site of injection in a few cases there were no local reactions. None of the patients showed any constitutional disturbance and all claimed to feel definitely better within 7 to 10 days of commencing treatment. By the time the final injection was given there was in all cases a significant increase in the hæmoglobin level. Details are given of 5 cases. S. L. W.

2-Diethanolamino-5-nitropyridine, Amœbicidal Action of. R. A. Neal and P. Vincent. (*Brit. J. Pharmacol.*, 1955, **10**, 434.) The amoebicidal activity of 2-diethanolamino-5-nitropyridine (263C49), was equal to that of chiniofon on rats experimentally infected with an invasive strain of *Entamœba histolytica*. With non-invasive strains of *E. histolytica* chiniofon was more effective, and 263C49 less effective, than on the invasive strains. 263C49 had no effect on experimentally-induced amœbic liver abcesses in the hamster. Toxicity of 263C49 was low and the drug was rapidly excreted in the urine. *In vitro* the new drug had a direct action, in the same minimal concentrations as chiniofon, on amœbæ grown on a horse serum—eggwhite medium; on one strain 263C49 was ten times more active than chinifon. In a small clinical trial with large doses of 263C49 (51 g. given orally over ten days) all cases were rapidly cleared of symptoms, but a proportion showed parasitological relapse after termination of treatment. G. P.

Glutethimide (Doriden), Clinical Trial of. M. Rushbrooke, E. S. B. Wilson, J. D. Acland and G. M. Wilson. (*Brit. med. J.*, 1956, **1**, 139.) Glutethimide (α-phenyl-α-ethyl-glutarimide), a new hypnotic, was investigated in a general practice, using ranking methods, and its effect compared with that of cyclobarbitone and an inert tablet. The trials were conducted on 18 patients, all of whom had previously been taking a barbiturate. Glutethimide 0·5 g., cyclobarbitone 0·2 g., and inert tablets were prepared so that they were identical in appearance, and all contained 3·6 mg. of quinine sulphate so that the taste was similar and the barbiturate could not be distinguished. In the dosage used glutethimide was found to

compare favourably with cyclobarbitone. Fourteen of the patients put the placebo in the third phase of preference, 10 chose glutethimide for first place and 6 cyclobarbitone. The estimates of the times in getting to sleep after each treatment were: glutethimide 71 ± 8·5 minutes; cyclobarbitone 84 ± 10·7 minutes; placebo, 138 ± 20·8 minutes. It was impossible to make any satisfactory analysis of the duration of sleep. Drowsiness on the following morning was noticed on 7 occasions after cyclobarbitone and on 4 occasions after glutethimide. After completion of this trial glutethimide was given as a hypnotic to 30 patients, either in hospital or general practice. The length of the courses varied from 3 to 80 days and the dose was 0·25 or 0·5 g. at night. No definite evidence of habituation or tolerance was obtained. In two patients a skin rash developed, but disappeared within 2 days after discontinuing the drug. In one patient mental confusion and excitement developed 1½ hours after giving 0·5 glutethimide and persisted for 3 hours; a second dose was given 4 days later with a similar result. Some statistical considerations in the use of ranking methods are appended to this paper. S. L. W.

Heparin, Function of. J. F. Riley, D. M. Shepherd, G. B. West and S. W. Stroud, (*Nature, Lond.*, 1955, **176**, 1123.) Both heparin and histamine are concentrated in tissue mast cells. In the dog release of histamine, caused by damage to the mast cells by peptone or by compound 48/80, is accompanied by the realease of heparin and a consequent increase in the clotting time of blood. This dual effect has been found only in the dog. To determine what happens in the rat following a maximal release of histamine the animals were given doses of 48/80 over a period of 5 days. At the end of this time there was widespread degranulation and disruption of the mast cells in the subcutaneous tissue. 94 per cent. of the histamine but only 53 per cent. of the tissue heparin was lost. This loss of heparin was unaccompanied by any sign of the release of the heparin into the circulating blood, since the clotting time remained normal.

Thus the almost complete release of histamine from the subcutis of the rat by compound 48/80 is accompanied by a loss of only half the associated heparin. Some of the metachromatic material from the disrupted mast cells may be disposed of locally by macrophages, some may adhere to adjacent connective tissue fibrils or cells, while some may be bound by the basic histamine-liberator itself. Although these same basic compounds can release active heparin into the blood stream of the dog, they fail to do so in the rat. The rabbit and the guinea-pig are similar to the rat. This suggests that the function of heparin may be concerned rather with events in the tissues than with the coagulability of the circulating blood. M. M.

Heparin Preparations. Assay of. J. E. Jorpes. (*Acta pharm. tox., Kbh.*, 1955, **11**, 367.) Experience of different methods for the determination of the anticoagulant activity of heparin preparations is summarised. Because the effects of heparin in the blood are both on thromboplastin formation in plasma and on thrombin action, fresh whole blood is considered to be the best medium for determining the relative strength of heparin preparations. Some drawbacks of the thrombin methods and the methods with recalcified oxalated or citrated plasma, or other artificial coagulation systems, are demonstrated. Details of an *in vivo* method are given, in which the heparin is injected intravenously into sheep and the subsequent coagulation time of the blood determined. Such a method gave results in good agreement with the fresh whole blood technique. For the assay of heparin samples to be used clinically, preference is given to the methods using fresh whole blood. M. M.

21-Hydroxypregnanedione Sodium Succinate, Pharmacological Properties of. S. Y. P'an, J. F. Gardocki, D. E. Hutcheon, H. Rudel, M. J. Kodet and G. D. Laubach (*J. Pharmacol.*, 1955, **115**, 432.) Hydroxydione, (21-hydroxypregnanedione sodium succinate, Viadril or P-55) a water-soluble steroid had pronounced central nervous system depressant activity in mice, rats, rabbits, dogs and monkeys. It produced surgical anæsthesia after either oral or intravenous administration. Onset of anæsthesia was smooth, without pre-anæsthetic excitement and recovery was rapid. Duration of anæsthesia varied with the dose given. With hydroxydione there was less cardiac or respiratory depression than with thiopentone sodium. Acute toxicity was low so that therapeutic indices after intravenous administration (11·6 in mice, 7·8 in rats and 6·3 in rabbits) were considerably greater than those of thiopentone sodium. There was no evidence or androgenic, œstrogenic, progestational, corticoid or gonadotrophic activity with the steroid. Also there was no sex specificity in anæsthetic activity of toxicity, as has been found in other cases of steroid anæsthesia. Liver damage or nephrectomy did not affect either intensity or duration of anæsthesia with hydroxydione. Preliminary results in over 100 human operations confirm the superiority of the steroid over the thiobarbiturates in clinical applications in basal or general anæsthesia. G. P.

Levallorphan Tartrate, Effects on Respiration of Rabbits given Morphine. J. W. Miller, T. M. Gilfoil and F. E. Shideman. (*J. Pharmacol.*, 1955, **115**, 350.) The duration and character of levallorphan antagonism of morphine-induced respiratory depression in unæsthetised male rabbits was examined. 4 mg./kg. morphine i.v. caused 50 per cent. reduction in respiratory minute volume, with only partial recovery in 6 hours. Levallorphan, 30 minutes after morphine, gave antagonism with degree and duration directly proportional to dosage employed. Antagonism by levallorphan was not maintained. 32 mg./kg. morphine had a respiratory stimulant component, the net resulting depression being less than with 4 mg./kg.; against this the antagonism of small doses (2·5 to 5·0 mg./kg.) of levallorphan was of short duration: at 2 hours the minute volume was less than that of animals not receiving the antagonist. Curves relating degree of antagonism and dose of levallorphan at 30 minutes indicate a relationship with morphine dose employed, rather than degree of respiratory depression. The levallorphan dose required to antagonise 4 mg./kg. morphine depression back to control levels, increased with time, reaching a maximum in 2 to 3 hours and declining thereafter. Respiratory rate was depressed 50 per cent., and tidal volume increased 20 per cent. in morphinised (4 mg./kg.) animals: the tidal volume was not greatly altered by levallorphan but respiratory rate increased significantly. Increased minute volume following levallorphan administration is primarily due to increased respiratory rate superimposed on pre-existing elevated tidal volume. Codeine phosphate also antagonised morphine respiratory depression. Levallorphan alone produced stimulation or depression of respiratory minute volume, depending on dosage and time after administration. Authors interpret their results on the basis of competition for receptor sites between two pharmacologically similar agents, the less potent (levallorphan) having the greater receptor affinity. The combined effect of levallorphan and morphine on respiration at any given time, is regarded as the summation of processes producing depression and stimulation; the sum in each case depending on the dose of each agent administered, balanced against its destruction, metabolism and excretion. G. P.

Mersoben, Clinical Evaluation of. R. H. Chaney and R. F. Maronde. (*Amer. J. med. Sci.*, 1956, **231**, 26.) This is a report of an investigation to determine the diuretic activity and side effects of the compound Mersoben, 3-[2-hydroxy-3(-D-gluco-pentahydroxyhexyl-mercaptomercuri)-propyl]-D-mannitol, dispensed as a lyophilised, white, amorphous, hygroscopic solid in 2 ml. ampoules, which is readily dissolved in distilled water immediately before intramuscular or subcutaneous injection. Eighty-five patients were given a single intramuscular injection of Mersoben; most of the patients were chronic cardiac cases, who had previously been receiving meralluride for many weeks. Another group of 32 patients was given injections of meralluride for comparison. The efficacy of diuretic activity was based on a loss of weight of over 3 pounds within 24 hours following injection. On this basis Mersoben was shown to be a potent diuretic comparing favourably with meralluride. A comparison of mercury levels in the organs of an additional 15 terminal patients who had received no mercury other than one injection of Mersoben with those of patients who had received meralluride showed the values of the former to be lower for all organs except the brain. Side-effects of Mersoben were minimal with the doses used, and there was no evidence of local, renal or systemic ill effects, except infrequent local pain and rare sensitivity reaction (a mild, generalised skin rash developed in 3 patients). The development of "low salt syndrome" should be guarded against with Mersoben as with other mercurial diuretics. S. L. W.

Nitrofurantoin in Urinary Tract Infections. W. A. Richards, E. Riss, E. H. Kass and M. Finland. (*Arch. intern. Med.*, 1955, **96**, 437.) Thirty-nine hospital patients with urinary tract infections were treated with nitrofurantoin in doses of 100 to 200 mg. four times daily for periods ranging from 2 to 13 days, and averaging 7 days. The patients ranged in age from 14 to 93 years. Favourable clinical results were obtained in the great majority of acute and uncomplicated infections, but in a much smaller proportion of chronic cases with underlying complicating conditions in the urinary tract. Only 1 of 17 infections with *E. coli* failed to show any improvement during treatment, whereas in 6 of 13 cases of infection with *Aerobacter aerogenes* and 6 of 9 cases with *Proteus* infection there was no improvement. Of the 12 patients in the series without recognised complication only 1 failed to show any improvement and 8 showed complete subsidence of the clinical manifestation while on treatment. No consistent effectiveness was noted in the cases of acute pyelonephritis. Toxic effects were minor and mostly consisted of nausea and vomiting that was often related to the dose used. Sensitivity reactions were not encountered. Levels of nitrofurantoin in blood and urine, as determined by antibacterial action, indicated that effective concentrations are excreted in the urine but that useful levels are not attained in the blood on oral therapy. *In vitro* studies, including growth curves, indicate that the antibacterial action of nitrofurantoin is considerably depressed at the highly alkaline pH attainable in urine or when there is an excessive concentration of organisms. These studies also confirm the primary bacteriostatic action of the drug with a bactericidal effect on more susceptible strains at higher concentrations. S. L. W.

Pentamidine in the Treatment of Moniliasis. A. Stenderup, J. Bichel and F. Kissmeyer-Nielsen. (*Lancet*, 1956, **270**, 20.) *In vitro* experiments have shown that even low concentrations of stilbamidine and pentamidine often prevent the growth of *Candida albicans*. Three cases of moniliasis in patients with chronic malignant disease were successfully treated with pentamidine administered intramuscularly in a dose of 200 mg. twelve-hourly or daily for a week or ten days. S. L. W.

Prednisone and Prednisolone in Lupus Erythematosus. A. J. Bollet, S. Segal and J. J. Bunim. (*J. Amer. med. Ass.*, 1955, **159**, 1501.) This is a study of the effects of prednisone and prednisolone therapy (no difference in potency effectiveness or side-effects was noted between the two drugs) in 10 patients with systemic lupus erythematosus followed for an average period of 4 months. Nine of the 10 patients had been receiving hormone therapy (cortisone, hydrocortisone and corticotrophin) prior to admission without satisfactory control of the disease; they were having continual symptoms or periodic exacerbations of activity of the disease. The initial suppressive daily dose of prednisone varied between 20 and 60 mg. and averaged 35 mg. Maintenance doses varied between 5 and 30 mg. per day, averaging 18 mg. Comparison of this maintenance dose with the previous maintenance dose of steroid shows that prednisone has about 4 times the potency of cortisone and a little over 3 times that of hydrocortisone in controlling the manifestations of this disease. None of the patients had a complete and sustained remission, but when the maintenance dose had been reached 8 of the patients felt that they were stronger, free of annoying minor symptoms and functionally improved in contrast to their status on previous therapy. Prednisone was shown to be capable of diminishing the fever, chills, malaise, anorexia, arthritis, rash, mucous membrane lesions, cough, pleuritic and precordial pain, chest wall tenderness, pleural and pericardial friction rubs, pulmonary rates, abdominal pain and tenderness, headache, convulsive seizures, leucopenia, elevated sedimentation rate, and C-reactive protein. Renal abnormalities were in general not improved. Oedema diminished gradually during therapy. Anæmia did not improve, and alterations in serum albumin and globulin levels were only slight. Minor undesirable side-effects were seen in all 10 patients. The authors conclude that the effectiveness and limitations of prednisone and prednisolone in the treatment of systemic lupus erythematosus parallel closely those of cortisone and corticotrophin with the exception that the new steroids do not cause sodium and water retention or potassium loss when given in moderate therapeutic dosage.

S. L. W.

Probenecid, Cinchophen and Colchicine, in Gout. S. Gjørup and H. Poulsen. (*Acta pharm. tox., Kbh.*, 1955, **11**, 343.) The purpose of this paper is to investigate whether probenecid and cinchophen act on other oxypurines besides 2:6:8-trioxypurine (uric acid) and also whether colchicine acts on 2:6-dioxypurine (xanthine) and 6-monoxypurine (hypoxanthine). The uric acid and also the hypoxanthine and xanthine concentrations in the plasma and urine were determined by an enzymatic spectrophotometric method. It was found that probenecid and cinchophen caused an increase of the uric acid excretion in gouty patients. At the same time the uric acid plasma level fell. The action of these substances was of the same order as in normal individuals. Colchicine had no influence on uric acid excretion or on the level of uric acid in the plasma. The excretion and plasma levels of hypoxanthine-xanthine remained unchanged after administration of both probenecid, cinchophen and colchicine. None of the three drugs influenced the glomerular filtration rate.

M. M.

Reserpine and Chlorpromazine, Effects of, on Gastric Secretion. B. J. Haverback, T. D. Stevenson, A. Sjoerdsma and L. L. Terry. (*Amer. J. med. Sci.*, 1955, **230**, 601.) Studies in patients have shown that reserpine given orally and intravenously increases the volume and acidity of gastric secretion in man. There is also evidence that reserpine stimulates the motor function of the gastro-intestinal tract. Chlorpromazine given intramuscularly reduced

the volume of gastric secretion but did not change the free acidity—results similar to those following the administration of atropine. It is suggested that chlorpromazine is the tranquillising agent of choice when stimulation of gastric secretion is contraindicated. G. F. S.

***NNNN'*-Tetraethyl-*N'N'*-dimethyl-3-oxapentane-1 : 5-diammonium Di-monohydrogen Tartrate, a new Ganglionic Blocking Agent, Pharmacology of.** J. Fakstorp, E. Poulsen, W. Richter and M. Schilling. (*Acta pharm. tox., Kbh.*, 1955, **11**, 319.) The acute toxicity of this bis-quaternary ammonium salt was determined by intraperitoneal injection into mice. The LD50 was found to be 183 mg./kg. Intravenous administration to rabbits of 10 times the expected therapeutic dosage, for three months, caused no pathological changes other than those associated with ganglion blocking action. The ganglion blocking action of the compound, as determined on the nictitating membrane of the cat and on the isolated ileum of the guinea-pig, was found to be somewhat greater than that of hexamethonium. It had very little anticholinergic, antihistaminic or musculotropic spasmolytic activity. In the cat and rabbit it caused a marked hypotensive effect. The blood pressure response to adrenaline was increased and the response to carotid artery occlusion was diminished. The absorption and excretion were studied by the reineckate method, after intravenous, subcutaneous and oral administration in rabbits. M. M.

BACTERIOLOGY AND CLINICAL TESTS

Bacteria-excluding Filters for Oils. K. E. Avis and L. Gershenfeld. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 682.) Various filters were tested by passing corn oil continuously through them for 84 days using alternating vacuum and gravity filtration. The filters examined included unglazed porcelain and diatomaceous earth candles, an asbestos pad and a sintered glass filter. Certain of the candles developed a relatively high rate of flow under vacuum and a decreased carbon-dioxide-water bubbling pressure during the course of the experiment. The increase in permeability was accompanied by lowering of effectiveness of the filters in retaining micro-organisms. Prolonged contact with corn oil rendered the diatomaceous earth filter candles more fragile and unreliable for the retention of *Bacillus cereus* spores and *Serratia marescens* organisms. A Selas 015 porcelain candle also appeared to be unsuitable for the sterilisation of corn oil by filtration. G. B.

***Bacteroides*, Sensitivity of Four Species of, to Antibiotics.** L. P. Garrod. (*Brit. med. J.*, 1955, **2**, 1529.) Sensitivity tests were made using plates prepared from meat extract peptone agar with added antibiotic solution and horse blood freshly lysed with saponin. The surface of the medium was divided into 12 compartments, each of which was inoculated with a different strain of *Bacteroides*. Plates were examined after incubation anærobically with 5 per cent. of carbon dioxide for 2–5 days. The minimum inhibitory concentration of penicillin, streptomycin, oxytetracycline, erythromycin, polymixin and bacitracin was determined for a total of 55 strains of *Bacteroides*. Penicillin was active against *Bacteroides fusiformis*, *necrophorus* and *melaninogenicum*. *Bacteroides fragilis* was the species most sensitive to oxytetracycline, but was resistant to penicillin. Chloramphenicol was moderately active against all species, but less active than oxytetracycline. All species were relatively resistant to streptomycin. For the treatment of infections in which the organisms have not been isolated and tested for sensitivity, penicillin is the most suitable antibiotic, except for infections of the lower bowel. These are commonly due to *Bacteroides fragilis*, for which oxytetracycline is suitable. G. B.

BOOK REVIEW

TEEANALYSE, by L. Hörhammer. 75 pages + 60 full page plates comprising 556 photographs. Institut für Pharmazeutische Arzneimittellehre der Univesität, Munich, 1955. DM.58.00.

The identification of vegetable drugs in the chopped condition calls for a detailed and applied pharmacognostical knowledge. The Atlas under review describes and figures those morphological characters used to identify 275 crude drugs which have been found in herbal teas occurring in current German pharmaceutical practice. All are normal drugs prepared in tea form by chopping and admixture. The following are described: 30 leaves, 77 herbs, 34 flowers, 24 fruits, 8 seeds, 7 woods, 25 barks, 36 roots, 12 rhizomes and 22 miscellaneous drugs. Each drug is photographed in the chopped condition to show its diagnostic characters at a magnification × 2; for comparison a photograph of the entire drug × 1 is given and in this way the significance of the diagnostic characters is emphasised. Adulterants are shown in some of the photographs. The 60 full page plates comprising 556 photographs are unbound and contained in a folder. The text to the Atlas is a book of 75 pages containing for each drug the Latin and common German name, biological sources, a concise description of the chopped sample, together with chemical tests and common adulterants where applicable, also brief references to diagnostic microscopical characters; drug constituents, actions and uses are recorded and finally detailed legends to the Atlas photographs are given. Arrangement of drugs is in morphological groups; the text contains a brief introduction to each group showing the classification employed within that group, e.g., colour, surface characters, shape. The author claims that chopped drugs which are superficially alike and may be confused are in this way considered side by side and so by picture and by description they are more certainly differentiated.

The photographs comprising the plates are of excellent quality with good definition showing every detail. As many as 20 to 30 pieces of one chopped drug are photographed and these have been well selected to illustrate the complete range of diagnostic characters; they alone form a most valuable record of the morphology of these crude drugs. The author is probably wise in maintaining the one fixed magnification of × 2 for all photographs of chopped drugs, but with such excellent photography certain of the smaller specimens, e.g., coriander, cardamom seed, mustard seed, would have displayed their diagnostic characters to still better advantage at a higher magnificaion. The majority of the drugs considered could be identified by means of the excellent photographs alone, but the text is also valuable in describing the drug characters and in outlining the methods of subdivision within the groups. These subdivisions are not always those normally applied to the entire drugs; thus flowers are classified according to their colour and in consequence such natural groups as the Composites and the Labiates are scattered throughout the three plates of photographs. In consequence the author might with advantage have incorporated definite analytical keys, especially for the larger groups of drugs. Such criticisms are, however, minor ones and apart from its high price and the necessity to read German the Atlas can be fully recommended as an excellent compilation and photographic record of this group of drugs.

J. M. ROWSON.