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SYMPOSIUM*

PLASTICS IN PHARMACY

NATURE AND PROPERTIES OF PLASTICS

By C. L. Child, B.Sc., Ph.D.

Plastics Division, Imperial Chemical Industries Ltd., Welwyn Garden City, Herts.

The group of materials known as "plastics" is hard to define unambiguously and yet comprehensively, but easier to describe. It is a group of new materials of construction, numbering several hundreds described in the literature and in patents, but of which only about a score are as yet of commercial importance.

A member of this group may have some properties which differ, not only in degree, but in kind from those of another, but in certain respects each member differs from the others only in a small degree. It is this essential similarity which enables us to group them together under the description "Plastics."

These are their important similarities; 1. Plastics are synthetic, of high molecular weight and with complex molecules; 2. Plastics are usually organic chemicals. There is the important exception of the silicone family of plastics which essentially is based on the inorganic element silicon; 3. Plastics are sensitive to temperature, and at some period in their history there is a temperature at which they can be made to flow in a controlled manner under applied pressure. Some retain this property throughout their history, others, having reached this temperature, undergo a chemical change and become infusible; 4. Plastics have limited temperature ranges of usefulness, becoming more brittle as the temperature decreases, softer as it rises. The lower limit may be $-100°$ C. or even much lower; the upper limit never exceeds $350°$ C. and generally lies in the range 70 to $90°$ C.; 5. Plastics are of low specific gravity—generally in the range sp.gr. 0·9 to 2·5; 6. Plastics have mechanical strengths approaching those of metals measured on a strength to weight basis, but much lower stiffness; 7. Plastics are electrical insulators; 8. Plastics have low thermal conductivity; 9. Plastics are generally resistant to inorganic chemicals, but in general are attacked or dissolved by organic chemicals.

It will be seen from 1 and 2 that there are no clear cut dividing lines between plastics on the one hand, and fibres and textiles, or rubbers, or paints and varnishes on the other. It is this overlap that is responsible for the difficulties in defining the term "Plastics."

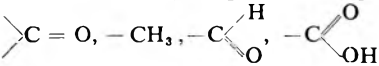
The Nature of Plastics

It is sometimes said that plastics can now be "tailor-made" by the chemist to meet a particular application. This is an over-statement. It

* Held on the occasion of the British Pharmaceutical Conference, Aberdeen, 1955, with Professor J. P. Todd in the chair.

is true that modern physico-chemical theories enable us to correlate some of the properties of a plastic material and specific features of its molecular "make-up" and from this knowledge we can forecast to some extent the kind of molecule and molecular arrangements which must be obtained in order to give a product with certain properties. To this extent the plastics chemist can "build-in" desirable properties in a new material, but our ability to do this is still limited by the complexity of the molecules and their arrangements. This can be illustrated by a consideration of the problems encountered with the (chemically) simplest plastic, polythene.

This plastic is made by polymerising ethylene of high purity at high temperature and pressure. The chemical process is often illustrated thus: $m(CH_2 = CH_2) \rightarrow -CH_2-CH_2-CH_2 \ldots . CH_2-CH_2-$. The polymer chains so formed can be of any desired length—hundreds or thousands of ethylene units long. By assuming this kind of picture for a polythene molecule, we can account for some of the properties of the polymer. For example, it is a simple straight chain hydrocarbon, therefore we may expect a material which is chemically rather inert, electrically highly insulating, with a crystalline structure and a sharp melting point, and which is rather brittle—in short, resembling any other paraffin. In fact the material behaves chemically as we expect, and it is an outstandingly good electrical insulator. It does have a crystalline structure—but only in part; partly it is amorphous. It therefore has a sharp melting point, and also a wide softening temperature range. Furthermore, it is flexible and remarkably tough, becoming brittle only at low temperatures, sometimes lower than $-100°C$.

It is apparent that our simple picture of a molecule, $-(CH_2)_{\overline{m}}$, is incomplete. Some of the factors which we have not considered—but must—are (1) the value of "m," that is, the length of the chain, (2) the terminal groups of each chain, (3) the possibility of chain-branching, its frequency and the lengths of any side chains, and (4) the presence of groups, even in minute quantities, such as  , and so on, arising from the presence of the catalyst or traces of impurities. And, of course, even if all the molecules were simple straight chains, we would not expect "m" (the molecular weight, in effect) to be constant for every molecule in a given sample. We should, on the contrary, expect to find a normal frequency distribution curve applying, so that we have to replace factor (1) above by, (1a) average chain length (or molecular weight), and (1b) chain length spread, (or molecular weight spread). It is unnecessary here to pursue these complexities further, so long as their existence is remembered, and it is appreciated that these and many related difficulties severely limit our ability to forecast, or fully understand, the properties of plastics.

With this qualification we can make some generalisations. Thus we can safely say that plastics owe their importance to the fact that they consist of macro-molecules—long chains which are entangled with one another. If the chains are flexible and have little attraction for one

another, then, under the influence of heat the chains can easily slide over one another, and this is manifested in a low softening point. If we want to raise the softening point, we can (i) increase the interchain attractions, for example, by introducing polar groups into the molecules; or (ii) we can stiffen the chains, for example, by introducing large groups which impede free rotation about C — C links in the molecule, or (iii), we can do both. Finally, we can modify the properties of the material drastically by chemical substitution and by linking chains together by strong "chemical" bonds, so giving a vast irregular network which is in effect a super giant molecule, weighing perhaps several pounds. If the links are numerous as well as strong, and the chains stiff, we can raise the softening point to such an extent that the material becomes infusible, decomposing before it can soften.

On the other hand, it is possible to lower the softening temperature of a given polymer by what may be called "external chemical means." This is the process of "plasticisation," in which polymer molecules are partly "solvated" by a poor solvent for the material. The effect of the solvation is to reduce interchain attraction directly, and also (indirectly) by increasing interchain distances. As a result, the polymer molecules can more easily slide over one another, and this is shown by increased flexibility, lower strength, lower softening temperature and retention of flexibility to much lower temperatures. Most polymers can be plasticised, and the choice of plasticiser in any instance is governed primarily by the chemical nature of the polymer. The application of these general principles will be illustrated in the consideration of individual materials later.

The Processing of Plastics

Much of the importance of plastics lies in the fact that their behaviour towards heat makes possible mass production to close tolerances at relatively low cost. Thus, with plastics such as polythene, consisting essentially of long entangled chains with no strong forces between them, heating to a moderate temperature produces a soft mass which flows very easily under pressure and which hardens again on cooling. If the material is cooled while it is held in an impressed shape, then that shape is retained when the plastic is cold. Materials behaving in this way are "thermoplastic." There is a second group of plastics which behave differently when heated, in that again they first soften and flow under pressure, but they then undergo a chemical reaction which leads to the linking of one molecular chain to another giving a rigid infusible material. Such materials are "thermohardening" or "thermosetting."

In commercial practice, thermoplastics are handled in the following basic ways; 1. Extrusion, in which the cold material is fed into a heated barrel, along which it is forced by an Archimedean screw. The material heats by friction and by contact with the hot cylinder, softens and squirts out of an orifice (die) at the end of the barrel, taking the shape of the orifice as its cross-section. The extrudate is quickly cooled in air or in water before it can deform; 2. Injection moulding, in which again the plastic is fed into a heated cylinder through which, this time, it is forced by a ram.

On emerging from the nozzle at the end of the cylinder, the material fills a cavity in a relatively cold mould, and takes the shape of the cavity; 3. Blow moulding, in which a tube of softened plastic made by extrusion or injection, is blown by internal air pressure against the walls of a cold mould cavity to produce hollow articles, such as containers; 4. Rolling and calendering, in which the material is squeezed through heated metal rollers until it is soft, when it forms a homogeneous skin round one of the rollers, and can be cut and pulled off, cooling in air or in water to give a sheet or film of determined thickness and width; 5. Spreading, in which rollers or scraper blades spread the softened material over the surface of a "base," such as paper or fabric; 6. Bending and stretching, in which sheets of heat softened thermoplastic material are shaped over formers or by pressing with male tools or by applying different air pressures on opposite faces, and then allowed to cool while held in the desired shape. There are many variations and combinations of these, and also some other methods used in special problems.

Thermosetting materials, however, can be heated in a hot metal mould, pressed and as soon as the chemical reaction (cross-linking) is completed the mould can be opened and the hot mouldings extracted and allowed to cool. Sometimes catalysts (usually in this connection called "hardeners") can be used to promote the cross-linking at lower temperatures, even down to room temperature.

Some Plastic Materials

18 major types of plastics have been selected for further discussion and it will be most convenient to make a somewhat arbitrary division of these into the following 5 classes.

1. *Semi-synthetic Thermoplastics*

Cellulose nitrate, cellulose acetate, cellulose acetobutyrate, ethyl cellulose, and regenerated cellulose.

2. *Synthetic Thermoplastics*

Polythene, P.V.C. and copolymers, polymethyl methacrylate, nylon, polytetrafluoroethylene, polystyrene, and polyvinylpyrrolidone.

3. *Synthetic Thermosets*

Phenol formaldehyde, aniline formaldehyde, urea formaldehyde, and melamine formaldehyde.

4. *Polyesters*

5. *Silicones*

Semi-synthetic Thermoplastics

The important members of this class are all based upon cellulose which is chemically modified to form either esters or ethers.

Cellulose nitrate. Cellulose nitrate, conventionally plasticised with camphor and castor oil, is the oldest of the plastics of present-day industry, having been discovered independently in England and America in the middle of the 19th century. It is best known under one of its trade

names, "Celluloid," and is variously described as "cellulose nitrate," "nitrocellulose," "N.C." and "pyroxylin." Its uses for dress ornaments and toilet accessories, such as combs, brush backs, spectacle frames, etc. are well known, and it is probably the most widely used material for knife handles. Another very important, but perhaps less known use, has been the covering of base fabrics for the production of leathercloth. In all these applications the toughness of the material is outstanding, but because of its very high inflammability it has been replaced in most of these applications in recent years. There remains one application which no other material has yet succeeded in capturing from cellulose nitrate, and that is in the manufacture of table-tennis balls, where the toughness and resilience are quite outstanding. In the form of cast film it provides an excellent base for photographic film and in solution form has been used for collodions.

Cellulose acetate. The first attempt to overcome the inflammability hazard of cellulose nitrate was to modify the cellulose with acetic acid instead of nitric acid. The product retains many of the advantages of cellulose nitrate, and overcomes its extreme inflammability, but cellulose acetate suffers from being much more sensitive to water and water vapour, so that its dimensional stability is inferior to that of cellulose nitrate. This is a disadvantage in such applications as manufacture of slide rules and other scales, where dimensional accuracy is required, and also in the manufacture of containers and components where screw threads or sliding fits are required. Cellulose acetate is plasticised with organic esters such as phthalates and because of its non-inflammability, its attractive colours, its stiffness and its toughness, it has found a major application in the manufacture of toys, and in transparent foil form for packaging. Cellulose acetate is used as the basis of "non-flam" photographic film.

Cellulose acetobutyrate. The mixed ester of acetic and butyric acids was prepared in an attempt to overcome the moisture sensitivity and consequent dimensional instability of cellulose acetate. While the product is in this respect superior to cellulose acetate it remains inferior to cellulose nitrate, but it is extremely tough and much less inflammable than cellulose nitrate, and has, therefore, been used for applications requiring dimensional stability, electrical insulation, toughness and non-inflammability—e.g. screw-driver handles. It suffers from the drawback that in certain circumstances the odour of butyric derivatives is perceptible.

Ethyl cellulose. As the various esters of cellulose were prepared, each in turn showing some advantages but some disadvantages compared with its forerunners, an attempt was made to overcome the disadvantages by forming cellulose ethers instead of esters. The only product of commercial importance in this group is the ethyl ester—ethyl cellulose—which is characterised by having a very high strength and general toughness. It is employed mainly in industrial applications, but has been used for the manufacture of hammer heads.

Regenerated cellulose. Regenerated cellulose is not perhaps, strictly speaking, included within the products of the plastics industry, but is

mentioned here as an illustration of yet another attempt to utilise the long chain molecules of cellulose. It has attained importance as the best known and one of the most useful of all transparent packaging films, particularly under the name of "Cellophane." It is made by converting the cellulose into cellulose xanthate, extruding this in the form of a flat ribbon, coagulating it and then decomposing it so as to reform the cellulose, which is now in the physical state of a flat film. Regenerated cellulose film can be used either unplasticised or plasticised with glycerine. Its resistance to water and water vapour can be improved by coating one surface of the film with a compound of wax, urea-formaldehyde resin and nitrocellulose. Its particular importance lies in its clarity, strength, stiffness and ease of printing.

Synthetic Thermoplastics

It is in this branch of the plastics industry that the greatest advance has been made in the last 25 years. In fact, it is only during this period that the synthetic thermoplastics have become of commercial importance. They are made either by polymerisation of compounds containing an ethylenic double bond, or by condensation of bi-functional molecules, e.g. the condensation of di-acids and di-amines, di-acids and di-alcohols, or the self-condensation of amino-carboxylic acids.

Polythene. Reference has been made earlier to the chemistry of this polymer. Its production is of interest not only because polythene is one of the few polymers made by the bulk polymerisation technique, but because the reaction is carried out at extremely high pressure, as high as 30,000 pounds per square inch and at high temperature, as high as 250° C. The polymer combines flexibility with toughness; low water absorption and low water vapour permeability with good resistance to inorganic chemicals; good electrical insulation characteristics at all frequencies with durability. It does not rust or corrode, and is of very low specific gravity —it floats on water. Its most important fields of use are in the insulation of low and medium voltage power cables (up to 11 kilovolts); in the insulation of high frequency communication cables (radar, television, submarine telegraph and submarine telephone); in sheathing underground cables operating in corrosive soils; in tubing for chemical plant and domestic, industrial and agricultural cold water supplies, and industrial effluent drains; in packaging films, for individual packs and drum liners; in surface coating papers for packaging; in domestic ware (bowls, baths, brush holders, racks, etc.); and in flexible and unbreakable containers, ranging from very small to 55 gallon capacity.

Polyvinyl chloride and vinyl chloride copolymers

Polyvinyl chloride (or P.V.C.) is chemically very closely akin to polythene, differing from it in having one hydrogen on every second carbon atom replaced by a chlorine atom. This modification, however, has profound effects on the properties, and hence the uses, of the polymer. The effects of the chlorine atoms in the molecule are to hinder regular packing of the

chains, thus preventing crystallite formation, to increase the stiffness of the chains, to increase the interchain attraction, to make the molecules electrically unsymmetrical, and to make the product non-inflammable. Thus P.V.C. is generally wholly amorphous, and it has a wide softening range with no sharp melting point. The product is hard, tough and horny, less flexible and "waxy" than polythene. The electrical unbalance of the molecules leads to a high electrical loss factor at high frequencies, and other factors reduce the insulation properties of P.V.C. considerably in comparison with those of polythene. Most commercial grades of polymer do not flow easily under pressure at temperatures less than about 160° C., and at this temperature it is difficult to prevent decomposition, with evolution of hydrochloric acid, discolouration and loss of strength. Grades of lower molecular weight are now available which, correctly stabilised, can be processed in sheet form by pressing, blowing or vacuum techniques at temperatures of 100° C. to 130° C., and these sheets are used for trays, refrigerator parts, drawer linings, signs and fluorescent lighting fitting reflectors. The higher molecular weight grades are used with plasticisers, so that their working temperature is reduced to a safe level below their decomposition temperature. Depending on the nature and quantity of plasticiser used, the products can vary from hard and horny to soft, flexible and rubbery. Although P.V.C. is unsuitable for high frequency insulation, at low frequencies it is comparable with rubber compounds, and indeed low voltage, low frequency cable insulation and sheathing are two of the major uses of plasticised P.V.C.

Its high loss characteristics at high frequencies allow it to be welded by the "high frequency" or "R.F." or "electronic" technique. Plasticised P.V.C. calendered sheets and films are welded in this way to make raincoats, babies' pants, sponge bags, racquet covers, quilted card table tops, pouches and shampoo packs. Protective clothing is often made from textile fabric coated with P.V.C., either by calendering or by spreading a paste made by cold mixing polymer and plasticiser and which is subsequently gelled by heating. The pastes are also used to make toys, stethoscopes, etc. by moulding and dipping techniques. The plasticised compounds are used for a variety of extrusions—straps, belts, braces, draught excluders, upholstery beading, and so on. For certain purposes it is necessary to modify the flow properties of the polymer under heat and pressure, that is, during processing, without having recourse to the addition of extra plasticiser. This can be done by co-polymerisation of vinyl chloride with a minor proportion of, for example, vinyl acetate or vinylidene chloride. The resultant copolymers are generally easier to process, less chemically resistant and hence more easily soluble. Because of the effect of the second component on the flow properties of the polymer, it is sometimes called an "internal plasticiser." Copolymers of vinyl chloride/vinyl acetate are used, for example, in the making of long-playing gramophone records where the requirement is for a material with the desirable properties of P.V.C., but which can be used satisfactorily in existing equipment, originally developed for handling slate-dust filled shellac.

Polymethyl methacrylate. Chemically, methyl methacrylate can be regarded as derived from ethylene by substitution of both hydrogens on one carbon atom, $CH_2=C(CH_3)(CO\cdot OCH_3)$. The polymers are remarkable for their clarity and colourlessness, and for retaining these properties on exposure. They can be prepared in an unlimited range of colours, in the physical forms of powders, sheets, rods and tubes. They have a low specific gravity and are rigid and strong. Electrically, they are moderate insulators, and chemically are resistant to inorganic and some classes of organic materials. The sheets are used for glazing of aircraft, and, in corrugated forms, for glazing of buildings; for instrument panels, dials and scales; for lightweight crockery; for sinks, baths and wash basins; for lighting fittings (reflectors, refractors and diffusers) used with filament, fluorescent, mercury and sodium lamps; for signs, advertisements and display stands; for machine guards and goggles and visors. Polymethyl methacrylate moulding powder is used principally for motor car rear light covers and reflectors, where resistance to outdoor exposure, to oil and petrol and their fumes, and to impact shocks, combined with high light transmission and suitable refraction characteristics are the essential properties; for car horn buttons and insignia, refrigerator name plates, combs, brush-backs and other toilet accessories, where clarity and a sparkling appearance are required; for coloured telephones, where brightness, strength, electrical insulation and permanence are wanted. Fine powders of polymethyl methacrylate are used, either by injection moulding or by making a partial solution in catalysed methyl methacrylate monomer, for the manufacture of dental plates and false teeth and eyes.

Nylon. There are 4 basic grades of nylon in commercial production at the present time, and intermediate and special combinations of properties can be obtained by mixing polymers, co-condensing mixtures of primary reactants, and by plasticisation. Chemically, nylons can be regarded as polythene in which, at regular intervals in each molecular chain, the peptide group $-C(=O)-N(H)-$ has been inserted. As a consequence of the regularity of the structure the molecules can easily pack into orderly arrangements, and the 4 basic nylon polymers are indeed highly crystalline. The most notable effect of the introduction of the peptide group is to increase the interchain attraction considerably (probably by hydrogen bonding), so that the melting point is considerably increased (nylon 66 melts at 264° C.), and the material is stiffer and stronger than polythene. In fact, if the orderliness of the molecules is increased by orientation, that is by making most of the crystallites lie in a given direction, tensile strengths of over 50,000 pounds per square inch can be obtained, compared with 2000 to 3000 pounds per square inch for polythene.

Nylon plastics resulted from a research designed to produce a protein-like fibre—an object triumphantly achieved. It followed, naturally, that the first use of nylon outside the textile industry was in the form of thicker

filaments. The success achieved by nylon monofil in domestic, toilet and industrial brushes is well known. It is perhaps not so well known that considerable progress has now been made in developing a tapered filament, with fibrilated ends, suitable for use in paint and distemper brushes. The outstanding property of nylon monofilament in these applications is its long life. Allied to this are the facts that the fibres are smooth surfaced solid rods, easily kept clean and aseptic, that the chemical resistance is good, and that the water absorption is low, so that the stiffness of the brush varies but little with humidity. Other uses of nylon monofil include fishing lines, sports racquet stringing and non-absorbable sutures.

The first development of nylon in forms other than monofils was as an injection moulding material, and in this form it is proving to be a competitor to light metals. In light engineering equipment, such as for example, calculating machines, meters, and textile machines, there are many bearings and gears now made from nylon. Nylon is silent in use, it generally requires no lubrication, and where a lubricant is necessary, water can be used, so that contamination of foods and textiles with oil can be eliminated, and it will outlast metal parts.

Whereas the 4 basic types of nylon have entered fields previously the preserve of light metals, the newer co-polymers and plasticised compositions have leathery characteristics, and one of the most recent applications for nylon is in moulding bicycle saddles. More recently, nylon grades suitable for extrusion have been produced. Tubing, such as for blood transfusion and car pressure lubrication, and sheathing, as on wire ropes and army communication cables, demand toughness, abrasion resistance and resistance to chemicals and temperatures above 100° C. and these are provided by nylon. Nylon type 66 can be chemically modified by methoxy methylation so as to render it soluble in mixtures of alcohol with water, and from the solutions water vapour permeable films can be cast. Nylon plastics, particularly type 66, are notable for their high melting points, rendering them more amenable to conventional sterilisation techniques. This is made use of in the surgeon's nailbrush, with a moulded nylon stock and nylon monofil tufts; in a light weight, unbreakable syringe; and in bottles and containers.

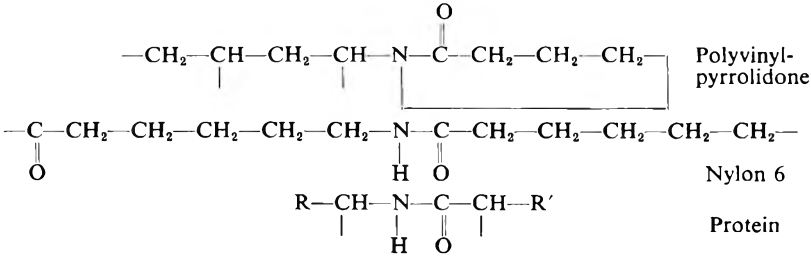
Polytetrafluoroethylene. This high-priced material is of great interest because of its unique properties which are unexpected in an organic compound, and it illustrates dramatically the effect of substituting all the hydrogen atoms in polythene by fluorine atoms.

First of all, it has the highest density of any organic polymer (2·2 cf. polythene 0·92). Secondly it is unaffected by any chemical at temperatures up to 280° C. except molten alkali metals and sometimes fluorine. Thirdly, it can be used continuously at temperatures of 280° C. for months, without decomposition, and, in fact, must be heated to above 327° C. in processing. Fourthly, it has the lowest reported coefficient of friction—less than that of wet ice on wet ice. The facts that it is in the same class as polythene as an electrical insulator, and that it exhibits remarkable non-sticking properties may be expected from, respectively, its chemical constitution and its chemical inertness.

The chief uses of P.T.F.E. are in high-frequency electrical insulation in situations where high temperatures and corrosive chemicals are met; in chemical plant (tank linings, gaskets); in bearings where low coefficient of friction is required, and loads and speeds of rotation are fairly low; and in food processing plant (bakeries) where surfaces to which doughs and other mixes will not stick are required.

Polystyrene. This material is again a hydrocarbon, and can be regarded as derived from polythene by substitution of one hydrogen atom in each ethylene unit by the phenyl radicle. Its electrical insulation properties are of the same order of magnitude as those of polythene, and again, as with polythene, the softening point is low, indicating low interchain attraction and low stiffness of the chains. The presence of the phenyl group in the molecule hinders crystallisation, and the polymer is glassy in type. It is colourless, rigid and brittle. Its important properties are, firstly its low cost, and secondly the ease with which it is injection moulded so that, although it has come into industrial production only recently, it has become the most important injection moulding material on a tonnage basis. It has been used for a great range of domestic, toilet and industrial mouldings, where rigidity and clarity or brightness of colour are of importance. One of its biggest uses has been in refrigerator components, such as crisper dishes. Copolymers, mixtures of polymers and plasticised polymers have been produced for special purposes, for example to give extra flexibility for such applications as battery boxes, or to reduce the accumulation of static electricity which leads to dust collection on display articles or dress hangers.

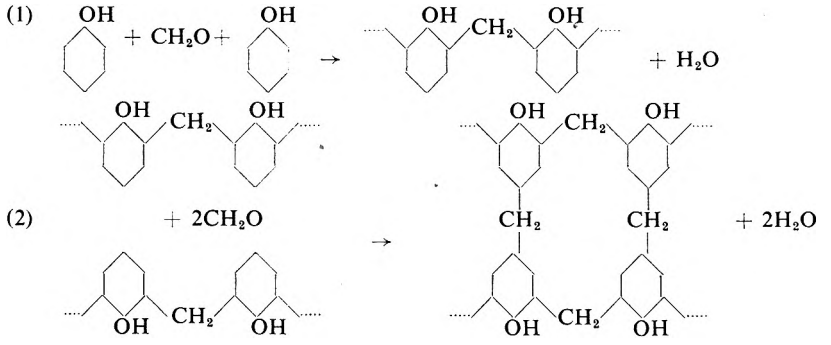
Polyvinylpyrrolidone. This material is of unusual interest because its major uses so far established lie in the field of medicine and surgery and not in the conventional fields of plastics applications. Its uses in medicine and pharmacy are dealt with elsewhere in this Symposium, and here it is sufficient to note the similarity in molecular structure of the polymer and a typical protein, as this doubtless accounts for its usefulness as, for example, a blood plasma substitute. Comparison of its molcular structure with that of a typical nylon is also interesting, and illustrates how molecular modification influences properties and uses.



Synthetic Thermosetting Products

The important products of this group are made by the condensation reaction of formaldehyde with a compound which can react chemically in at least 2 places. Examples are, aniline, phenols, urea, and melamine.

Phenol-formaldehyde. The term "phenol formaldehyde plastic" covers resins and moulding powders made not only with phenol, but also any cresol or mixture of them. The reaction can be typified in two stages as follows:—



The first stage produces moderately long thermoplastic chains which, in the second stage, react with further formaldehyde to form methylene links from one chain to another. As this process occurs in all directions a vast network is formed, rendering the product rigid and infusible.

The resins are dark coloured powders or liquids, and are used as adhesives for many purposes. They are unaffected by moulds or bacteria, or by moisture, and phenolic bonded plywood will withstand years of exposure even in the tropics. Because of their water resistance, phenolic glues are also used for marine work. Besides wood glues, phenolic resins are used to bond layers of paper or fabric together to make the so-called laminates for industrial and electrical work. These are employed in building as wall panels and corrugated corrosion-proof roofing sheets, and in special hollow forms and sandwich structures as partitions and bulk-heads on ships and where strength and insulation are required combined with light weight.

The best known use of the resins is as the binder for a variety of fillers to make moulding powders of the type generally referred to as "Bakelite" (a trade name derived from that of Dr. Baekeland, who first controlled the reaction of formaldehyde with phenols). The uses of these powders require no elaboration, being so widespread in the home and all branches of industry.

Phenolic powders of a special type can be prepared which are compatible with foodstuffs and beverages, but in general they are unsuitable for this purpose because they cause tainting of the taste of the food or drink. Their dimensional stability, however, makes them suitable for closures for many purposes.

Amino-plastics. This group includes the condensation products of formaldehyde with aniline, urea and melamine. Aniline formaldehyde resins are used to make paper and fabric laminates for the electrical industry. The laminates have very good insulation properties and high strength.

Urea resins are used in the same applications as phenolic resins, but

differ in several ways from them. The urea resins are pale straw coloured to colourless, and hence permit laminates and powders to be produced in a complete range of colours, whereas phenolic products, because of the basic colour of the resins, are perforce restricted to dark colours such as black and brown. The urea resins are cheaper, but more sensitive to moisture than their phenolic counterparts. On the other hand, they are odourless and tasteless and can be used in contact with foodstuffs and drinks.

Urea moulding powders are used for lighting fittings, closures, domestic and toilet ware, electrical fittings and cosmetic containers, and the laminates are used for decorative purposes. In this last application advantage can be taken of the dimensional stability and resistance to humidity of phenolic resins, and the attractive appearance of clear urea resins by making laminates with phenolic bonded cores and urea bonded surfaces. These are finding wide use for table tops, and restaurant panelling, and cigarette proof grades are made by incorporating a layer of metal foil in the laminate to conduct away the heat of the burning cigarette end. Melamine plastics combine many of the merits of both phenolic and urea plastics, but they cost more. They are used in the same ways as phenolic and urea resins and powders wherever their special combination of properties justifies their extra cost.

Polyesters

Polyesters, derived in effect from polyhydric alcohols and polycarboxylic acids, are yielding a rich harvest of new polymers. Not all are plastics—indeed the most famous of them all is a textile, "Terylene," but even this one, polyethyleneterephthalate, can be made into a film with unusual properties of electrical insulation, resistance to water and water vapour, and strength. Indeed, films as thin as ·00025 in. thick are stronger than many films from other materials in thicknesses 10 times greater. It is a new product, and its possibilities remain largely unexplored, but it is certain that much will be heard of it in future years. Other classes of polyesters containing conjugated double bonds yield resins which cross-link—that is, thermoset—by polymerisation, and this process can be carried out often at pressures and temperatures near atmospheric. These resins have found particular application in making glass fibre laminates, which are gaining prominence as structural materials for small boats, yachts and motor car bodies. Some polyesters yield foams which are of value in insulation against heat and sound, and for upholstery and vibration damping. The chemistry of this class of resins is particularly adapted to the new techniques of "block" and "graft" polymerisation which have given the polymer chemist two new tools to use, and many new plastics, films, textiles and rubbers, each with special properties, can be expected from the polyesters.

Silicones

In one sense, the silicones are "the odd man out" of the plastics family, in so far as their molecular chain is not composed of a carbon backbone,

but of silicon and oxygen. The different members of the family are formed by attaching different organic radicles to the silicon atoms of the backbone. Although these organic pendant groups place some limitation on the thermal resistance of the silicones, the main silicon-oxygen chain proves to confer more heat resistance on the product than the carbon-carbon chain of other plastics except P.T.F.E., which may be regarded as so well protected by the fluorine atoms as to be "hors concours." The presence of the organic side groups adds water-repellency, flexibility and solubility in organic solvents to the mineral silicate—like chemical inertness, resistance to heat and resistance to oxidation of the siloxane backbone. An important property of the silicones is that their viscosity changes but little with temperature, so that their temperature range of usefulness is very wide—in some instances from $-$ 85° C. to $+$ 285° C.

Their uses lie in water repellent finishes for masonry, textiles, etc.; in providing anti-sticking surfaces in the food and confectionery industries, and in organic plastics moulding; in chemical and heat resistant rubbery tubes and mouldings; in electrical insulation of wires; in foam suppressors; in polishes; and in medical and surgical applications.

They are available in the forms of oils, greases and rubber and can be either thermoplastic or thermosetting in type.

PLASTICS IN CONTAINERS AND EQUIPMENT

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The subject of the symposium at the 1953 meeting of this Conference was "Containers and Closures." At that time Stephenson[1] described some experiences with containers and closures in the pharmaceutical industry. His contribution included many references to the use of plastics as packaging components. Boardman[2] at an earlier symposium on the Storage of Drugs and Medicines also mentioned plastic closures.

In selecting a pack the object is to protect the contents under every condition and in any environment to which the pack may be exposed.

The pack must function efficiently over a wide range of temperature and humidity, be resistant to the effects of light and ageing, have adequate mechanical strength, prevent penetration by liquids and gases (including water vapour), be resistant to mould and insect attack and also be unaffected by the product it contains.

The many plastics which are now available offer to the packer materials with valuable properties which must be known and understood for efficient functional design. Plastics must be regarded as specialised products which should be judged by their established properties and not on superficial appearances. It is essential to realise that familiar names like polystyrene, nylon, polythene and so on, do not refer to clearly defined and standardised products. One name may cover several grades of material each with properties different in degree one from the other. This makes it difficult to give useful quantitative data on the properties of plastics.

For example, transparent tube packs in polystyrene and in cellulose acetate superficially resemble similar tube packs in glass but all three differ radically in gas permeability, resistance to organic liquids and ability to stand heat treatment.

Some of the main uses of plastic components in containers for pharmaceutical products will now be considered.

Closures and Caps

One of the earliest uses of plastics in the packaging of pharmaceutical products was as screw-fitting caps. These are mainly made from phenol-formaldehyde plastics, urea-formaldehyde plastics and more recently, polythene.

The thermosetting phenol-formaldehyde and urea-formaldehyde plastics used for closure manufacture contain fillers the composition of which can materially influence the properties of the finished component. For example, wood filled and paper filled urea plastics show considerable water absorption if exposed to wet or humid conditions, and under fluctuating conditions of temperature and humidity the dimensional changes in a closure made of such material may result in loosening to an

extent that leakage or loss of volatile constituents from the container may occur. If, for example, chloroform, which is present as a preservative is lost, secondary deterioration in the form of fungal or bacterial contamination is liable to occur. Alternatively if a closure contracts it may bind on the bottle neck making removal difficult. In extreme cases the physical stress causes the cap to crack. These dimensional changes are particularly undesirable in packs destined for export where considerable climatic variations are often experienced both en route and in the destination. Ordinary wood filled thermo-setting plastics also show poor resistance to alkaline products such as Cream of Magnesia and Aluminium Hydroxide Gel which may cause distortion, cracking and on long exposure disintegration of the closure. By attention to the composition of the moulding powder, thermo-setting plastics can be made which are resistant to alkaline preparations. When purchasing plastic caps for products of the type mentioned it is advisable to specify an alkali resistant grade.

The finish of a plastic cap must be of good quality. It is important that particles of "flash" from the moulding are not present as these may contaminate the product. "Flash" on a black plastic cap used on a container for a white cream or ointment may result in unsightly black specks on the surface. It is equally important that the container itself should be well finished. Pronounced mould marks on a bottle neck or threads may cause abrasion of particles from the plastic closure and in this way contaminate the product. Also, a poor seal may result if the plastic liners, discs or facing are cut.

Most phenol-formaldehyde mouldings can be sterilised by autoclaving but this does not apply to the urea-formaldehyde plastics which can be badly distorted by such treatment. The phenol-formaldehyde plastics are restricted in their colour range and are mostly used as black or dark brown.

Closures for collapsible tubes in thermo-setting plastics are available which make an effective seal without a liner disc or facing. This is achieved by skilful design of the cap moulding and by attention to the shape of the nozzle of the tube itself. Such closures eliminate the nuisance of a small liner disc sticking to the end of the tube and possibly being lost or discarded and also ensure an effective reseal. Wadless polythene caps for eye ointment applicator tubes have been described in the literature and are finding increasing acceptance. Most polythene closures do not use liner discs or secondary seals.

Polythene closures may be either screw-fitting, snap-on, or plug-fitting. With plug closures and snap-on closures the dimensions of the container neck should not vary appreciably. Because of the precision limits to which polystyrene containers can be produced a polystyrene tube closed by a polythene plug is being used for packaging pharmaceuticals such as tablets. In considering such a pack it must be remembered that both polystyrene and polythene are not completely impervious to gases including water vapour and that although for many purposes they afford perfectly adequate protection they do not give it to the same degree as an

efficiently sealed glass container. To cope with any slight permeability of the pack to moisture vapour, hollow polythene plugs have been made containing a dessicant such as silica gel granules; the base of the plug is perforated. One such plug is used as the closure for a pack of multi-vitamin pellets. Screw fitting polythene caps are available for winchester bottles, and this pack is in use for concentrated hydrochloric acid.

Many auxilliary closure devices in polythene have been the subject of patent applications. A polythene insert is available which greatly improves the pouring qualities of aqueous preparations from the usual screw necked glass bottle. Our own tests with such a device have shown that it is not so effective with alcoholic or oily products as with aqueous preparations. A similar device on a larger scale is incorporated in at least one well-known make of vacuum flask. Another polythene device which can be inserted in a bottle neck enables drops to be measured with ease and accuracy. This has been used in packaging oily vitamin-containing preparations, the dose of which is measured in drops. A polythene stopper with three flexible prongs has been evolved as a closure for a tube pack of tablets. The tablets are prevented from rattling by the prongs without need of cotton wool or other padding which may act as a reservoir for moisture.

Captive closures moulded as one with the container have been produced in polythene. Reversible spouts in polythene are available for use as a pouring aid from metal drum packs. Many devices of which the above are only a few examples have been described in the patent literature and in the literature on packaging.

Cap Liners

The cap liner is a most important component of a pack because it frequently effects the seal of the container. The use of plastic bonding for composite cork liners gives improved resistance to mould attack which so frequently occurs on composite cork liners bonded with glue of animal origin. Many plastics and plastic laminates find application in cap liners which are widely used under trade names such as Resistol, Crystalcap, Blackol, and Vinylite.

Resistol is paper coated with melamine-formaldehyde resin plasticised with alkyd resin. It is not suitable for strongly alkaline or acid products and is effected by a number of solvents.

Crystalcap is similar in general properties to Resistol but is white in colour this being obtained by pigmentation.

Blackol, which is widely used in pharmacy, consists of paper impregnated with a resin prepared from polymerised cashew nut-shell oil and is coloured with carbon black. It has quite good resistance to acid and alkali and resists some organic solvents but is softened by others. A metal foil liner facing is more satisfactory for solvents.

Vinylite consists of paper coated with white pigmented vinyl acetate, vinyl chloride copolymers. It has good resistance to acid or alkali but poor resistance to organic solvents or heat.

Polythene, polyvinyl chloride and various laminated forms using a

plastic film backed by a resilient wad are also in use in cap liners. Polythene and pliofilm are typical plastic materials used as facings for such liners. Pliofilm laminated to paper by a thermoplastic adhesive has low water vapour permeability and is useful as a closure liner for many alcoholic preparations but is unsuitable for oily products. A useful oil resistant film is provided by regenerated cellulose.

Some plastic caps are fitted with liners which are fixed in the cap by adhesive—frequently a plastic based adhesive is used—and with some products the liner may stick to the bottle neck. If this occurs with a faced composite cork wad which is stuck in the cap it is possible on unscrewing the cap to break up the cork backing, resulting in an unsightly appearance and in loss of sealing efficiency on reclosure.

An ingenious polythene cap liner in the form of a plug which fits into the bottle neck has been designed such that when the cap is screwed down, pressure of the cap on the centre of the liner disc forces the sides outwards thus ensuring a really tight closure.

Rigid Containers

Rigid containers fabricated from plastics include bottles, tubes, jars and boxes. The plastics most widely employed for this purpose are polythene, polystyrene and cellulose acetate for bottles and tubes with phenolic and urea formaldehyde plastics also being used for boxes. Nylon bottles have recently been developed which are stated to be capable of repeated sterilisation by autoclaving at 120° C.

Plastic containers have many advantages. They are available in a wide range of colours and opacities and are attractive in appearance; their light weight reduces freight charges and their mechanical durability reduces loss from breakage. The flexibility of a material such as polythene enables many types of container to be fabricated with ease. Offset against the advantages are increased cost compared with containers of glass or metal, together with the greater possibility of chemical or physical actions affecting the contents. Therefore in choosing the plastic container much consideration must be given to the properties of the product and to those of the container.

The increasing uses of plastic bottles has drawn attention to Section 23 (1) (*b*) of the Poisons Rules which specifies that a glass container of capacity not more than 120 fl. oz. used for packing poisons, not being medicines made up ready for internal treatment of human ailments or local anæsthetics for injection in the treatment of human or animal ailments, shall have the outer surface of the bottle fluted vertically with ribs or grooves recognisable by touch. At present this requirement does not apply to plastic bottles. The matter has been referred to the Poisons Board for consideration.

Polystyrene is used to a considerable extent for producing containers by injection moulding. It is a hard rigid material which gives a typical sounding ring when dropped onto a hard surface. It breaks with a sharp fracture and is stable to temperature about 170° F. It is free from odour

and taste and is available in a wide range of colours in addition to a transparent finish. It has excellent dimensional stability and components can be produced within fine limits of accuracy. This is important when the closure is a plug as is often the case. Although polystyrene is water proof and has a very low moisture absorption it is not water vapour proof if subjected to varying conditions of humidity and temperature. In the course of work in the author's laboratory to test the suitability of a wide variety of containers for packaging penicillin tablets it was found that polystyrene tubes were very much less satisfactory than glass bottles. By sealing the opening of the polystyrene tube with a cork and dipping in wax and similarly treating glass controls it was shown that after treatment for several weeks in a climatic testing cabinet set to give fluctuating temperatures and humidities over a wide range, the resulting deterioration of the product in the plastic pack was due to water vapour passing through the walls of the polystyrene tube. The pack which was finally chosen to ensure greatest protection to each separate tablet was a strip foil pack in heat-sealing aluminium cellulose acetate laminate, each tablet being hermetically sealed in a separate pocket.

The peculiar properties of polystyrene in transmitting water vapour have been described in the literature[3]. It has been stated that the water resistance of polystyrene is about the same as polythene or polyvinylidine chloride but that the rate of water vapour transmission through polystyrene is incomparably greater than through the other two plastics. The water vapour transmission rate of nylon is also very high.

Polystyrene is not affected by acids, alkalis and alcohols but has poor resistance to chlorinated hydrocarbons and certain organic liquids. Polystyrene is used for one well-known photographic developing tank and has been shown to stand up well to the alkaline solutions used for developers. Polystyrene containers are used for cosmetic products, for example a double shelled jar for creams can be made with a great degree of accuracy and looks very well. Lipstick cases may also be made of polystyrene but these sometimes show surface marking and "crazing" after contact with many essential oils and materials used in perfume.[4]

Polystyrene readily becomes charged with static electricity and attracts to it dust and fibres much more readily than does a glass or metal container. Dusting such containers with a dry cloth merely aggravates the problem. They can be cleaned with a cloth damped with water or with a very dilute solution of a cationic detergent. Antistatic agents are now available for incorporation in plastics including polystyrene. These appreciably reduce the problem of charge. Several years ago the writer observed an incompatibility of plastics—a container was made, the base of polystyrene and the lid from cellulose acetate. After storage for a little time the fine surface finish of the polystyrene base became dulled and pitted and the lid had tightened to such an extent that the container was quite unacceptable. It is possible that the plasticiser in the cellulose acetate reacted with the polystyrene. This effect of plasticisers on plastics is of importance to pharmacists as dimethylphthalate and dibutylphthalate which are widely used in insect repellent preparations are also used in the plastics industry

as plasticisers and may soften or dull the surface of plastic articles which come in contact with them. Spectacle frames are particularly vulnerable.

Polythene is used for bottles and tubes and also for collapsible tubes. The bottles can be finished with a variety of separate fitments for delivering a liquid as a spray, as a jet, or as drops; large bottles and containers in sizes up to 50 gallons capacity can be obtained for specialised packaging; puffer containers for powders are also widely employed, and double shell jars are used for cosmetic creams to provide light, unbreakable containers suitable for travelling.

Among the virtues of the material are flexibility, excellent resistance to chemicals (polythene containers have been found suitable for packaging hydrofluoric acid), and low water vapour transmission rates. Although polythene has good resistance to permeation by water vapour it has a relatively high rate of transmission compared with some other plastics, for oxygen, carbon dioxide and many organic vapours. This permeability to gases may result in spoilage of the contents of a polythene container if it is stored with odourous articles, and also the loss of volatile constituents including preservatives from the contents may occur. Pinsky, Nielson and Parliman[5] have carried out a long-term study of 67 chemicals in polythene bottles and have recorded data concerning permeability and physical effects.

Affixing labels by the usual adhesives is unsatisfactory with polythene containers. One useful device on a cylindrical container is to have a recessed panel which will take an all-round band-label stuck to itself. Polythene tubes for pastes and ointments, now beginning to make an appearance in this country, set problems in filling and sealing at speeds comparable with those used for metal tubes. Also on prolonged exposure to sunlight polythene may become oxidised, this gives rise to an odour, and a taste develops which would be imparted to the contents of the container.

Each product must be specifically tested for suitability for packing in plastic tubes. For example, experimental toothpastes packed in polythene were found to lose flavour very quickly, while controls in tin remained perfectly satisfactory.

Bottles of irradiated polythene have been produced on an experimental scale. It is claimed that these can be sterilised with heat[6] as the material does not melt at temperatures up to 350° F. and retains the toughness and flexibility of polythene itself. One possible use for such bottles is as containers for milk, where advantages of lightness and shock-proof qualities apply. The initial cost of the bottles would undoubtedly be higher, but the life of each container longer than its glass counterpart. Facilities for producing containers in irradiated polythene are at present limited.

Transparent polyvinyl chloride has been used to provide an outer safety covering for glass bottles used for pressurised aerosols. Tests have shown that even if the glass bottle bursts the polyvinyl chloride "skin" prevents flying glass. Plastic containers and jars are considerably lighter than their glass counterparts, and when put through conventional bottle-washing machines designed for glass they may be blown off the holders by the jets.

Plastic Films and Laminates

The plastic films most widely used in this country in containers for pharmaceuticals are cellulose acetate, polythene, rubber hydrochloride (pliofilm) and polyvinyl chloride. Other plastic films which are potentially useful but not yet available in quantity in this country include polyvinylidine chloride (Saran), ethyl cellulose and nylon. Regenerated cellulose although not truly a plastic is also used widely as a component of containers.

These plastic films can be used alone or laminated to other materials. Laminates with each other may give a useful combination of properties. Plastic films are frequently employed as components of packs in the form of laminates with non-plastic materials such as paper, aluminium foil, hessian and fibre board. In considering the use of plastic films and laminates as potential containers for pharmaceuticals it is essential to take into account the permeability of the materials to water vapour and gases Riddell[7] has pointed out that, apart from metal foil free from holes, no commonly used flexible packaging material is absolutely vapour proof. Polythene gives good resistance to moisture vapour but has a high transmission rate for oxygen compared with say regenerated cellulose film. Riddell[8] quotes polythene as having approximately 100 times the permeability to oxygen as dry cellulose film of equivalent gauge. In this case, therefore, it might give better product protection to use a moisture-proof cellulose film rather than polythene even though on grounds of water vapour permeability the latter is more efficient.

The films or laminates are used for making bags, liners for drums or sacks and for "sachet" containers for liquids and powders. Polythene liners to metal and fibre board drums enable chemicals to be packaged without actual contact with the outer. The lined drums are free from fibres and foreign bits and provide excellent containers for chemicals required to give "particle free" solutions, and also provide excellent storage and transport containers for soft capsules which in card or fibreboard boxes tend to dull and pick up foreign particles. Many liquid shampoos are packed in plastic or plastic laminated sachets. Materials used for such products include polyvinyl chloride and pliofilm/cellulose acetate laminates where the pliofilm gives good water and water vapour resistance and the cellulose acetate good protection to the perfume. A disadvantage of pliofilm, however, is that it deteriorates with age, particularly if exposed to sunlight, when the film becomes brittle and very readily broken, so destroying the water-resisting barrier which it was intended to provide.

Polyvinyl chloride bags or sachets made of sheet or tubing are in use for a variety of liquid products from horticultural spray concentrates to shampoos. The plastic is readily printed by accepted methods. It is resistant to water and water vapour, it is relatively unaffected by sunlight, has good ageing properties, and is resistant to inorganic chemicals including strong acids and alkalis but is attacked by some organic liquids. Petrols, oils and greases may extract plasticiser and cause embrittlement, however, oil- and grease-resistant grades are available. The material is

preferably sealed by high frequency heating, the heat-sealing temperature being in the range 280° to 320° F. (140° to 150° C.).

Many of the thermoplastic films and laminates incorporating them can be sealed or welded by heat. Polythene and pliofilm are readily sealed by heat. Regenerated cellulose is not thermoplastic; the so-called moisture proof heat-sealing variety being dependent on a plastic coating, so there is no true weld, the strength of bond between two sheets being only equal to the strength of adhesion of the coating to the film. In addition to direct pressure heat-sealing, high frequency welding, impulse welding and flame sealing may be applicable according to the properties of the particular plastic film. The optimum temperature range for heat sealing the various plastics is critical. It is important to realise that the actual temperature of the plastic itself must fall within this range and that the temperature of the sealer jaws or plate is no direct indication that this will be achieved. The time of contact with the heated surface and the pressure applied also influence the thermostat setting on the sealing apparatus. A certain amount of experience and trial and error is necessary in establishing optimum settings for a particular piece of equipment. With polythene film, polyvinyl chloride and some other plastics the plastics sometimes stick to the hot sealing jaws. Coating of the jaws with polytetrafluoroethylene eliminates this difficulty. Polytetrafluoroethylene is a good insulator and jaws covered with this material must be applied for two to three times as long as with uncoated jaws to achieve a seal.

Polytetrafluoroethylene is at present very expensive but it is a most interesting plastic. It resists attack by all known solvents and chemicals other than gaseous fluorine and the molten alkali metals. It has anti-adhesive properties which make it useful for coating hoppers and slides on packaging machinery. Bearing surfaces coated with this plastic need no lubricant.

Tapes

The use of self-adhesive tapes on pharmaceutical containers is widespread. Different types of tapes are available according to the properties required. Several are covered by British Standards[9]. Among these are tapes using plastics as the foundation. They include plasticised polyvinyl chloride which gives pliable and extensible characteristics with good resistance to water, oil, acids and alkalis. Polythene based tapes remain pliable, soft and flexible even at very low temperatures. Ethyl cellulose is also used as the base for sealing tapes as also is the widely used non-plastic regenerated cellulose.

Miscellaneous Devices

Miscellaneous devices used in conjunction with pharmaceutical packs include applicators of various sorts. These may be in polythene which is soft and flexible, for example those on tube packs for hæmorrhoidal ointments. Polythene appliator rods are used for corn paints. Plastic measures in polystyrene are used with some products, for example, a spoon is packed with one well known oral penicillin suspension to ensure

that the proper dose is given in view of the very considerable variation in the capacity of the domestic teaspoon.

Both nylon and polythene have been used in valves for pressurised aerosol packs.

Platforms and holding devices for irregularly shaped materials are made by vacuum forming from sheet plastic.

Silicone elastomer components are used in order to meet specialised packing requirements where contact with rubber may cause deterioration of the product or where flexibility is required over a wide temperature range.

Plastics in Equipment

Many plastics find application in equipment used in processing or packaging pharmaceuticals. It is not possible to deal with every application of plastics to pharmaceutical equipment and the examples which follow are more direct applications as opposed for example to the use of plastics in electrical equipment used in pharmaceutical machinery. Much the same criteria apply in selecting plastics for use in equipment as apply to their selection for containers. The properties of the plastic must be known in relation to the materials and conditions with which it will come in contact.

Fabrics woven from plastic filaments such as nylon are used for sieves and filter cloths. Microporous polyvinyl chloride sheet provides an excellent filter medium. It is prepared in an ingenious manner by mixing the plastic with starch, casting in sheets and then removing the starch grains by enzymatic digestion to give a porous structure. Polythene funnels and buckets provide non-breakable apparatus for handling corrosive materials. Laminated phenol plastics with fibre have been used for gears in pumps for handling many chemicals corrosive to metal. Similar laminated material has been used to replace teak in carriers used in plating baths where prolonged contact with nitric and other acids is involved.

Amino-formaldehyde plastics are well known under trade names in the form of laminated surfacing for table tops and dispensory benches. They provide a heat resistant, chemical resistant washable surface. Transparent polymethyl methacrylate is used in making dispensing screens for aseptic working. It is also useful in making safety guards on machinery. Tablet counting devices are available embodying polymethyl methacrylate which is also used as a non-splintering alternative to glass in safety goggles and visors.

Plastics in sheet form have been used to line metal equipment where corrosion would result by direct contact with the product. One of the problems in such work is to stick the plastic to the metal, another is the very much greater expansion coefficient of most plastics compared with metal. The temperature at which such lined equipment may be used is limited by the properties of the plastic in relation to heat. With materials such as polythene and polyvinyl chloride extensive areas can be covered by welding several sheets together.

Nylon is used where toughness and durability is of value. On filling machines nylon nozzles reduce the risk of glass chipping which might occur with metal nozzles when a bottle is presented slightly off centre. Engraved nylon face plates have been used in soap stamping machinery. Link chain belting has been made in nylon—it is hard wearing, self lubricating and kinder to glass containers than metal link belting.

The handling of strong solutions of hydrogen peroxide has been helped materially by development of a polyvinyl chloride hose reinforced externally with fabric woven from polyester filaments. Polyvinyl chloride sheets are used for covering pans during manufacture and also for protective aprons. Polytetrafluorethylene has already been mentioned as a facing on heat sealing equipment to prevent sticking of plastic films to the jaws. Due to its very low friction coefficient it is used on feed slides and dough rollers to prevent sticking.

Polythene and polyvinyl chloride piping and tubing find many applications in pharmaceutical plant.

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PRODUCTS CONTAINING PLASTICS

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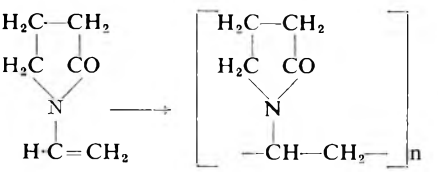
The introduction of plastic materials and chemically related polymers into medicine and surgery is largely a development of the Second World War and the post-war period. Before 1940, only a few semi-synthetic products such as nitrocellulose, celluloid and hardened rubber had been used in any medical connection. Of these, nitrocellulose formed the basis for medicated collodions, while celluloid and hardened rubber were the normal materials for dental plates, the artificial teeth themselves being of porcelain. Shortly before the war, an important advance in dentistry was made in Britain with the introduction of dental plates made of polymethyl methacrylate. By 1941 these were in regular use, although still with porcelain teeth, while in 1946 they were replaced by the now familiar "complete" acrylic denture. The war also provided a stimulus to the exploitation of plastics in several branches of medicine and surgery and led to, *inter alia*, numerous improvements in surgical instruments, a synthetic plasma substitute, and a simplified process for the manufacture of prostheses such as eyes, noses, ears or hands. After the war medical uses were found for newer polymers such as the silicones, while fresh outlets continue to be discovered for other plastics, particularly as they become available in a wider variety of physical forms. The total use of plastics for medical, dental, and related purposes is, of course, extremely small in comparison with that of other arts and industries, and with the possible exception of polyvinylpyrrolidone, no single polymer has its main outlet as a pharmaceutical product. The main advantages which plastics offer to the surgeon are that, in the solid state, they are light, easily worked and moulded, are not cold to the touch, and do not conduct electricity. Some have the additional advantage of transparency. They can usually remain implanted in living tissue for long periods without causing irritation. This has made possible many developments in which plastics have replaced metal and other materials in surgical instruments, in surgical repair and in the fabrication of prostheses. Much of this work is still at the experimental stage, however, and it may well take several years of further investigation before the safety and efficacy of the new materials are fully established. Their optical properties have permitted them to replace glass and have, for example, made practicable the "contact" lens. The fact that many polymers can be converted into mono-filaments of accurately controlled diameter has led to the partial replacement of animal and vegetable fibres in surgical sutures.

Plastics still suffer from certain disadvantages and one of the main difficulties from the pharmacist's viewpoint is that very few can be sterilised by autoclaving and none by dry heat. Comparatively few polymers have been used in medicine, either as active agents or excipients, and, as might be expected, their value lies chiefly in their chemical stability

coupled with a high molecular weight. These properties have been utilised in "slow-release" or "depot-dose" therapy. Numerous other medical uses have been suggested and some are now being explored in the laboratory. It may well be that the availability of non-toxic compounds of high molecular weight and controlled purity may provide in the near future some extremely interesting developments in both medicine and pharmacy.

1. *Plastics in Medicine*

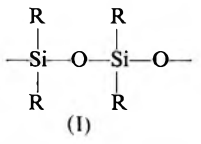
Polyvinylpyrrolidone (PVP). The use of this substance as a plasma substitute was developed in Germany during the Second World War, and it has since been introduced into several other countries under a variety of trade names (Periston, Subtosan, Plasmosan, PVP-Macrose, etc.). A comprehensive review of its properties and uses has recently been compiled by Reppe[1]. The chemical synthesis requires only simple starting materials (acetylene, formaldehyde, ammonia) and the final stage is the polymerisation of *N*-vinyl-α-pyrrolidone in aqueous solution.



This yields a mixture of polymers covering a wide range of molecular weight, and fractionation is necessary to provide material of uniform properties suitable for medicinal use. The approximate molecular size of any given fraction is usually expressed as the "K value"[2], which is derived from measurement of viscosity of a PVP solution. A K-value of 16, for example, corresponds to a mean molecular weight of about 10,000. For use as a plasma substitute a fraction having K-value in the range 28 to 32 is preferred. A concentration of 3·5 per cent. w/v of this material gives a colloidal osmotic pressure approximating to that of whole blood. PVP itself is a stable, white powder which can be stored indefinitely. It is readily soluble in water and in many organic solvents, but is almost insoluble in ether. An aqueous solution can be sterilised by autoclaving, the only change being development of a slight yellow colour caused by fractions of low molecular weight. The solution is slightly acid in reaction (about *p*H 5) and this is usually neutralised by sodium bicarbonate when PVP is formulated for intravenous injection. There appears to be ample evidence that it is non-toxic to man; the usual dose is 500 to 1000 ml. of PVP-saline but, in extreme cases, doses of over 3 litres have been administered. Its ultimate fate in the body is not known, but excretion takes place via the kidneys and normally up to 75 per cent. can be recovered from the urine. In addition to its original war-time use as a plasma substitute, it has been tested extensively as a vehicle for "delayed-release" or "depot-dose" preparations. For this purpose, small volumes of 10 to 20 per cent. solutions containing fractions of K-value 50 and over have been used in conjunction with various drugs such as local anæsthetics, hormones, and antibiotics. This work has been carried out chiefly in France. Most workers report significant retarding effects but no satisfactory

theory has been advanced to explain the mechanism by which PVP operates. A third use arose from the observation that it showed the so-called "embathic effect" characteristic of serum, namely the ability to peptise coarsely dispersed dyes such as Congo Red. It was thereafter shown in experimental animals that PVP exerted a protective or detoxifying action by removing certain toxins and toxic dyestuffs, possibly by diverting them from the liver and causing excretion via the kidney. It is thought that fractions of low molecular weight are the most effective for this purpose. A recent innovation has been the introduction of a complex formed between PVP and iodine. It is claimed that combination with iodine renders the latter safe, even for internal use, without reducing its bactericidal power and that successful results have been obtained using "PVP-iodine" in the treatment of skin infection cases which had failed to respond to conventional treatment. Systemic infections have also shown rapid and marked improvement under this treatment, no harmful side effects being observed in any of the cases. Besides detoxifying the iodine PVP appears to give a longer duration of effect.

Silicones. There has been a recent revival of interest in the chemistry of silicon due to the discovery of a series of organic/inorganic polymers containing both carbon and silicon and having the essential structure of a substituted siloxane or "silicone," (I). The substituted organic groups, R, are commonly methyl or phenyl, and one of the best known series of fluid silicones is composed of polymerised dimethyl siloxane. These polydimethylsiloxanes comprise the silicones which have so far been used most extensively in pharmacy and medicine, and their use has recently been reviewed by Levin[3]. Individual silicones in the series are usually distinguished from one another by their viscosity, expressed in centistokes, and by a manufacturer's identifying number. Silicone polymers of different composition have been obtained as solids, e.g., as elastomers and resins, and have other industrial applications. The value of silicones lies in an almost unique combination of chemical and physical characteristics. In the first place they are extremely stable in the chemical sense, that is to say they are unaffected by water, acid, alkali or other common reagents, and are not decomposed by heat. Fluid silicones, for example, can safely be heated to temperatures at which hydrocarbons would char. As an additional sign of inertia, they are odourless and tasteless and appear to have no pharmacological action. Coupled with this high degree of stability they have several extremely valuable physical properties, notably water-repellancy, lubrication and anti-foaming action.



(I)

There have been a large number of investigations into the pharmacology of silicones. Independent clinical tests in several countries on unformulated silicone oils and on various barrier creams have shown that there is no danger of skin irritation, and even when rubbed into the eyes of experimental animals they only produce a transitory irritation of the conjunctiva with no permanent damage. Silicone oils have also been tested extensively by oral administration to laboratory animals; this has been done to ensure

their safety in the food and confectionery industries, e.g., for glazing bread-baking tins and for coating paper on which confectionery is placed. Other laboratory tests have been made on the administration of silicones by intradermal, intraperitoneal and subcutaneous injection, and also by inhalation of vapour from the heated oils. The general conclusion seems to be that their toxicity is extremely low by all routes of administration, although their eventual disposal in the body is not yet fully known. The first feature to attract interest in pharmacy and medicine was water-repellancy, and this has led to the formulating of many types of barrier cream. It has been claimed that silicone fluids yield a water-repellent film, free from unpleasant greasiness, and that they are effective even when applied in an emulsified or "vanishing-cream" base. Quite apart from their use as "barriers," silicones have been studied as alternative excipients to paraffins or vegetable oils in various ointments, and in liniments. They appear to compare favourably in pharmaceutical properties with the more conventional formulæ but no outstanding advantages have been claimed. On account of their antifoaming action silicones are used in veterinary medicine as a remedy for "frothy bloat" (tympanites) of cattle. In this condition there is a persistent froth in the animal rumen due to saponins in grass and legumes; it is relieved by administering an antifoam preparation, either by mouth or directly into the rumen by canula. It has been suggested that silicone antifoaming preparations might also be used in human medicine to break the froth which develops in the lungs in extreme cases of lobar pneumonia. A clinical report has been published on the use of silicone aerosols in the treatment of pulmonary œdema. There has naturally been much speculation about the future of silicones in medicine, although many of the ideas put forward will require very careful clinical assessment. A favourite theme is the replacement of vegetable or mineral oils in preparations where these do not provide the ideal vehicle. Preliminary work has already been carried out using silicone oils as a depot-dose vehicle (i.e., as an alternative to arachis oil) but the release of drug was quite rapid. Another suggestion is that silicones should be used for infiltration in and about malignant growths, i.e., as vehicles for anti-carcinoma agents. In all probability the use of silicones as vehicles for drugs under conditions which do not require their specific water-repellent or defoaming properties, will be postponed until more is known of the effect of administering relatively large quantities by mouth or by injection.

Polyvinyl Alcohol. Like PVP, polyvinyl alcohol is not a precise chemical entity but is a mixture of polymers of different molecular weight. It is manufactured by controlled hydrolysis of polyvinyl acetate and is available commercially in several grades or fractions, each suited to a particular purpose. Essentially the grades differ in molecular size (the approximate molecular weight is 30,000 and upwards) and in the proportion of unchanged acetate which they contain (up to 20 per cent.). Polyvinyl alcohol is normally isolated as a white or cream-coloured powder which is soluble in water but insoluble in most organic solvents. The powder is hygroscopic and may absorb up to 10 per cent. of water on

prolonged exposure to an atmosphere corresponding to a relative humidity of 75 per cent. at normal room temperature. Aqueous solutions are neutral, or almost so, but those prepared from grades containing unchanged acetate may develop acidity on storage, particularly at high temperature. The solutions are viscous and mucilaginous and have hence found uses in pharmacy and cosmetics as thickeners, and as protective colloids to stabilise emulsions. Evaporation of an alcoholic or aqueous solution leaves a plastic film which may be used in the manner of a collodion to bring drugs into contact with the skin without the need of bandaging. A study of polyvinyl alcohol as an emulsifying agent has been made by Biehn and Ernsberger[4] who found that the most effective grades for this purpose were those of high viscosity, containing about 20 per cent. of unchanged polyvinyl acetate. The efficiency of polyvinyl alcohol was compared with that of common emulsifying agents such as sodium oleate, sodium dodecyl sulphate, sodium alginate and methyl cellulose, and it was found that polyvinyl alcohol at a final concentration of 1 per cent. showed good emulsifying properties but its value in relation to the "controls" varied considerably with the water-immiscible liquid used. Some work has also been done on the injection of polyvinyl alcohol, and Loubatières[5] has reported its use as a plasma substitute, both in experimental animals and human patients.

Polyvinyl acetate. In the preparation of polyvinyl acetate, vinyl acetate is first made by a catalysed interaction of acetylene and acetic acid in the vapour phase. The ester is then polymerised in the presence of a catalyst such as benzoyl peroxide or hydrogen peroxide. Polymerisation may be carried out in an aqueous dispersion or emulsion and the resultant emulsion containing 50 per cent. of polymer is one of the common forms in which polyvinyl acetate is commercially available. One of the most useful properties of this polymer is its ability to form a durable thermoplastic film of good clarity and, for this reason, it is used extensively in varnishes and adhesives, and as an interlayer in safety-glass. The film is also non-irritant and has therefore been tested as a flexible first-aid dressing. Until quite recently there was little or no interest in polyvinyl acetate in pharmacy or medicine, but in 1954 Piney[6] reported a significant prolongation of blood-level in drugs administered by mouth using polyvinyl acetate as excipient. Aspirin and sodium aminosalicylate were tested in this way and it was suggested that a "long-acting" aspirin tablet might be developed by combining "free" aspirin with aspirin intimately associated with polyvinyl acetate. This suggests interesting possibilities of various depot-dose formulations based on the polymer, but will obviously require considerable collaborative clinical and formulating work before any clear idea of its efficacy can be obtained.

2. *Plastics in Surgery*

(*a*) *Internal Use*

The search for materials suitable for use in surgical repair has been in progress for many years but the criteria for an ideal substance are extremely difficult to meet. Substitution for bone, for example, obviously

demands mechanical strength and, if a joint is involved, freedom of movement. Many metals are unsuitable on account of electrolytic reaction which they induce in presence of blood serum, and even stainless steel has the disadvantages of a high specific gravity coupled with the fact that it cannot be fashioned at the time of the operation. The use of synthetic materials is less than twenty years old but already it seems likely that they will go a long way towards replacing metals, and possibly also bone grafts. These and other possibilities have been discussed in several reviews[7,8]. The first point which had to be established was the freedom of plastic materials from any tendency to produce tissue reaction. The polymers themselves are extremely inert but dangers might result either from unchanged monomer or from various added substances such as plasticisers, lubricants, fillers, pigments and stabilisers. Another factor which must be taken into account is abrasion due to friction and the consequent release of fine particles which may produce tissue irritation due to their physical properties. The two plastics which have been used most frequently in surgical repair are polythene and polymethyl methacrylate the former for flexibility and the latter where mechanical strength and rigidity are required. In addition, nylon monofilament is used as a suture material on account of its high tensile strength and nylon tubing for ureteric and other catheters. "Pure" polythene, i.e., the polymer free from any added substance, appears to exert no harmful effect on living tissue even over long periods of contact. A variety of experiments have been carried out on laboratory animals in the course of which polythene has been in contact with brain, thoracic cavity, abdomen, blood vessels and other tissues without producing any inflammatory or foreign body response. On one occasion, polythene was found to produce a fibrous tissue reaction but this was later traced to a small percentage of dicetylphosphate introduced during manufacture of the polymer. The special properties of polythene containing this substance have since been used advantageously in the treatment of syphilitic aneurisms of the aorta[9]. Its normally inert nature has made flexible polythene tubing of value in common bile duct surgery, in urological surgery and as an aid to intravenous infusion of fluids or blood[10], particularly over long periods of time. Another interesting application of polythene is in thoracic surgery. In chronic pulmonary tuberculosis, cavities of varying size may be formed in the lung and will not heal until their walls are brought in apposition. This has been accomplished by artificial pneumothorax and by thoracoplasty. The latter is a painful operation which may require to be done in several stages, and a less drastic means of achieving collapse of the lung is by the polythene pack operation (polythene plombage)[11]. The procedure consists essentially of inserting a bag made of polythene sheet and stuffed with polythene ribbon so as to form a cushion between lung and ribs. A small quantity of iodised oil is added before the bag is sealed in order that its position may subsequently be verified by X-ray. Polythene has not been used extensively for replacement of bone but some examples of such use have been reported, such as the replacement of part of the shaft of the femur[12]. An extruded film 0·002 in. thick has been used to replace gaps

in the dura mater in brain surgery and a thicker form is used to cover skull defects.

The main use of polymethyl methacrylate has been in replacement of bone, a well known example being arthroplasty of the hip joint, described by Judet[13]. For this purpose, a pylon of pure acrylic is made with a bulbous "mushroom" head and containing, for additional strength, a stainless steel shaft which is embedded before polymerisation. The same acrylic polymer has also been used in cranial surgery for the fabrication of plates to replace defective bone. It is non-irritant to brain tissue and has an advantage over vitallium in being transparent to X-rays. It has been used for thoracic implants in a manner similar to that already described for polythene, except that balls of 2·5 cm. diameter were used instead of tape. Artificial lenticuli of polymethyl methacrylate have been inserted after cataract extraction; the polymer is much preferred to glass for this purpose on account of its light weight. Nylon was the first plastic to be used extensively for surgical repair and was introduced as a suture material. It has since been used also as nylon mesh, nylon floss, and nylon tubing. Nylon filaments have a high tensile strength and are very tough; they are also of uniform diameter and are hence easily withdrawn. The filaments are prepared in a range of sizes from ·004 in. to ·020 in. in diameter, each having a distinctive colour. The repair of large herniæ by nylon mesh has been reported[14], while nylon floss has been used for herniæ and for closure of abdominal incisions[15].

The sterilisation of plastics before surgical use is still a problem. None can be sterilised by dry heat at 150° C. and only nylon will withstand autoclaving. Polythene can be boiled, but tubing and other fabricated articles will undergo deformation unless supported mechanically while hot. Colker and Norman[16] have described a method for sterilising polythene canulæ by enclosing them in glass tubes and autoclaving at 104° C. for 30 minutes. Immersion in solutions of antiseptics, such as quaternary ammonium salts, has been recommended by Farquhar and Lewis[10] but these authors also point out that polythene will increase in weight after prolonged immersion in solution of chloroxylenol, presumably due to absorption of certain constituents of the antiseptic.

(*b*) *Surgical Dressings*

Nylon film. Although conventional dressings of bandage, gauze, and cotton wool will, when dry, prevent infection of a wound, in practice it is difficult to avoid wetting the dressing either from external contact with water or as a result of exudation from the wound. Under such conditions, infection will rapidly penetrate from outside. A completely waterproof dressing is not the answer to this problem since it causes an accumulation of condensed skin perspiration. Human perspiration proceeds at the approximate rate of 15 g./sq. metre/hour, and a suitable dressing material would thus be one which permitted passage of water vapour at this rate but, at the same time, acted as a barrier to liquid water and to micro-organisms. It has been found that a nylon film of thickness 2/1000 in. meets this requirement, and this material has been used in a

variety of ways, including gauntlets for the protection of burns of the hand and arms. The progress of the wound can be followed visually by the surgeon without changing dressings, and much lighter protective dressings can be used.

Paints and sprays. Synthetic polymers have led to several improvements on the original nitrocellulose collodions. Acrylic polymers are most suited to this method of presentation since they are soluble in a range of organic solvents. The first product of this type to be introduced was a solution of an acrylic resin in a mixture of acetic esters which, on evaporation, left an elastic transparent film. Like nylon film, this prevented the entry of micro-organisms but allowed free passage of air and of water vapour. Its use for covering clean surgical wounds has been described by Rob and Eastcott[17]. More recently, a solution of similar properties based on a different polymer has been made available as an aerosol dispenser or "bomb." It is claimed that a burn covering half of the body area can be treated in 5 minutes using spray dressings, compared with the 60 minutes which would be required for applying ointment and bandage. In emergencies, the spray dressings can be applied by semi-skilled persons.

(*c*) *Splints and Orthopædic Appliances*

The use of plastics in this field has been reviewed by Scales[18]. Their chief advantages lie in their ease and speed of manipulation, lightness, durability and resistance to chemicals. As an alternative to plaster of Paris for arm and leg splints, a knitted bandage has been developed containing 1 part of glass fibre and 4 parts of cellulose acetate. This is immersed before use in a setting fluid consisting of a mixture of volatile organic solvents in which the cellulose acetate is partly soluble. The damp bandage is then wound around the limb and after "setting," forms a light, porous water-impermeable splint which is also radio-translucent. Polythene is useful where a semi-rigid support is required such as in forearm splints, cervical collars, and spinal jackets. It can be obtained in sheet form, and on heating to 120° C. this becomes semi-viscous and can be applied to a plaster cast. If allowed to cool slowly on the case over about $\frac{3}{4}$ hour it will harden without wrinkling and thus provides a light and easily cleaned support which is much easier to make than a similar appliance from moulded leather or cellulose acetate reinforced with steel. Polymethyl methacrylate may be used in a similar manner but requires a higher temperature for moulding; it is less pliable than polythene and is therefore used for smaller splints and in simpler shapes. An interesting recent development in this field is the use of plastic appliances moulded direct to the patient and hence eliminating the need for a previously prepared plaster cast. This has been accomplished by using polythene together with polyurethane; polythene sheet reinforced by polythene strip provides rigidity while polyurethane supplies a heat-insulating layer during moulding and afterwards forms a comfortable padding. The technique has been described by Brennan[19].

(*d*) *Instruments*

The optical properties of polymethyl methacrylate have led to improvements in the design of diagnostic instruments such as the sigmoidoscope and laryngoscope. Owing to the high degree of internal reflection from the polished surface it is possible to direct "cold" concentrated light from a distance at any point or angle without risk of explosion from inflammable vapour or of tissue damage through burning. The construction of transparent instruments to various designs was made possible by the introduction in 1940 of methacrylate polymer in block form, from which the required shape can be cut without necessity of heat-moulding. Due to the "memory" of the polymer, a heat-moulded article tends to revert to its original shape when reheated, and instruments made in this way would thus become deformed when sterilised by boiling. The newer instruments made from "block" are free from this defect and can be sterilised repeatedly. Their introduction has greatly increased the safety of many surgical operations, particularly on the brain, due to elimination of heat. On account of its heat resistance and mechanical strength, nylon has been used to replace glass in the manufacture of syringes. These are now available in capacities up to 20 ml. and the plungers and barrels of each size are interchangeable, which facilitates reassembly after sterilisation. The syringe is virtually indestructible under ordinary conditions of use and can be sterilised by autoclaving up to 120° C.

3. *Plastic Prostheses*

Dental uses. Modern dentures are made entirely of polymethyl methacrylate since this possesses a suitable mechanical strength combined with ease of moulding and ability to take up inorganic pigments for colouring. The absence of any gap between teeth and plate makes the "complete" acrylic denture more hygienic than its precursor which had porcelain teeth on an acrylic plate. To prepare the denture the powdered polymer is mixed with a suitable amount of liquid monomer to produce a "dough," which is then transferred to a plaster mould contained in a metal flask. The latter is sealed and heated to a suitable temperature to cause complete polymerisation. The acrylic polymer is clean and easy to work with and seems to meet nearly all the requirements of both dental mechanic and patient. During the war, however, it was found that it would not resist mechanical shock of the severity experienced by paratroopers on landing, and nylon is therefore being investigated as an alternative of increased toughness.

Eyes. The making of artificial eyes was originally the secret of a small number of European glass-blowers. Their skill lay mainly in the blending of coloured glass to form the iris, and no attempt was made to fit the individual socket with any degree of accuracy. The first step in the use of plastics, during the early part of World War II, was to fit a glass iris on an acrylic eyeball. The modern method is to print irises on paper patterns and to insert these into complete acrylic eyes. An impression is first taken of the patient's socket and then transferred to a two-part plaster mould. The mould is used for the preparation of a wax

eye which is checked for ease of fitting and then inscribed with the position of the pupil and the size of the iris. Using the wax model, a second plaster mould is prepared in a dental flask and an artificial acrylic eye is prepared in the same manner as a dental plate. The final stage is the cementing in of the iris, previously painted or printed on paper or cellulose acetate film.

Facial and other restorations. In spite of the considerable advances which have been made in plastic surgery there is still a demand for a material which can be fabricated into prostheses, either for temporary use during a protracted series of surgical operations, or as a permanency if inoperable. Polyvinyl chloride is suitable for this purpose, since it is easily moulded and tinted, is not cold to the touch and has a texture not unlike that of natural soft tissue. For theatrical and film use it has the special advantage of not melting as wax may under strong artificial light. For facial and other repairs an impression of the injured area is first taken, using either plaster of Paris or dental impression material, and this is used to build a model. The missing part is made up in wax or plasticine and, when satisfactory, is impressed in plaster of Paris in a dental flask. A pourable cream is then made by addition of a plasticiser (e.g., phthalic esters) to polyvinyl chloride, and "foundation" colouring by inorganic pigments is also carried out at this stage. The coloured plastic cream is then poured into the dental flask mould and heated at 150° C. for one hour. After removal, it is trimmed and finally tinted, using organic dyes dissolved in liquid plasticiser.

4. *Miscellaneous Products of Some Interest to Pharmacists*

Spectacle frames and lenses. The "horn-rims" which were introduced about 1920 were celluloid and had the drawback of inflammability; they were later replaced by cellulose acetate frames of similar appearance, which were non-inflammable but less tough. An important post-World War II development was the frame made from polymethyl methacrylate, either by moulding or by cutting from sheet. This material is particularly suitable for the more decorative type of frame. Cheaper frames, such as those used in sunglasses, are still made of cellulose acetate. Another development has been the use of nylon, which is extremely tough, for sturdier articles such as safety goggles. Plastic lenses are almost entirely a post-war feature. Acrylic polymers are used and are either injection-moulded or roughly machined and then stamped out to give the correct curvature. Their main advantage over glass is that they are non-splintering; they are thus useful in sports or under any conditions where there is risk of breakage and hence damage to the eyes. Bifocal lenses are easier to fabricate in plastic than in glass, while contact lenses are really only practicable in plastic material. The latter are moulded to fit the patient's eye in a manner similar to that used for dentures.

Tooth brushes. Tufts are now usually made of nylon while the handle may be polymethyl methacrylate, polystyrene, or cellulose acetate. The brush should not be placed in hot water since this softens the cavity in which the tufts are embedded and thereafter allows them to splay out.

Unlike natural bristle, nylon does not soften when wet and the user should therefore be advised to start with a relatively soft nylon tuft to avoid damaging dental enamel. Having no central channel or cavity, nylon is more hygienic than natural bristle.

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DISCUSSION

MR. R. L. STEPHENS (Brighton) asked Dr. Child where the field of plastics ended and resin chemistry started. An interesting application referred to in the *Lancet* recently was a combination of melamine resin with plaster of Paris, to give a plaster cast which was strong, light and completely waterproof. Was the absorption of chemicals likely by plastic apparatus, with the possibility of contamination of one material with another when a change was made?

MR. W. H. STEPHENSON (Nottingham) asked whether the authors had any comment to make on the future development of the newer plastics for wound protection.

MR. T. D. WHITTET (London) said that some surgical dressings of the older type had almost entirely been replaced by plastic ones in his hospital. Jaconet and battiste were no longer employed, and the theatre staff found plastics particularly useful for covering couches and for aprons. Polyvinyl alcohol was used in buffered gels, but difficulty was experienced in obtaining a standard product. He understood plastic eye drop bottles were used in America. Tests had been carried out with nylon syringes and it was found that they had distinct advantages from the point of view of breakage. Unfortunately they were jerky in use and after repeated sterilisation they became opaque and dirty brown in colour.

MR. N. J. VAN ABBÉ (Loughborough) said he was particularly interested in the possibilities of polyvinylpyrrolidone dissolved in chloroform or alcohol for preparing tablet granules of readily hydrolysable substances. It did not prolong the disintegration time of the tablets. The non-sticking properties of P.T.F.E. suggested that if it could be bonded to

the surface of tablet punches it might assist in overcoming sticking problems.

MR. J. D. KULKARNI (Nottingham) said that soon after the war plastic watch straps were very popular in India, but the wearers suffered from skin diseases. Could the authors explain this occurrence?

MR. R. W. GILLHAM (Leeds) said that the use of flexible plastic wash bottles in the laboratory was accepted practice. It would be of great advantage if a plastic were developed which could stand up to sterilisation at 150° C., for use in packing sterile powders, such as cord powder.

MR. A. R. ROGERS (Brighton) said it would be interesting to know whether any of the plastics were good transmitters of ultra-violet light.

DR. K. R. CAPPER (London) stressed the necessity for plastic and other containers to be tested with the particular solution which it was intended to pack in them to ensure the constituents were not adversely affected.

MR. J. A. FREEMAN (Hounslow) pleaded for closer tolerances of the necks of glass bottles which were to be sealed with plastic plugs. How could such plugs be sterilised? There were plastic caps which were satisfactory in this country and others which were satisfactory in the tropics, but he had not found one which was satisfactory under both conditions.

MISS M. J. HINCKS (Slough) said it was often very difficult to remove plastic stoppers from bottles.

MR. D. STEPHENSON (Dartford) in commenting on the statement that plastics could not be sterilised by dry heat, said that some types of nylon could be heated to 180° C. without any deformation and with only slight shrinkage. Would not such heat treatment sterilise the nylon and was it not rather the shrinkage that occurred which rendered such nylon apparatus unsatisfactory.

MR. N. BRUDNEY (London) asked whether the transfer of carbon dioxide through polythene was sufficiently great to vitiate the storage of standard alkali solutions in polythene containers.

MR. W. P. LEGGETT (Liverpool) enquired whether there was any move afoot to introduce standards for plastics used in pharmacy and to standardise their nomenclature.

DR. C. L. CHILD, in reply, said that the ill-effects arising from plastic watch straps were due to polyvinyl chloride which had been plasticised, stabilised and lubricated for use in insulating cables, being employed. The material which was now sold for watch straps was suitable for that purpose. It was almost impossible to define plastics and ion exchange resins could be considered as coming within their range. With regard to the absorption of chemicals into plastic tubing, it was always necessary to ensure that the material was suitable for the particular purpose in view. As to the difficulty of obtaining a standard polyvinyl alcohol, it had hitherto been imported into this country and only recently had British manufacture of industrial grades commenced. The point that repeated sterilisation of nylon syringes resulted in discoloration did not surprise him. Grades of nylon varied. They had a melting point range from 180° C. to 264° C., but if heated in air at about 150° C.

there was fairly rapid oxidation. He would expect oxidation accompanied by discoloration and embrittlement to occur over a fairly short period at 120° C. On the question of bonding P.T.F.E. on to tablet punches, work had been done with only partial success. One of the difficulties was that P.T.F.E. did not stick to anything very easily. As to the prospect of transparent plastic bags withstanding sterilisation at 150° C., that might happen in the near future but it was not possible at the moment. He had no figures for the transmission of ultra-violet light through plastics. He agreed with the desirability of making full tests on containers against the contents to be placed in them, though a certain amount of generalisation was possible. It was surprising to hear that some stoppers had been found to be suitable in this country but not in the tropics and vice versa. If they were satisfactory in the tropics one would have expected them to be satisfactory in this country. On the question of heating nylon at temperatures of 180°, it was not just the dimensions which changed at that temperature; oxidation occurred accompanied by discoloration and embrittlement. The effect of the carbon dioxide transmitted on alkali solutions stored in polythene containers depended on the vapour pressure difference, the wall thickness and the length of time it was left in those particular conditions. He agreed that the standardisation of nomenclature was long overdue.

MR. A. W. BULL, in reply, emphasised that each product must be tested specificially for its use in the container which was proposed. In the case of eye drops, for example, there was a possibility that gases might pass through the polythene into the bottle. It was fundamental to the process by which glass bottles were made that it was impossible to work to extremely fine limits, and it was for that reason he had mentioned polystyrene containers which could be made to much finer limits than glass. But it must be remembered that with polystyrene there were water vapour transmission and other effects so that again it was necessary to test the product in the proposed container. With regard to possible absorption of carbon dioxide by alkali in polythene bottles the volume of atmosphere over the solution was an important factor. The less solution there was in the bottle the more the likelihood of absorption. One could visualise a closure not proving satisfactory in the tropics, but it was difficult to visualise a closure which was satisfactory in the tropics but unsatisfactory in this country. Dimensional changes could be appreciable and this could cause a loosening of the stopper. There was also the susceptibility of some plastics to alkaline preparations, and again temperature was a factor which would accelerate the production of defects.

MR. A. G. FISHBURN, in reply, said that collodion was one of the first plastic wound dressings. It was now claimed that in the case, for example, of extensive burns, rapid protection could be given by a sprayed on plastic dressing. A second advance in plastic wound dressings was by using their water vapour transmission properties to improve on the rather heavy cotton wool pads. A third advance was the incorporation of several different plastics in a first-aid dressing. The general problem

of how to sterilise the plastic normally arose in the sterlisation of the fabricated plastic article. To the best of his knowledge nylon was the most heat resistant and could be autoclaved. Polythene could be sterilised by boiling either alone or with chemicals, but the article required mechanical support against deformation. The sterilisation of plastic material by bactericidal gases had also been suggested. He agreed that a transparent plastic material which could be repeatedly sterilised at 150° C. would be a great advantage. P.V.P. certainly had many interesting possibilities. A monograph for a plastic would not be easy to write because of the difficulty of defining the mixed polymers, but the job would have to be done sooner or later and at the same time an approved name would have to be coined.

BRITISH PHARMACEUTICAL CONFERENCE ABERDEEN, 1955

SCIENCE PAPERS AND DISCUSSIONS

(*continued from page 773*)

THE SPECTROPHOTOMETRIC ASSAY OF INJECTION SOLUTIONS CONTAINING CHLOROCRESOL

By L. Brealey and K. A. Proctor

From the Physical Assay Division, Standards Department, Boots Pure Drug Co., Ltd., Nottingham

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Spectrophotometric methods of assay are usually simple and rapid and for these reasons are used extensively. These methods are most useful for simple preparations which contain only one ingredient which absorbs light in the ultra-violet or visible regions. When two ingredients absorb then the problem of determining the concentration of one or both is less simple and often impossible without some preliminary separation.

Many of the injection solutions listed in the British Pharmacopœia contain two main ingredients—the active principle and an antibacterial agent. The latter is usually phenylmercuric nitrate, chlorbutol, phenol or chlorocresol, and of these the last two absorb in the ultra-violet. One of the more difficult injection solutions to assay by any method is Apomorphine Hydrochloride Injection B.P. with chlorocresol as the antibacterial agent, and whilst devising a spectrophotometric method for this preparation it seemed probable that other injections containing chlorocresol might also be assayed by similar techniques.

A single method by which any injection containing any bacteriostatic could be determined would have been an ideal solution but this is still impractical and eight preparations have been assayed by three fundamentally different methods.

The Direct Method (I)

A direct method is applicable when the absorption of the active ingredient is so much higher than that of the chlorocresol that the latter can be ignored. Here, the determination of the active ingredient requires only a simple dilution and measurement at a suitable wavelength. Injection of Procaine and Adrenaline B.P. provides an example.

Injection of Procaine and Adrenaline B.P. Procaine hydrochloride shows a maximum absorption at 290 mμ and at this wavelength in water the absorptions of all the ingredients in the concentration present are as follows. Procaine hydrochloride $E^{2\ \text{per cent.}}_{1\ \text{cm.}}$ 290 mμ = 1360; adrenaline

hydrochloride $E^{0.002\ \text{per cent.}}_{1\ \text{cm.}}$ 290 mμ = 0·09; chlorocresol $E^{0.1\ \text{per cent.}}_{1\ \text{cm.}}$ 290 mμ = 4·0.

As the total absorption of all other ingredients is so small compared with that of the procaine hydrochloride, the latter is determined directly by making a 2000 times dilution in water and reading the maximum absorption at about 290 mμ.

$$\text{Per cent. procaine hydrochloride} = \frac{\text{absorption at 290 m}\mu}{680} \times 2000$$

The Simultaneous Determination Method (II)

This method can be used when the absorption characteristics of chlorocresol and the active ingredient are so different as to allow both to be determined by making measurements at two wavelengths. To achieve a sufficient degree of accuracy the absorption of the active principle should be at least as great as that of the chlorocresol. A suitable example is provided by Injection of Pethidine Hydrochloride B.P.

Injection of Pethidine Hydrochloride B.P. In 0·1N hydrochloric acid at 279 mμ chlorocresol exhibits maximum absorption while pethidine hydrochloride has none. At 257 mμ pethidine hydrochloride has maximum absorption and knowing the concentration of the chlorocresol a suitable correction can be made and the former determined. In order that both spectrophotometric measurements are made at suitable absorption values it may be necessary to use different dilutions for the two readings, depending on the relative concentrations of the pethidine and chlorocresol in the original solution. The calculations are as follows. In 0·1N hydrochloric acid for chlorocresol, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 257 mμ = 20, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 279 mμ = 105; for pethidine hydrochloride, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 257 mμ = 7·3, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 279 mμ = zero.

If the observed absorptions are, at 257 mμ = A at dilution factor = x, 279 mμ = B at dilution factor = y, then per cent. chlorocresol = $\frac{By}{105}$. At 257 mμ contribution of chlorocresol to total absorption = $\frac{20B}{105} \times \frac{y}{x}$ = C, per cent. pethidine hydrochloride = $\frac{(A - C)x}{7{\cdot}3}$.

Injection of methylamphetamine hydrochloride. A second example is provided by injection of methylamphetamine hydrochloride. When dissolved in 0·1N hydrochloric acid methylamphetamine hydrochloride has the following absorption characteristic. Maximum $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 257 mμ = 10·1, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 279 mμ = zero. In mixtures containing chlorocresol both may be determined in the same way as injection of pethidine hydrochloride.

Separation Methods (III)

Preliminary separation is necessary when the absorption curves of the two ingredients are so similar that simultaneous determination is inaccurate, or when the absorption of the active ingredient is much less than that of the chlorocresol. An example is Injection of Atropine Sulphate B.P.

Injection of Atropine Sulphate B.P. The official preparation contains 0·06 per cent. atropine sulphate and when chlorocresol is used as a preservative its usual concentration is 0·2 per cent. In 0·1N acid the absorption characteristics are as follows. Atropine sulphate $E^{1\%}_{1\,cm.}$ 257 mμ = 6·25 (max), $E^{1\%}_{1\,cm.}$ 279 mμ = zero; chlorocresol $E^{1\%}_{1\,cm.}$ 257 mμ = 20, $E^{1\%}_{1\,cm.}$ 279 mμ = 105.

It might appear at first sight that the simultaneous method could be used for this preparation but it would give rise to large errors in the determination of atropine sulphate. In the concentrations at which the two are present in the original injection the absorption at 257 mμ is 0·38 for atropine sulphate and 4·0 for chlorocresol. The chlorocresol can be determined directly by diluting and reading the absorption at 279 mμ, but if this determination is used as the basis for a correction at 257 mμ as in the simultaneous method, the absolute error in determining the atropine sulphate will not be less than the absolute error in determining the chlorocresol. If the latter is 1 per cent. then it will contribute a 10 per cent. error to the atropine sulphate result. It is desirable therefore to separate the atropine sulphate from the chlorocresol and determine it directly.

Whether separation was possible was tried with 5 ml. of a solution containing 0·12 per cent. atropine sulphate and 0·2 per cent. chlorocresol. This was made alkaline with sodium hydroxide and extracted with four 15 ml. portions of chloroform. The chloroform extracts were filtered through a paper previously moistened with chloroform, and evaporated to dryness. The residue was dissolved in 10 ml. 0·1N sulphuric acid and the absorption curve measured, using in the comparison cuvette a solution obtained by evaporating to dryness 30 ml. of chloroform and dissolving the residue in 5 ml. 0·1N sulphuric acid. A comparison of this curve with that of pure atropine sulphate showed that the alkaloid was extracted quantitatively and in a reasonably pure form. The purity of the extracted alkaloid was important in order that the absorption could be used for a direct determination. Table I shows the ratios obtained on the absorptions of the four maxima of atropine sulphate both in direct solution and after extraction.

TABLE I

THE RATIOS OF ABSORPTIONS AT PEAK WAVELENGTHS FOR ATROPINE SULPHATE IN 0·1N·H_2SO_4. (*a*) DIRECTLY, (*b*) AFTER CHLOROFORM EXTRACTION

	(*a*) Direct solution	(*b*) After extraction
257/246 mμ	1·48	1·41
257/251 mμ	1·21	1·18
257/263 mμ	1·29	1·29

Solutions containing 0·06 per cent. and 0·12 per cent. atropine sulphate and 0·2 per cent. chlorocresol were prepared. Five ml. was extracted as described and the atropine sulphate content determined by dissolving the residue in 0·1N sulphuric acid, the absorption being measured at 257 mμ. A further 2 ml. of the injection was diluted to 100 ml. with 0·1N hydrochloric acid and its absorption at 279 mμ used to determine chlorocresol.

Injection of Strychnine Hydrochloride B.P. A similar method was applied to strychnine hydrochloride solutions for injection. A solution was made containing 0·2 per cent. strychnine hydrochloride and 0·1 per

cent. chlorocresol, and 5 ml. of this solution diluted to 50 ml. with water. 10 ml. of this diluted solution was made alkaline with 1 ml. N sodium hydroxide and extracted as described for Injection of Atropine Sulphate. The residue was dissolved in 50 ml. 0·1N sulphuric acid and 15 ml. of this solution diluted to 50 ml. also with 0·1N sulphuric acid. The absorption of the final solution was measured at 255 mμ using 0·1N sulphuric acid in the comparison cuvette. In 0·1N sulphuric acid strychnine hydrochloride has maximum absorption at 255 mμ, $E^{1\text{ per cent.}}_{1\text{ cm.}}$ 255 mμ = 315. Using this information the strychnine hydrochloride of the original solution was calculated.

The alkaline solution remaining after the original chloroform extraction was diluted to 100 ml. with 0·1N sodium hydroxide and the absorption of this solution measured at 296 mμ. In 0·1N sodium hydroxide chlorocresol shows maximum absorption at 296 mμ, $E^{1\text{ per cent.}}_{1\text{ cm.}}$ 296 mμ = 183. The chlorocresol content of the original solution was determined. The simultaneous determination method may be used for chlorocresol and strychnine, but the extraction method gives a more accurate determination of both components.

Apomorphine Hydrochloride Injection B.P. This is an example in which the absorption curves of the ingredients are so similar as to make a separation necessary despite the fact that the apomorphine hydrochloride absorbs much more strongly than the chlorocresol. The absorption curves of the two ingredients are shown in Figure 1 and their similarity is obvious.

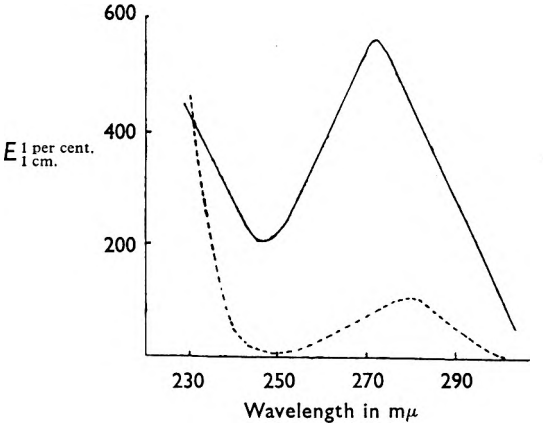

FIG. 1. Absorption curves in 0·1N hydrochloric acid of:—
——— Apomorphine hydrochloride.
– – – – Chlorocresol.

A partition chromatographic method of separating *para*- and *meta*-cresols on a buffered kieselguhr column has been described[1] and was found successful in removing chlorocresol from the injection solution. The sample after dilution is put onto a column buffered at *p*H 11·4, and eluted with *cyclo*hexane. Chlorocresol is eluted first and can be estimated in the *cyclo*hexane solution while the apomorphine is retained on the column. The total absorption of the original injection solution is measured and a correction for the chlorocresol applied. As the apomorphine hydrochloride absorbs the more strongly the method is accurate. The results summarised in Table II shows chlorocresol to be removed quantitatively and in a pure form.

The method for a solution containing 0·3 per cent. apomorphine hydrochloride and 0·2 per cent. chlorocresol is described. Dilute 2 ml. to 10 ml. with water and add 2 ml. of this solution to a beaker containing 5 g. of kieselguhr previously mixed with 5 ml. of acetate buffer solution of *p*H 11·4, and mix well. Transfer the mixture in two portions to a chromatographic tube 12 inches long, and 1 inch in diameter pressing down each portion lightly with a flat-ended glass rod. Scrub out the beaker first with 1 g. kieselguhr and then with a small piece of cotton wool, adding each to the top of the column. Elute the column with *cyclo*hexane collecting the first 25 ml. and measure the maximum absorption of the solution at about 280 mμ, using in the compensation cuvette *cyclo*hexane which has passed through the column after the first 25 ml. Determine

TABLE II

THE ABSORPTIONS OF SOLUTIONS OF APOMORPHINE HYDROCHLORIDE IN *cyclo*HEXANE (*a*) AFTER ELUTION FROM A BUFFERED KIESELGUHR COLUMN, (*b*) DIRECTLY

	λ (mμ)	Absorption of eluted solution	Absorption of direct solution
Maxima	280	0·256	0·258
	288	0·240	0·240
Minima	247	0·020	0·028
	285	0·206	0·208

TABLE III

SUMMARY OF ALL RESULTS INVOLVING THE DIFFERENT TECHNIQUES DESCRIBED. ALL SOLUTIONS USED WERE MADE UP IN THE LABORATORY FROM STANDARD MATERIALS

Active ingredient	Method	Per cent. active ingredient		Per cent. chlorocresol	
		Theory	Found	Theory	Found
Procaine hydrochloride ..	I	2·0	2·01		
Pethidine hydrochloride ..	II	2·5	2·46	0·20	0·210
		2·5	2·58	0·10	0·107
		7·5	7·54	0·20	0·206
Methylamphetamine HCl ..	II	1·0	1·05	0·10	0·108
		1·0	1·07	0·20	0·205
Atropine sulphate	III	0·06	0·062	0·20	0·200
		0·12	0·124	0·20	0·200
Strychnine hydrochloride ..	III	0·2	0·203	0·10	0·092
		0·2	0·206	0·20	0·197
Apomorphine hydrochloride	III	0·10	0·107	0·10	0·104
		0·30	0·307	0·20	0·190
Morphine sulphate	III	1·0	0·989	0·20	0·213
		3·0	2·89	0·20	0·220
Morphine sulphate (with atropine)	III	1·0	0·988	0·10	0·100
		1·0	0·990	0·20	0·224

the chlorocresol in the injection knowing that in *cyclo*hexane $E^{1\ per\ cent.}_{1\ cm.}$ 280 mμ = 129. Dilute 5 ml. of the injection to 100 ml. and a further 5 ml. to 100 ml. using 0·1N hydrochloric acid for both dilutions. Measure the absorption at 272 mμ and subtract the chlorocresol contribution. The net absorption is then directly proportional to the apomorphine hydrochloride content and the latter is calculated. The method of calculation is similar to that described for Injection of Pethidine Hydrochloride, using the following values. Chlorocresol in *cyclo*hexane $E^{1\ per\ cent.}_{1\ cm.}$ 280 mμ = 129, in 0·1N hydrochloric acid $E^{1\ per\ cent.}_{1\ cm.}$ 272 mμ = 80, apomorphine hydrochloride in 0·1N hydrochloric acid $E^{1\ per\ cent.}_{1\ cm.}$ 272 mμ = 552.

Injection of Morphine Sulphate B.P. and Injection Morphine and Atropine B.P. Morphine sulphate is retained on a kieselguhr column buffered at *p*H 11·4 when *cyclo*hexane is used for elution, and these injections can be assayed for morphine sulphate and chlorocresol by the same method as is used for Injection of Apomorphine Hydrochloride.

SUMMARY

1. Spectrophotometric methods have been used to assay eight injection solutions containing chlorocresol as an antibacterial agent.

2. The methods are accurate and are more rapid than chemical estimations.

REFERENCES

1. White and Grant, *Nature, Lond.*, 1955, **175**, 513.

DISCUSSION

The paper was presented by MR. K. A. PROCTOR.

DR. F. HARTLEY (London) said that in many official assays of injections interference might be caused by some of the permissible bacteriostatics. He hoped that the authors would go on to study the effect of other bacteriostatics in addition to chlorocresol in their recommended procedures.

DR. R. E. STUCKEY (London) said it would be of interest to know whether the figures quoted for chlorocresol were for a chemically pure sample, or one of pharmacopœial quality, what difference was found, and whether this difference could be allowed for in assaying mixtures where the absorption due to chlorocresol was as great as that due to the active principle. He also asked whether the authors had any further information as to the *p*H at which the absorption of chlorocresol started to change, as they had quoted results showing that absorption was markedly different in alkaline and acid solutions.

DR. A. H. BECKETT (London) asked for further information on the separation method III. The authors had extracted atropine with chloroform. In extracting phenols, he had experienced trouble when taking the chloroform to dryness. Had the authors done a series of blank extractions and had they obtained reproducible results?

MR. K. A. PROCTOR, in reply, said work was proceeding on other bacteriostatics. The chlorocresol used was of B.P. standard. No great variation was found with different samples, and the figure used was a mean. He had no further information of the effect of *p*H changes on the absorption. No difficulty had been encountered with chloroform in the determination of atropine sulphate by the method described.

THE SEPARATION AND VOLUMETRIC DETERMINATIONS OF ALUMINIUM, BISMUTH, CALCIUM AND MAGNESIUM, IN PHARMACEUTICAL PREPARATIONS

By H. E. Brookes and C. A. Johnson

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During recent years the number of pharmaceutical preparations containing aluminium, bismuth, calcium and magnesium in various combinations has increased considerably, and thus the problem of the rapid determination of these elements in the presence of one another has become correspondingly important. The wealth of literature describing the determination of individual metal ions with ethylenediaminetetra-acetic acid naturally led to a consideration of this reagent.

As ethylenediaminetetra-acetic acid (EDTA) is capable of chelating with a large number of metal ions care must be taken that the conditions under which it is used are so chosen that the interference with the determination of one ion by others present is a minimum. The published data on stability constants of the various metal complexes may assist in devising methods for selective determinations, but the constants will almost certainly have been determined in an arbitrarily arranged set of conditions, and they may be considerably modified in practice. There are three main lines of approach to the problem, namely (i) control of *p*H, (ii) use of specific indicators and (iii) use of masking agents; that is, reagents which will, under given conditions, react with certain metal ions to form complexes more stable than those formed with EDTA under the same conditions, thus leaving other ions free to be titrated. Much work remains to be done especially in the search for specific indicators, but considerable advances have been made, particularly as a result of the systematic studies of Přibil and others.

These considerations have now been applied to the analysis of pharmaceutical preparations containing aluminium, bismuth, calcium and magnesium. The determinations of calcium and magnesium in the absence of other metals are already well known and require only passing comment. It was necessary, however, to examine the various methods which have been suggested for the determination of aluminium and of bismuth to select those most suitable for inclusion. Certain difficulties, not mentioned in the literature, were encountered, necessitating a more detailed study of the conditions of reaction. These two metals are therefore dealt with individually before the problem of separation is discussed.

Aluminium

Most methods which have been suggested for the volumetric determination of aluminium with EDTA depend upon the addition of a known excess of the reagent to a solution of the metal followed by titration of the excess with a standard solution of some other metal for which a

reliable indicator is available; ferric chloride[1,2], thorium nitrate[3,4], and zinc chloride[5] have all been used for this purpose. More recently a method of direct titration using hæmatoxylin as indicator has been suggested by Taylor[6].

For routine application to the present work it seemed that the thorium nitrate method showed the greatest promise, because it could also be used to determine aluminium and bismuth together, and because thorium nitrate solution is stable and reliable for use as a standard. It was therefore decided to examine ter Haar and Bazen's[4] procedure in detail, despite the fact that they reported the reaction between aluminium and EDTA to be nonstoichiometric and to yield results which were consistently low to the order of about 1 per cent.

Experimental

A number of standard solutions of aluminium prepared from different grades of the metal were analysed by the slightly modified ter Haar and Bazen's procedure described below and by the gravimetric oxine method[7] (Table I). The effects of varying the excess of complexing agent added (Table II), the time for which the reaction was allowed to proceed (Table III) and the temperature of the reaction were also examined. In addition, two of the solutions were used to examine the direct titration method proposed by Taylor[6].

TABLE I

APPLICATION OF THE METHOD OF TER HAAR AND BAZEN TO STANDARD ALUMINIUM SOLUTIONS

	Theoretical Al content (g. per litre)	Al found		Per cent. recovered by EDTA method
		By EDTA*	By oxine	
Solution 1 (prepared from 99·99 per cent. Al)	1·666	1·665		99·9
Solution 2 (prepared from 99·99 per cent. Al)	1·636	1·638	1·652 1·640	100·1
Solution 3 (prepared from 99·8 per cent. Al)	1·623	1·623	1·626 1·641	100·0
Solution 4 (prepared from 99·5 per cent. Al)	1·781	1·776†	1·785 1·791	99·7

* The results obtained using EDTA are based on the mean values obtained from four titrations on each solution. In no case did a burette reading in any of the groups differ by more than 0·05 ml. from the mean value.

† Corrected for the presence of 0·25 per cent. of Fe.

*Method of ter Haar and Bazen**

To a quantity of solution containing from 15 to 20 mg. of aluminium add 25 ml. of 0·1N EDTA and 80 ml. of water; neutralise to congo red paper by the dropwise addition of solution of sodium hydroxide and add 5 ml. of 2M monochloroacetic acid, 10 ml. of 1M sodium acetate and 1·5 ml. of alizarin S indicator. Titrate with 0·1N thorium nitrate to the

* The reagents used in the above and all subsequent determinations described are classified as an Appendix.

TABLE II

EFFECT OF VARYING THE EXCESS OF EDTA ADDED IN THE METHOD OF TER HAAR AND BAZEN

0·1N EDTA added (ml.)	Amount theoretically required (ml.)	Amount used (ml.)	Per cent. deviation
50	6·74	6·70	−0·6
40	13·48	13·44	−0·3
35	13·48	13·46	−0·2
25	13·48	13·44	−0·3
25	6·74	6·76	+0·3
25	12·92	12·92	0
20	12·92	12·88	−0·3
15	12·92	12·70	−1·7
15	13·48	13·22	−1·9

bluish-red end-point; each ml. of 0·1N EDTA is equivalent to 0·001349 g. of Al.

In order to examine the effect of temperature on the reaction, solutions were boiled and maintained as near the boiling point as possible during titration. They gave almost identical results with those quoted in Table I.

TABLE III

THE EFFECT OF TIME OF REACTION BETWEEN AL AND EDTA

Time*	Per cent. deviation of the result from the theoretical value	
	Using a twofold excess of EDTA	Using only a slight excess of EDTA
30 seconds	−3·9	−9·1
1 minute	−0·6	−2·2
4 minutes	+0·3	−1·5
15 minutes	−0·1	−0·7
30 minutes	+0·1	+0·2

* The time recorded here is from the end of the addition of EDTA to the commencement of the titration. In practice this time would be somewhat more than a minute; the above figures were obtained by having all solutions measured for addition before commencement of the reaction and by knowing in advance the exact amount of sodium hydroxide required for neutralisation.

The effect of certain ions likely to be present in pharmaceutical preparations when determining aluminium was examined experimentally. Under suitable conditions bismuth reacts quantitatively and reference is made to this in a later section; calcium and magnesium do not interfere; sulphates cause interference with the end-point when present in large excess; phosphates, which may be present in certain tablets, cause precipitation of thorium and must therefore be removed; the effect of silicate in solution has not yet been examined, although it has been removed by the classical gravimetric procedure before determination of the metal ions. Common excipients such as lactose, sucrose, starch, tragacanth and acacia are without effect on the determination, but it was found to be easier to remove insoluble matter by filtration or by ignition before proceeding to the titration.

Direct Titration Method of Taylor

A number of determinations by the method of Taylor[6] were made on two of the aluminium solutions prepared during the examination of the thorium nitrate method. This direct titration procedure is carried out by adding the almost neutral aluminium solution, the strength of which is to be

determined, from a burette to standard EDTA containing hæmatoxylin indicator until a permanent pinkish-purple colour is obtained. The titration liquid is then buffered to *p*H 6·0, boiled and titrated with the aluminium solution to the return of the pinkish-purple end-point, the temperature being maintained above 70° C. It was found that a sharper and more satisfactory end-point could be obtained by titration with the aluminium solution to within 0·5 ml. of the equivalence point before addition of the buffer solutions and boiling. This demands an approximate knowledge of the titre to be expected, obtained by a preliminary titration carried out as described by Taylor. The series of results listed in Table IV were obtained in this manner.

TABLE IV

APPLICATION OF THE METHOD OF TAYLOR TO STANDARD ALUMINIUM SOLUTIONS

	Theory	Content of aluminium (g./l.)	
		Found	Per cent. deviation
Solution 1	1·666	1·658	−0·5
		1·673	+0·4
		1·662	−0·2
		1·676	+0·6
		1·653	−0·8
Solution 2	1·636	1·632	−0·2
		1·623	−0·8
		1·628	−0·5
		1·633	−0·2

Discussion

The results listed in Table I show that, under the conditions proposed, theoretical amounts of aluminium have been found. This observation is at variance with that of ter Haar and Bazen who reported results which were consistently low to the order of about 1 per cent. It has been demonstrated that low results may be obtained if an insufficient excess of complexing agent is added, or if the time of reaction is insufficient, and, following from this, the smaller the excess of EDTA added the longer the time required for the reaction to proceed to completion. These results show that the reaction between aluminium and EDTA is a relatively slow one, and explain one of the principal difficulties which has been encountered in devising a suitable direct titration. If a twofold excess of EDTA is added to the aluminium solution the reaction may be assumed to be complete well within the time required to neutralise and add the buffer solutions and indicator.

The direct titration using hæmatoxylin as indicator yields results which may vary within about 1 per cent. of the theoretical figure, but there is no indication that they are consistently low. For routine purposes the method suffers from the disadvantages that the sample solution must be placed in the burette, that the titration must be carried out in hot solution and that so unsatisfactory a material as hæmatoxylin must be used as indicator. In one respect the use of hæmatoxylin as indicator is of value in that the formation of the aluminium-hæmatoxylin lake at the end-point confers a degree of specificity. A reasonable balance between accuracy, convenience and specificity must be maintained, however, and it is therefore considered that the thorium nitrate method should be applied in general routine practice, whilst the direct titration procedure is of value in the

occasional instances when a pharmaceutical preparation of aluminium also contains sulphate or phosphate.

Bismuth

Methods which have been suggested for the titration of bismuth with EDTA include amperometric titration[8], back-titration with standard magnesium solution at *p*H 10[9], direct titration using the thiourea complex as indicator[10,11], back-titration with thorium nitrate at *p*H 3·5[12], direct titration using potassium iodide as indicator[13] and direct titration using catechol violet as indicator[14]. Since a high degree of accuracy was claimed for the majority of these methods it was decided to concentrate on those which are more likely to be specific. Pre-eminent in this category is the method using catechol violet, an acid-base indicator[15] which is yellow in acid, and deep blue in moderately alkaline media, forming a deep blue complex with bismuth at *p*H 1 to 2; few other metals chelate with EDTA at this degree of acidity and the method is therefore considerably selective; the colour change is specific for bismuth at this *p*H. The procedure described by Grönkvist[10] was also selected for examination since the formation of the yellow-coloured bismuth-thiourea complex also serves to confirm the presence of bismuth; this method suffers from the disadvantage that, since the titration is carried out at *p*H 2·5 to 4·0, other metals may also chelate with the titrant. More recently Fritz[11] has suggested modifications to the method which render it more selective. Some determinations were also made by the back-titration method using thorium nitrate as described for aluminium.

Experimental

A standard solution of pure bismuth metal (99·995 per cent.) in nitric acid was prepared for an initial comparison of the methods. The thiourea procedures of Grönkvist and of Fritz were applied exactly as stated by the authors. Considerable modifications were necessary, however, to the outline procedure suggested by Malát, Suk and Ryba[14] using catechol violet before a satisfactory titration, free from precipitation of bismuth and fading of the indicator, yielding a sharp end-point, could be obtained. Attention was drawn by these authors to the instability of catechol violet in strongly alkaline solutions, but no mention was made of its instability in strongly acid media or its destruction by strong oxidising agents; these features, taken in conjunction with the facts that the end-point is much sharper in relatively dilute solutions, but that precipitation of bismuth is likely to take place in all but quite acid solutions, has made it necessary to adopt the following procedure:—

Dilute a suitable aliquot of the solution in nitric acid, containing about 120 mg. of bismuth, to 50 ml. with water ensuring that only sufficient acid is present to prevent precipitation of the metal. Add 2 drops of catechol violet indicator and, if the solution is violet in colour, add dilute solution of ammonia drop by drop until the characteristic deep blue colour is obtained. Titrate with 0·1N EDTA until the solution turns violet-red, dilute with 200 ml. of water, adding a further 6 to 8 drops of the indicator

solution (the solution reverts to its original blue colour on dilution) and continue the titration. The colour of the solution changes through violet, red and orange, followed by a sharp change to bright yellow at the end-point; each ml. of 0·1N EDTA is equivalent to 0·01045 g. of Bi.

By adopting this procedure the necessary dilution required for a sharp end-point may be made without danger of precipitating the bismuth, since the majority of this is complexed by the time the additional water is added. Table V records the results obtained by these various methods on a standard bismuth solution.

TABLE V

THE DETERMINATION OF A STANDARD BISMUTH SOLUTION BY VARIOUS METHODS

	Content of Bi (mg. per 10 ml.)
Theoretical figure	124·6
By precipitation as phosphate[18]	124·9
By Catechol Violet method	124·0
By method of ter Haar and Bazen	124·0
By method of Grönkvist	124·3
By method of Fritz	124·0

The determination with catechol violet suffers from the disadvantage that even small quantities of strongly oxidising agents seriously impair the end-point; chlorides, for example, must be absent from the nitric acid solutions, organic materials giving rise to oxides of nitrogen when dissolved in nitric acid are deleterious and so is the presence of hydrogen peroxide. In general, however, solutions may be prepared from most pharmaceutical preparations so that these interfering substances can be avoided. The method of Fritz gives a good end-point, although not so sharp as that with catechol violet, but the precise *p*H adjustment necessary makes this determination a somewhat lengthy one compared with the catechol violet method. Grönkvist's procedure suffers from a number of disadvantages:

(i) Since it is carried out at *p*H 2·5 to 4·0 it is subject to interference from a number of other metal ions.

(ii) The end-point is by no means as definite as in the other methods described.

(iii) Precipitation of phthalic acid has been encountered during titration on a number of occasions and this may make the end-point more difficult to detect.

Discussion

These considerations point to a clear choice of the catechol violet method for the majority of pharmaceutical materials. Only in those instances where an oxidising mixture cannot be avoided is it suggested that the thiourea method be employed; as will be seen from the applications in a later section this has not been necessary in any of the work carried out.

The determination of Aluminium, Bismuth, Calcium and Magnesium in the presence of each other

The methods which have been employed in this scheme of separation are based upon the above procedures and upon joint determinations, that is, determination of two metals together, and masking techniques. For

example, bismuth and aluminium may be determined together by the thorium nitrate method, bismuth and magnesium or bismuth and calcium, by a modification of the usual solochrome black titration in alkaline solution. If both calcium and magnesium are present as well as bismuth, the bismuth may be removed rapidly by precipitation as oxychloride. Several attempts were made to mask bismuth so that magnesium and calcium might be obtained without having to carry out a separation, but these have so far been unsuccessful. Very recently, however, Přibil and Roubal have described the use of 2:3-dimercaptopropanol for this purpose[17] and the possibility of applying this method to the present scheme is now under consideration.

Aluminium in the presence of magnesium or of calcium is more easily masked since, under suitable conditions, it is effectively complexed with triethanolamine[18,19]. One or two cautionary notes must be added concerning the methods, however, and these are mentioned below with the recommended procedures. Detailed methods for the various separations and joint titrations are described, together with results of some of the experimental work. The method given for the removal of bismuth is considered to be an improvement on that described in the British Pharmaceutical Codex for Compound Lozenges of Bismuth, since traces of bismuth may remain in solution in the official method causing an unsatisfactory solochrome end-point and, possibly, slightly high results. If precipitated bismuth oxychloride remains in the titration liquid after addition of EDTA it is slowly soluble, resulting in erroneous results unless the titration is carried out rapidly after addition of the complexing agent. The detailed methods are followed by a suggested scheme of separation.

Method I

The determination of Aluminium in the presence of Bismuth

To a quantity of solution believed to contain sufficient bismuth and aluminium to react with about 12 ml. of the complexing solution add 25 ml. of 0·1N EDTA and 80 ml. of water and proceed by the method described for the determination of aluminium, commencing with the words "neutralise to congo red paper. . . ." From the quantity of EDTA used subtract the amount required to titrate the bismuth in the same volume of solution using the catechol violet method; each ml. of 0·1N EDTA is equivalent to 0·001349 g. of Al.

NOTE: If too large an excess of EDTA is added, precipitation of bismuth-EDTA complex may occur causing an unsatisfactory end-point. If too small an amount is added there will be a danger that the aluminium will be incompletely complexed.

Using the above conditions, satisfactory recoveries of aluminium in the presence of bismuth were obtained.

Method II

The determination of Magnesium or Calcium in the presence of Bismuth

To a suitable aliquot of the slightly acid solution containing bismuth and magnesium or calcium, add 25 ml. of 0·1N EDTA, 100 ml. of water

and a few drops of solochrome black indicator; add dilute solution of ammonium hydroxide drop by drop until just blue, and then 1 ml. in excess; add more indicator as required and titrate with 0·1N calcium chloride. From the quantity of EDTA used subtract the amount required to titrate the bismuth in the same volume of solution using the catechol violet method; each ml. of 0·1N EDTA used is equivalent to 0·001216 g. of Mg. (or 0·002004 g. of Ca).

NOTES: 1. If only bismuth and calcium are being determined a little magnesium-EDTA complex should be added to the solution to sharpen the end-point.

2. It is important that the amount of ammonia added should not exceed that stated; at higher *p*H values the full blue colour of the indicator does not develop satisfactorily, a reddish-blue colour being obtained which seriously masks the end-point.

Some results obtained by this method are included in Table VI.

TABLE VI

DETERMINATION OF A STANDARD MAGNESIUM* SOLUTION BY EDTA TITRATION

		g. of Mg per litre
1.	No other metals present	1·509
2.	By method IV, but without added Al	1·508
3.	By method IV, fivefold equivalent of Al present	1·504
	" " " "	1·510
	" " " "	1·509
4.	By method IV, 2 minutes standing	1·510
5.	" " " 5 " "	1·520
6.	" " " 10 " "	1·535
7.	" " " 15 " "	1·549
8.	" " " 30 " "	1·600
9.	By method II, one equivalent of Bi present	1·511
10.	" " " two equivalents " "	1·509
11.	" " " three " " "	1·505
12.	By method III, three equivalents of Bi removed	1·509

* The magnesium was first determined as pyrophosphate[21] and, in duplicate determinations, the solution was found to contain 1·507 g. and 1·511 g. of Mg per litre.

Method III

The removal of Bismuth prior to the determination of Magnesium and Calcium

To a suitable aliquot containing sufficient calcium and magnesium for the determination of each metal, add 2 ml. of dilute hydrochloric acid, 150 ml. of water and sufficient dilute solution of ammonia until the mixture is only just acid to litmus paper; heat to boiling and boil for 2 minutes, stirring to prevent bumping; cool in ice, confirm that precipitation is complete by the addition of a few drops of brine and filter through a No. 42 Whatman filter paper, washing with cold water; adjust the combined filtrate and washings to a suitable volume with water.

To one aliquot add 10 drops of solution of potassium cyanide, 10 ml. of ammonia buffer solution and sufficient solochrome black indicator, and titrate with 0·1N EDTA to give the combined titre for calcium and magnesium.

To a second aliquot add 25 ml. of 0·1N EDTA, 5 ml. of solution of sodium hydroxide and 0·2 g. of murexide indicator, and titrate the excess of EDTA with 0·1N calcium chloride to give the titre for calcium.

NOTE: The solochrome black end-point is considerably improved by the addition of the cyanide, which serves to complex any trace of heavy metals left in solution.

Some results obtained for a magnesium solution by this method are included in Table VI.

Method IV

The determination of Magnesium or Calcium in the presence of Aluminium

To a suitable aliquot of the slightly acid solution containing aluminium and magnesium (or calcium) add 1 g. of ammonium chloride, sufficient solution of triethanolamine so that the precipitate which at first forms completely redissolves (about 25 to 40 ml.) 200 ml. of water, 5 ml. of ammonia buffer solution and sufficient solochrome black indicator to give a full red colour; titrate immediately with 0·1N EDTA to the formation of a full blue colour; each ml. of 0·1N EDTA is equivalent to 0·001216 g. of Mg (or 0·002004 g. of Ca).

NOTES: 1. The red colour changes to purple about $\frac{1}{2}$ to 1 ml. before the end-point and thus provides a useful indication that the end-point is near.

2. Whilst it is agreed that the blue end-point fades back to purple on standing, no difficulty with fading before the end-point, as has been found by Ritchie[20], has ever been encountered using the above procedure. He suggests that the titration be carried out at a temperature below 5° C., but at the dilution used in the proposed method this modification would appear to be unnecessary.

On carrying out a number of determinations by the procedure described it was observed that high results were obtained if solutions were allowed to stand before titration, probably due to a gradual hydrolysis of the aluminium-triethanolamine complex. A standard magnesium solution was therefore prepared and a number of determinations were made to find the rate and extent of the breakdown under the conditions stated. Some determinations of the magnesium in the presence of bismuth, both by the titration of the two metals together (Method II) and the separation procedure (Method III) were also made. The results are listed together in Table VI.

It is obvious from results 3 to 8 that Method IV is only satisfactory if the titration is carried out as soon as possible after addition of the triethanolamine and dilution with water.

Method V

The determination of Magnesium and Calcium in the presence of Aluminium

The magnesium and calcium are determined together using Method IV. The calcium is then determined as follows:—

To a suitable aliquot of the slightly acid solution containing aluminium, calcium and magnesium add sufficient solution of triethanolamine followed by 5 ml. of N sodium hydroxide; if a gelatinous precipitate forms at this stage filter off and wash the filter with water, collecting about 200 ml. of combined filtrate and washings; if no precipitate forms, dilute with water

to 200 ml.; to the resulting solution add 0·2 g. of murexide indicator and titrate with 0·1N EDTA; each ml. of 0·1N EDTA is equivalent to 0·002004 g. of Ca.

NOTE: The end-point in this determination is not as well-defined as others described in this work, but is quite satisfactory for routine application.

These methods are classified below into a scheme for dealing with mixtures.

Section I. Bi present

Titrate Bi by Catechol Violet method and then proceed as described below according to the other metals present.

A. If Al is present. Titrate Bi and Al together by Method I; on another portion of solution precipitate Bi and most of the Al by Method III, and determine Mg and/or Ca by Methods IV and V.

B. If Al is absent. If either Mg or Ca is present, determine together with Bi by Method IV; if both Mg and Ca are present, remove Bi and determine Mg and Ca by Method III.

Section II. Bi absent

A. If Al is present. Titrate Al by the thorium nitrate method; determine Mg and/or Ca by Methods IV and V.

B. If Al is absent. Titrate Mg and Ca by the solochrome black method; titrate Ca by the murexide method.

APPLICATION OF THE METHODS TO SOME TYPICAL PHARMACEUTICAL PRODUCTS

1. *Materials containing Bismuth*

Unless otherwise directed in the footnotes, the general method to be adopted is as follows:—

Dissolve the stated amount of material in a sufficient quantity of dilute nitric acid to give a clear solution when diluted to 50 ml. and proceed by the method described for the determination of bismuth, commencing with the words "add 2 drops of catechol violet indicator. . . ."

Table VII shows the application of this procedure to the determination of bismuth in various pharmaceutical materials.

Some examples are now given in which bismuth is associated with other metals mentioned in the scheme to demonstrate the general method of application.

Mixture of bismuth, N.F. 1939. Assay for bismuth. Dissolve 10 ml. in just sufficient nitric acid to give a clear solution when diluted to 50 ml. with water and proceed by the method described for the determination of bismuth commencing with the words "add 2 drops of catechol violet indicator. . . ." Assay for magnesium. Dissolve 10 ml. in nitric acid and dilute to exactly 50 ml. with water; take 5 ml. of the dilution and proceed by Method II, subtracting from the volume of 0·1N EDTA used one tenth of the volume required in the determination of bismuth. Results obtained are shown in Table VIII.

TABLE VII

THE DETERMINATION OF BISMUTH IN VARIOUS PHARMACEUTICAL MATERIALS

Product	Amount taken for determination	See footnote	Result (per cent. Bi) By EDTA	Result (per cent. Bi) By reference method
Bismuth Carbonate, B.P.	0·2 g.		80·9	81·4 (Ignition)
Bismuth Citrate, B.P.C. 1949	0·3 g.		51·9	52·4 (Ignition)
Bismuth Oxyiodide	0·35 g.	(*a*)	56·7	57·0 (Phosphate)
Bismuth Precipitated, B.P.	0·2 g.			
Bismuth Salicylate, B.P.	0·3 g.		57·4	57·3 (Ignition)
Bismuth Sodium Tartrate, B.P.	0·4 g.		40·7	40·7 (Phosphate)
Bismuth Subgallate, B.P.	0·3 g.	(*b*)	47·1	47·4 (Ignition)
Bismuth Subnitrate, B.P.C.	0·25 g.		72·9	73·4 (Ignition)
Bismuth Tribromphenate, B.P.C. 1949	0·35 g.	(*c*)	52·6	52·9 (Phosphate)
Injection of Bismuth, B.P.	Equivalent of 0·2 g. Bi	(*d*)		
Injection of Bismuth Salicylate, B.P.C.	5 ml.	(*e*)		
Mixture of Bismuth with Pepsin, Compound, B.P.C. 1934	5 ml.	(*f*)	5·08	5·09 (Phosphate)
Ointment of Resorcinol Compound, B.P.C.	6 g.	(*g*)	5·71	5·80 (B.P.C. method)
Paste of Bismuth Subnitrate and Iodoform, B.P.C.	1 g.	(*h*)	18·3	18·4 (B.P.C. method)
Solution of Bismuth and Ammonium Citrate, B.P.C. 1949	20 ml.	(*f*)	4·65	4·64 (Phosphate)
Solution of Bismuth, Concentrated, B.P.C. 1949	10 ml.	(*i*)	9·42	9·42 (Ignition)

(*a*) Treat with excess of nitric acid and heat to dryness on a water bath, repeating if necessary until all iodine is expelled. Dissolve the residue in the minimum amount of dilute nitric acid and proceed by the general method.

(*b*) Gently ignite at a temperature not exceeding 500° C., cool, dissolve the residue in the minimum quantity of warm nitric acid and proceed by the general method.

(c) Add 10 ml. of dilute nitric acid to the sample, keeping the mixture cool to prevent nitration; shake with 10 ml. of ether, separate and wash the ether with 2 portions, each of 5 ml. of dilute nitric acid; combine the acid fractions, dilute to 50 ml. with water acidified with nitric acid if necessary and proceed by the general method.

(*d*) Prepare a solution as described in the assay of the B.P. and use an accurately measured volume, equivalent to about 0·2 g. of bismuth for determination by the general method.

(*e*) Prepare a solution as described in the assay of the B.P.C. and continue the determination by the general method instead of precipitating bismuth as phosphate.

(*f*) Evaporate to dryness, ignite at a temperature not exceeding 500° C., dissolve the residue in the minimum amount of nitric acid, dilute to 50 ml. and proceed by the general method.

(*g*) Destroy the fat by gentle ignition, then continue the ignition at a temperature not exceeding 500° C.; dissolve the residue in nitric acid and dilute to 50 ml. To 20 ml. of this solution add 30 ml. of water and more nitric acid if necessary to maintain a clear solution, and proceed by the general method.

NOTE: The zinc has also been determined successfully in this preparation by treating 10 ml. of the solution obtained in the assay for bismuth by Method II or Method III. If Method III is used the addition of cyanide must, of course, be omitted.

(*h*) Digest with warm chloroform, filter, wash the residue with warm chloroform until the washings are free from iodoform and fat, dissolve the residue in the minimum amount of nitric acid and proceed by the general method.

(*i*) Dilute to 100 ml. and titrate 20 ml. of the dilution by the general method.

Compound lozenges of bismuth, B.P.C. Assay for bismuth. Take a sample of 20 lozenges and determine the average weight. Powder the sample and ignite an accurately weighed quantity of the powder, equivalent to about 4 lozenges, at a temperature not exceeding 500° C.; cool, moisten the residue with water, dissolve in the minimum quantity of nitric acid and dilute to 100 ml. with 0·1N nitric acid. Dilute 30 ml. to 50 ml. with water and proceed by the method described for the determination of bismuth commencing with the words "add 2 drops of catechol violet

TABLE VIII

THE DETERMINATION OF BISMUTH AND MAGNESIUM IN MIXTURE OF BISMUTH

Sample No.	Per cent.	By EDTA	By classical gravimetric separation
I	Bi	1·81	1·79
	Mg	1·28	1·29
II	Bi	1·83	1·84
	Mg	1·17	1·19

indicator . . ."; each ml. of 0·1N EDTA is equivalent to 0·01045 g. of Bi. Calculate the proportion of Bi in each lozenge of average weight.

Assay for calcium carbonate. To 10 ml. of the solution prepared in the assay for bismuth add 2 ml. of dilute hydrochloric acid, 150 ml. of distilled water and make just acid to litmus paper by using dilute solution of ammonia and dilute hydrochloric acid; heat to boiling and boil for 2 minutes, stirring to prevent bumping; cool in ice, confirm that precipitation is complete by addition of a few drops of brine, and filter through a No. 42 Whatman filter paper; wash with cold water and adjust the volume of the combined filtrate and washings to 250 ml. with water; to 100 ml. of this solution add 25 ml. of 0·1N EDTA, 5 ml. of solution of sodium hydroxide, 10 drops of solution of potassium cyanide and 0·2 g. of murexide indicator and titrate with 0·1N calcium chloride; each ml. of 0·1N EDTA is equivalent to 0·005004 g. of $CaCO_3$. Calculate the proportion of $CaCO_3$ in each lozenge of average weight. Assay for magnesium. To a further 100 ml. of the solution prepared in the assay for calcium carbonate add 10 ml. of ammonia buffer solution, 10 drops of solution of potassium cyanide and sufficient solochrome black indicator and titrate with 0·1N EDTA; each ml. of 0·1N EDTA, after the volume required in the determination of calcium carbonate has been deducted, is equivalent to 0·001216 g. of Mg. Calculate the proportion of Mg in each lozenge of average weight. Results obtained by this method are shown in Table IX.

Compound Powder of Bismuth, B.P.C., is clearly determinable by the same procedure, except that there is no necessity to ignite.

TABLE IX

THE DETERMINATION OF BISMUTH, CALCIUM AND MAGNESIUM IN COMPOUND LOZENGES OF BISMUTH, B.P.C.

	By proposed method	By B.P.C. 1954 method
Content of	g. per lozenge	g. per lozenge
Bi	0·127	0·126
$CaCO_3$	0·312	0·312
Mg	0·041	0·041

2. *Materials containing Aluminium*

The general method to be adopted is as follows:—

Dissolve the stated amount of material in just sufficient hydrochloric acid and dilute to 100 ml. with water. To 10 ml. of the solution add 25 ml. of 0·1N EDTA and 80 ml. of water and proceed by the method described for the determination of aluminium, commencing with the words "neutralise to congo red paper. . . ."

Table X shows the application of this method to the determination of aluminium in various pharmaceutical materials.

An example of application of the method to a mixture of aluminium and magnesium is provided by a proprietary product consisting of a suspension containing approximately 4 per cent. of Al_2O_3 and 1·2 per cent. of MgO. The method adopted was as follows:—

Dissolve 15 ml. of the mixture in a slight excess of dilute hydrochloric acid and dilute to 250 ml. with water.

Assay for aluminium. To 10 ml. of the dilution add 25 ml. of 0·1N EDTA and 80 ml. of water and proceed by the method described for the determination of aluminium, commencing with the words "neutralise to congo red paper. . . ."

TABLE X

THE DETERMINATION OF ALUMINIUM IN VARIOUS PHARMACEUTICAL MATERIALS

Product	Amount taken for determination (g.)	Result (per cent. Al_2O_3)	
		By EDTA	By reference method
Aluminium Hydroxide Gel, B.P.C.	0·6	50·3	50·0 (B.P.C. method)
Aluminium Hydroxide Gel, B.P.C.	7	3·76	3·80 (,, ,,)
Aluminium Hydroxide Paste, Concentrated (containing approximately 6 to 7 per cent. Al_2O_3)	4	7·53	7·46 (Oxine)
		7·43	7·47 ,,
		5·81	5·81 ,,
		6·59	6·65 ,,
		7·34	7·28 ,,

Assay for magnesium. To 50 ml. of the dilution add 1 g. of ammonium chloride, and proceed by Method IV commencing with the words "sufficient solution of triethanolamine. . . ."

Results obtained using this method are shown in Table XI.

TABLE XI

THE DETERMINATION OF ALUMINIUM AND MAGNESIUM IN A SUSPENSION OF THE MIXED HYDROXIDES

Sample No.	Aluminium (per cent. Al_2O_3)		Magnesium (per cent. MgO)	
	By EDTA	By hydroxide precipitation	By EDTA (Al masked)	By EDTA (Al removed)
1	4·05	4·08	1·26	1·27
2	3·92	3·98	1·29	1·30
3	4·10	4·06	1·21	1·20
4	4·06	4·04	1·19	1·19
5	3·88	3·97	1·27	1·27

One final example of separations using this scheme is that of a digestive powder containing aluminium hydroxide, magnesium trisilicate, magnesium oxide and about 4 per cent. of vegetable powders. This was assayed by first removing the silica by the gravimetric method described for magnesium trisilicate in the British Pharmacopœia, followed by determinations of aluminium and magnesium in the resulting solution. The quantity of vegetable material present was not sufficient to warrant its removal, but should interference be encountered the sample may be ignited as a preliminary measure.

APPENDIX

Reagents used throughout this work

For convenience the reagents used in the various determinations are listed below in alphabetical order. Where water is mentioned it is intended that distilled or deionised water be used.

Alizarin S indicator: 0·1 per cent. w/v of alizarin S in water.

Ammonia buffer solution: dissolve 13·5 g. of reagent grade ammonium chloride in 114 ml. of strong solution of ammonia and dilute to 200 ml. with water.

NOTE: This preparation is not the ammonia buffer solution of the B.P.C., which contains magnesium-EDTA complex.

Ammonia, dilute solution of: of the B.P.

Calcium chloride, 0·1N: a solution of hydrated calcium chloride in water, containing 10·96 g. of $CaCl_2,6H_2O$ per litre.

This solution is conveniently standardised by titration with 0·1N EDTA using solochrome black indicator.

Catechol violet indicator: 0·1 per cent. w/v of catechol violet in water.

EDTA, 0·1N: a solution of the disodium salt of ethylenediaminetetra-acetic acid in water, containing 18·61 g. of $C_{10}H_{14}O_8N_2Na_2,2H_2O$ per litre.

Standardise by titration of pure zinc metal according to the following procedure:—

Dissolve about 0·7 g., accurately weighed, of pure zinc metal in the minimum quantity of dilute hydrochloric acid and dilute to 250 ml. with water. Take 25 ml., dilute to 150 ml. with water, add 10 ml. of ammonia buffer solution, sufficient solochrome black indicator and titrate with the 0·1N EDTA to be standardised.

NOTE: It is important that, for general application to the methods described, the standard disodium ethylenediaminetetra-acetate solution of the B.P.C. should not be used. The latter solution, intended for the titration of calcium and magnesium only, contains a proportion of magnesium which would remain complexed under the conditions of standardisation given above, and during titrations in alkaline media. For the acid titrations of aluminium and bismuth, however, additional EDTA would be released from the magnesium-EDTA complex and the factor would be correspondingly low.

Hydrochloric acid, dilute: of the B.P.

Monochloroacetic acid, 2M: a solution of monochloroacetic acid in water, containing 189 g. of $CH_2Cl{\cdot}COOH$ per litre.

Murexide indicator: of the B.P.C. Appendix IV.

Nitric acid, dilute: of the B.P. Appendix I.

Potassium cyanide, solution of: of the B.P. Appendix I.

Sodium acetate, 1M: a solution of sodium acetate in water, containing 136 g. of $CH_3COONa,3H_2O$ per litre.

Sodium hydroxide, solution of: of the B.P. Appendix I.

Solochrome black indicator: a freshly prepared 0·5 per cent. solution of solochrome black in ethanol (95 per cent.).

In the opinion of the authors this freshly prepared solution is far superior to the various "stabilised" solutions, or to the salt diluted indicator of the B.P.C. The solution provides a very sharp end-point indicator up to a period of about 3 hours from the time of preparation.

Thorium nitrate, 0·1N: a solution of thorium nitrate in water, containing 27·6 g. of $Th(NO_3)_4,4H_2O$ per litre.

This solution is conveniently standardised by titration of standard EDTA using the conditions described for the determination of aluminium.

Triethanolamine, solution of: a 30 per cent. v/v solution of triethanolamine in water. For application to the macro-scale work described above, triethanolamine of B.P.C. quality has been found to be perfectly satisfactory.

With the exception of Solochrome Black Indicator all the above reagents have been found to be stable over a period of several months. 1M Sodium Acetate readily supports mould growth, but in the authors' experience this has had no effect on its buffering capacity. The mould growth may be suppressed, however, by preparation of the reagent with chloroform water.

Summary

1. Published methods for the volumetric determinations of aluminium and of bismuth with EDTA have been examined. The effect of variation of conditions of reaction on the accuracy of the determination of aluminium by back-titration with thorium nitrate has been investigated. Modifications to the procedure for the determination of bismuth using catechol violet as indicator have been proposed.

2. Methods of determining aluminium, bismuth, calcium and magnesium in the presence of each other have been described.

3. Examples have been given of applications of the methods to a number of commonly occurring pharmaceutical products to demonstrate their general applicability.

We wish to express our thanks to Mr. C. Vickers, for much of the experimental work and for the valuable suggestions he has made throughout.

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19. Přibil, *ibid.*, 1954, **19**, 465.
20. Ritchie, *Analyst*, 1955, **80**, 402.
21. Vogel, *Quantitative Inorganic Analysis*, 2nd Ed., Longmans, Green and Co., London, 1951, p. 416.

DISCUSSION

The paper was presented by MR. H. E. BROOKES.

DR. G. E. FOSTER (Dartford) said one minor criticism he wished to make was that the abbreviation "EDTA" had been used extensively, but at different times referred to the acid itself, the disodium salt and the tetra-sodium salt.

DR. D. C. GARRATT (Nottingham) hoped others would present papers in the future describing rapid methods of assay which were sufficiently accurate provided their limitations were realised.

MR. H. D. C. RAPSON (Dorking) said his colleague, Mrs. Mary Taylor, had altered her technique in respect of the aluminium determination to bring it very much in line with the method described in the paper.

DR. G. BROWNLEE (London) explained the difficulty in printing long chemical names which were repeated many times in a paper. It was the accepted practice in such cases for the author to use an abbreviation provided he explained its meaning properly, which in this case was so.

MR. C. A. JOHNSON, in reply, said that the only question raised had been answered by Dr. Brownlee.

ANTITUBERCULOSIS AGENTS

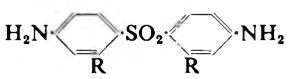
PART I. BISDIALKYLAMINOALKYL SULPHONES AND RELATED SUBSTANCES

BY D. EDWARDS and J. B. STENLAKE

From The School of Pharmacy, The Royal Technical College, Glasgow

Received June 23, 1955

THE use of aromatic amino-sulphones such as dapsone[1] (4:4′-diaminodiphenyl sulphone, I,R=H), solapsone[2,3,4,5] and related compounds in the treatment of tubercular infections and in leprosy is well established. Toxic effects which are often exhibited with dapsone have been circumvented by administration in graded doses.[6] Linnell and Stenlake[7,8] and others[9,10] have also shown that introduction of hydroxy groups into the molecule as in 2:2′-dihydroxy-4:4′-diaminodiphenyl sulphone (I,R=OH) leads to a marked reduction of toxicity, with retention of activity. Poor yields in the synthetic routes to such compounds, and the need to study structural variants have led us to examine a number of related aliphatic compounds.



(I)

Marked *in vitro* activity against *Myco. tuberculosis* is shown by many aliphatic amines[11,12,13,14,15,16], particularly by those with 16 to 20 carbon atoms. They are, however, inactive *in vivo*. A series of long chain aliphatic diamidines[17] also showed activity *in vitro* but proved too toxic for prolonged *in vivo* tests. Many aromatic amines similarly are active *in vitro*[18], but activity *in vivo* appears to be limited to those which are related either to *p*-aminosalicylic acid (PAS) or alternatively to the amino-sulphones (I). Eiseman[19] has shown that polyoxyethylene substituents in the amino groups increase the effective surface concentration of these substances, and some of these compounds possessed *in vitro* activities one thousand times greater than that of the parent sulphone. Peak and Watkins[20] examined the effect of introducing a sulphone group into the carbon chain of aliphatic amines. A series of compounds II ($R=C_4H_9$, C_8H_{17}, $C_{16}H_{33}$) and (III) were shown to exhibit only low *in vitro* activity, though in many cases the corresponding sulphides were appreciably

$Et_2N{\cdot}CH_2{\cdot}CH_2{\cdot}SO_2{\cdot}R$ (II)

$MeN(CH_2{\cdot}CH_2{\cdot}SO_2{\cdot}C_8H_{17})_2$ (III)

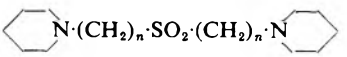
$Et_2N{\cdot}CH_2{\cdot}CH_2{\cdot}SO_2{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$ (IV)

$Et_2N{\cdot}(CH_2)_n{\cdot}SO_2{\cdot}(CH_2)_n NEt_2$ (V)

active. No compounds were examined in which the amino groups were separated by a long aliphatic chain, whilst the only example of an $\alpha\omega$-bisdialkylamino sulphone was (IV) which showed low activity.

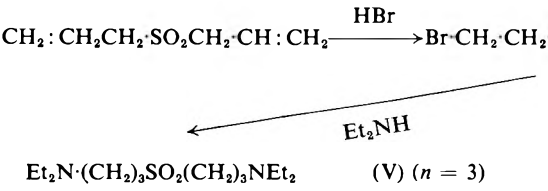
In view of the high activities reported for $\alpha\omega$-bisdialkylaminoalkanes[14] and for the polyoxyethylene derivatives of amino-sulphones[19] it was decided in the first instance to synthesise a series of $\alpha\omega$-bisdialkylamino-alkyl sulphones (V). Three compounds have been prepared in which

n = 3, 6 and 10 respectively (giving chain lengths of 7, 13 and 21 units) to survey the effect of varying chain length. The two latter compounds were obtained via the corresponding sulphides (VI), and in the light of the observation that such compounds can show considerable activity[20], these also have been tested against *Myco. tuberculosis*. Ames and Bowman[14] showed that piperidylalkanes generally were less toxic than the corresponding dialkylaminoalkanes, and we have accordingly extended the range of compounds examined to include the $\alpha\omega$-bis-(1′-piperidyl)-alkyl sulphones (VII, n = 6 and 10) and the corresponding sulphides (VIII).



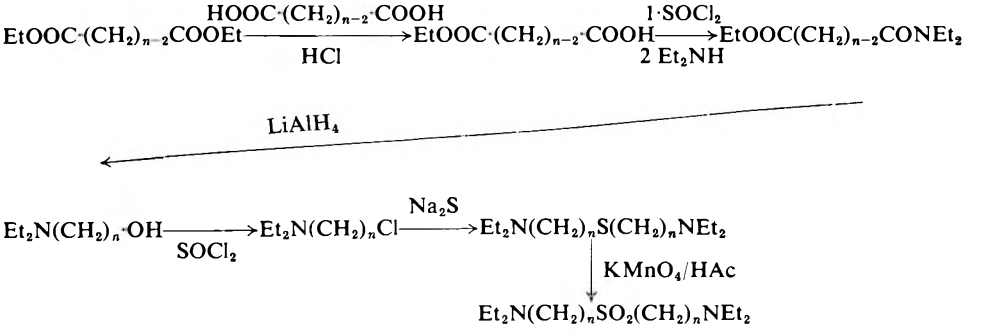
(VII)

Bis-3-diethylaminopropyl sulphone (V, n = 3) was obtained from diallyl sulphone according to the following scheme:



Considerable difficulty was experienced initially with the hydrobromination of diallyl sulphone to give bis-3-bromopropyl sulphone the yields varying eratically and without explanation. Consistent yields (43 per cent.) were finally obtained by passing dry hydrogen bromide slowly through a solution of diallyl sulphone in carbon tetrachloride continuously for 20 hours, the reaction mixture being raised to boiling initially and periodically every three hours. Continuous reaction at the boiling point of the solvent gave much resinous material, and small yields of product which was difficult to isolate. On one occasion small amounts of two monobromosulphones were isolated. Bis-3-bromopropyl sulphone was readily converted to the required bis-3-diethylaminopropyl sulphone V ($n = 3$) in 53 per cent. yield (isolated as the dihydrochloride) by reaction with diethylamine in hot benzene.

The medium and long chain compounds, V ($n = 6$ and 10), VI ($n = 6$ and 10), VII ($n = 6$ and 10), VIII ($n = 6$ and 10) were obtained by an adaptation of the methods described by Andrews, Bergel and Morrison[21]



for the preparation of the analagous $\alpha\omega$-trimethylalkylammonium sulphides, as outlined above. The sulphides were readily oxidised to the corresponding sulphones in good yield using potassium permanganate in glacial acetic acid.

Introduction of a hydroxy group into the molecule of aromatic amino-sulphones markedly reduces toxicity[7,8]. Examination of similarly hydroxylated aliphatic amino-sulphones was desirable, and we therefore considered the possibility of preparing a series of sulphones (IX), which in respect of the hydroxyl group are analagous to 2:2′-dihydroxy-4:4′-diaminodiphenyl sulphone. One route to such a series of compounds

$$Et_2N(CH_2)_nCHOH{\cdot}CH_2{\cdot}SO_2{\cdot}CH_2{\cdot}CHOH{\cdot}(CH_2)_nNEt_2 \qquad \text{(IX)}$$

via the corresponding $\alpha\beta$-unsaturated sulphones is particularly attractive in that there exists an analogy between these intermediates and certain of the long chain fatty acids which have been isolated from the tubercle bacillus[22,23,24,25,26]. Many of these acids are $\alpha\beta$-unsaturated, and it is known that they are largely responsible for tubercle formation, one of the characteristic signs of the disease. Little is known of their function in the metabolism of the organism, and it was felt that a study of some unsaturated compounds might provide a useful means of probing the importance of such unsaturation.

In an attempt to find a general method which would be applicable to all compounds, we examined the reaction between bromine, red phosphorus and dibutyl sulphone. No bromination occurred after refluxing for six hours. We therefore turned our attention to the preparation of the short chain compound (X) by the following route, based on a preparation of benzyl 3-bromoprop-1-enyl sulphone by Rothstein[27].

$$CH_2{:}CH{\cdot}CH_2{\cdot}SO_2{\cdot}CH_2{\cdot}CH{:}CH_2 \xrightarrow{Br_2} BrCH_2{\cdot}CHBr{\cdot}CH_2{\cdot}SO_2{\cdot}CH_2{\cdot}CHBr{\cdot}CH_2Br$$

$$BrCH_2{\cdot}CH{:}CH{\cdot}SO_2{\cdot}CH{:}CH{\cdot}CH_2Br \xrightarrow{Et_2NH} Et_2N{\cdot}CH_2CH{:}CH{\cdot}SO_2{\cdot}CH{:}CH{\cdot}CH_2NEt_2$$

(Pyridine)

(X)

Direct bromination of dialkyl sulphone gave bis-2:3-dibromopropyl sulphone which was readily dehydrobrominated with pyridine in hot benzene to bis-3-bromoprop-1-enyl sulphone. The latter on treatment with diethylamine gave bis-3-diethylamino-prop-1-enyl sulphone (X) which was isolated as its hydrochloride. The product decolourised potassium permanganate in alkaline solution, but otherwise showed complete lack of reactivity at the double bonds, giving no reaction with hydrobromic acid, bromine, iodine monochloride and tetranitromethane. With monoperphthalic and perbenzoic acids reaction was negligible at room temperature and no useful products could be isolated. Attempts to obtain the corresponding bis-3-diethylamino-2-hydroxypropyl sulphone

by this route were therefore abandoned. Alternative routes to these compounds are being examined.

Bacteriological Results

The preliminary bacteriological examination of the above compounds was kindly carried out by Dr. S. R. M. Bushby of the Wellcome Research Laboratories. The tuberculostatic activity was measured against *Myco. tuberculosis, var hominis* (CN3679) both in Dubos medium and in the egg-agar solid medium of Peizer and Schecter, and also against *Myco. tuberculosis var hominis*, H37 Rv in Peizer and Schecter medium only. The results for the more active compounds are shown in Table I. None

TABLE I

Tuberculostatic activity of bisdialkylaminoalkyl sulphones

Compound	Incubation (days)	Minimum inhibiting concentration μg./ml.					
		Dubos medium				Peizer and Schecter medium	
		Strain CN 3679				H 37Rv	CN 3679
		Expt. 1	Expt. 2	Expt. 3	Expt. 4		
Bis-3-diethylaminoprop-1-enyl sulphone dihydrochloride ..	7	8	8	16	8	—	—
	14	8	16	62	16	500	250
	21	—	—	—	—	500	500
Bis-10-diethylaminodecyl sulphide dihydrochloride	7	2	4	4	2	—	—
	14	4	8	4	4	62	125
	21	—	—	—	—	62	125
Bis-10-diethylaminodecyl sulphone dihydrochloride ..	7	62	62	31	62	—	—
	14	62	62	31	62	250	125
	21	—	—	—	—	250	250
Bis-10-(1′-piperidyl)-decyl sulphide dihydrochloride ..	7	4	8	<1	4	—	—
	14	4	8	4	8	125	125
	21	—	—	—	—	125	125
Bis-10-(1′-piperidyl)-decyl sulphone dihydrochloride ..	7	16	31	16	31	—	—
	14	16	31	16	62	125	125
	21	—	—	—	—	125	125
Isoniazid(*iso*nicotinic acid hydrazide)	7	0·06	0·06	0·06	0·06	—	—
	14	0·06	0·06	0·06	0·06	0·06	0·06
	21	—	—	—	—	0·06	0·06
Streptomycin sulphate	7	—	0·30	—	—	—	—
	14	—	0·30	—	—	—	2·50
	21	—	—	—	—	—	2·50

of the compounds examined showed activity of the same order as that of isoniazid or streptomycin. As expected the compounds with the longer chains were, in general, more active than those with short chains, whilst sulphides were more active than the corresponding sulphones. A surprisingly high level of activity was shown by the short chain unsaturated sulphone, bis-3-diethylamino-prop-1-enyl sulphone, this being the most active of the sulphones examined. It is intended that future work should be directed towards producing a series of unsaturated compounds, and related β-hydroxy compounds.

EXPERIMENTAL

Melting points are uncorrected.

Bis-2:3-*dibromopropyl sulphone* was prepared by the method of Lewin[22].

Bis-3-*bromoprop*-1-*enyl sulphone.* Bis-2:3-dibromopropyl sulphone (46·6 g.) was dissolved in hot benzene (160 ml.). Pyridine (17 ml.) was slowly added to the hot solution (15 min.) with continuous stirring, and the mixture refluxed for a further 30 minutes. When cold the benzene solution was washed with water, dried (Na_2SO_4) and evaporated to yield a yellowish mass of crystals (crude yield 61 per cent.). Recrystallisation from carbon tetrachloride gave colourless needles of *bis*-3-*bromoprop*-1-*enyl* sulphone, m.pt. 73 to 74° C. Found: C, 23·8; H, 2·9; Br, 52·7 per cent. $C_6H_8O_2SBr_2$ requires C, 23·7; H, 2·7; Br, 52·6 per cent.

Bis-3-*diethylaminoprop*-1-*enyl sulphone dihydrochloride.* Bis-3-bromoprop-1-enyl sulphone (3·65 g.) was dissolved in hot benzene (55 ml.). Diethylamine (7 ml.) was added and the mixture refluxed for 15 minutes. When cold the benzene solution was washed with water, dried (Na_2SO_4) and evaporated. The residual oily base was dissolved in dilute hydrochloric acid (10 per cent.; 9 ml.) and the solution cautiously evaporated. The semi-crystalline residue, recrystallised from absolute ethanol, gave colourless needles of *bis*-3-*diethylaminoprop*-1-*enyl sulphone dihydrochloride* (2·3 g.; 53 per cent.), m.pt. 192 to 193° C. Found: C, 46·5; H, 7·7; Cl, 19·8 per cent. $C_{14}H_{30}O_2N_2SCl_2$ requires C, 46·5; H, 8·4; Cl, 19·6 per cent. The corresponding *dipicrate* had m.pt. 188 to 189° C. (decomp.). Found: 42·2; H, 4·3 per cent. $C_{26}H_{34}O_{16}N_8S$ requires C, 41·8; H, 4·6 per cent.

Bis-3-*bromopropyl sulphone.* Diallyl sulphone (4·2 g.) was dissolved in carbon tetrachloride (100 ml.) and a crystal of benzoyl peroxide added. The solution was heated to boiling and dry hydrogen bromide passed in whilst the solution cooled, and thereafter for 19·5 hours. The solution was brought to the boil at 3 hourly intervals, being allowed to cool to room temperature during the intervening periods. The product separated as a yellow oil which formed a solid crystalline mass on standing overnight. Evaporation of the benzene and crystallisation of the residue from ether gave *bis*-3-*bromopropyl sulphone* as colourless plates, m.pt. 85 to 86° C. (3·7 g.; 43 per cent.). Found: C, 23·8; H, 4·1; Br, 51·8 per cent. $C_6H_{12}O_2SBr_2$ requires C, 23·4; H, 3·9; Br, 51·9 per cent. A subsequent experiment in which hydrogen bromide was only passed in for 8 hours gave the same product in 39·2 per cent. yield.

2-*Bromopropyl prop*-2′-*enyl sulphone* and 3-*bromopropyl prop*-2′-*enyl sulphone.* Diallyl sulphone (6·5 g.) was dissolved in carbon tetrachloride (100 ml.) and a crystal of benzoyl peroxide added. The solution was heated to boiling and dry hydrogen bromide passed in for 5 hours, whilst the solution was refluxed. Removal of the solvent gave a tarry residue, which was extracted with hot ether, benzene and ethanol to yield a liquid (1·48 g.). The liquid on further reaction in carbon tetrachloride with dry hydrogen bromide for 7 hours, followed by chromatography from benzene on a mixture of activated charcoal and powdered cellulose (1:1)

gave a crystalline solid (1·67 g.). Chromatography of this solid from benzene on alumina gave two fractions. The first small fraction recrystallised from ether gave *2-bromopropyl prop-2'-enyl sulphone*, m.pt. 102·5 to 103·5° C. Found: C, 32·1; H, 5·4 per cent. $C_6H_{10}O_2SBr$ requires C, 32·1; H, 5·3 per cent. The second much larger fraction after repeated recrystallisation from ether gave 3-bromopropyl prop-2'-enyl sulphone, m.pt. 72 to 74° C. Found: C, 31·8; H, 4·9 per cent. $C_6H_{10}O_2SBr$ requires C, 32·1; H, 5·3 per cent.

Bis-3-diethylaminopropyl sulphone. Bis-3-bromopropyl sulphone (4·64 g.) in benzene (100 ml.) was refluxed for 1 hour with diethylamine (8 ml.). When cold the benzene solution was washed with water, dried (Na_2SO_4) and evaporated. The residual oily base was dissolved in dilute hydrochloric acid (10 per cent.; 12·5 ml.) and the solution cautiously evaporated. The solid residue recrystallised from absolute ethanol (dried) gave colourless hygroscopic needles of *bis-3-diethylaminopropyl sulphone dihydrochloride* m.pt. 186·5 to 187° C. (2·93 g., 53 per cent.). Found: N, 7·4; Cl, 19·3 per cent. $C_{14}H_{34}O_2N_2SCl_2$ requires N, 7·7; Cl, 19·4 per cent.

Ethyl NN-*pentamethyleneadipamate* was prepared from ethyl hydrogen adipate[31] as described by Avison[32], b.pt. 169 to 172°C./3 mm. (literature b.pt. 148 to 152°C./0·5 mm.). Found: C, 64·8; H, 9·3; N, 6·2 per cent. Calc. for $C_{13}H_{23}O_3N$, C, 64·7; H, 9·6; N, 5·8 per cent.

Ethyl NN-*diethyladipamate.* Ethyl hydrogen adipate[31] (26·25 g.) was refluxed with excess thionyl chloride for 1·5 hours. After removal of excess thionyl chloride, the residue, in ether (200 ml.), was treated with a solution of diethylamine (35 ml.) in ether (50 ml.). The ethereal solution was extracted first with water (to remove diethylamine hydrochloride), then with aqueous sodium carbonate (to remove ethyl hydrogen adipate) and then washed with water. The resulting ethereal solution was dried (Na_2SO_4), evaporated, and the residual liquid distilled to yield *ethyl* NN-*diethyladipamate*, b.pt. 144° C./3 mm., $n_D^{17·5}$ 1·4572 (27·9 g., 81 per cent.). Found: N, 5·9 per cent. $C_{12}H_{23}O_3N$ requires N, 6·1 per cent.

Ethyl NN-*diethylsebacamate* was prepared from ethyl hydrogen sebacate[31] (31·7 g.) by the above method. *Ethyl* NN-*diethylsebacamate* was obtained as a colourless oil, b.pt. 183 to 190° C./3 mm., n_D^{19} 1·4571 (22·3 g., 57 per cent.). Found: C, 67·3; H, 10·9; N, 4·95 per cent. $C_{16}H_{31}O_3N$ requires C, 67·3; H, 10·9; N, 4·9 per cent.

Ethyl NN-*pentamethylenesebacamate* was prepared from ethyl hydrogen sebacate[31] (23·6 g.) by the above method. *Ethyl* NN-*pentamethylenesebacamate* was obtained as a colourless oil, b.pt. 208 to 211° C./3·5 mm., $n_D^{18·5}$ 1·4757 (20·1 g., 66 per cent.). Found: C, 68·2; H, 9·9; N, 4·5 per cent. $C_{17}H_{31}O_3N$ requires C, 68·6; H, 10·5; N, 4·7 per cent.

6-*Hydroxyhexyldiethylamine.* Ethyl *NN*-diethyladipamate (27·9 g.) in dry ether (60 ml.) was slowly run into a stirred hot suspension of lithium aluminium hydride (9 g.) in dry ether (400 ml.). The addition was continued at a rate which was just sufficient to keep the solution boiling, addition being complete within approximately 15 minutes. The reaction mixture was cooled in ice, and water added dropwise sufficient to decompose the excess lithium aluminium hydride. After treatment with sodium

hydroxide solution (20 per cent.; 200 ml.) the ethereal solution was evaporated and the residual oil distilled to give 6-hydroxyhexyldiethylamine, b.pt. 110° C./5·5 mm., n_D^{15} 1·4575 (18·9 g., 90 per cent.). Work[33] gives b.pt. 96 to 99° C./2 mm.

6-*Hydroxyhexlpiperidine* was prepared from ethyl *NN*-pentamethyleneadipamate (21·1 g.) by the above method, and was obtained as a colourless oil, b.pt. 123° C./3·5 mm., n_D^{19} 1·4781 (14·4 g., 89 per cent.). Sauer and Adkins[34] give b.pt. 96° C./1 mm., n_D^{25} 1·4730.

10-*Hydroxydecyldiethylamine* was prepared from ethyl *NN*-diethylsebacamate (22 g.) by the above method and was obtained as a colourless oil, b.pt. 146° C./3 mm., $n_D^{19·5}$ 1·4602 (15·5 g., 88 per cent.). Schinzel and Benoit[35] give b.pt. 178 to 183° C./16 mm.

10-*Hydroxydecylpiperidine* was prepared from ethyl *NN*-pentamethylenesebacamate (19·6 g.) by the above method and was obtained as colourless platelets (from ether), m.pt. 59·5° C. (14·3 g., 90 per cent.). Price, Guthrie, Herbrandson and Peel[36] gave m.pt. 60 to 61° C.

6-*Chlorohexyldiethylamine*. Thionyl chloride (8 ml.) in benzene (30 ml.) was slowly added to a solution of 6-hydroxyhexyldiethylamine (18·9 g.) in benzene (100 ml.). The greyish crystalline mass obtained after removing the solvent was dissolved in water (20 ml.), cooled to 0° C. and basified by the addition of sodium hydroxide solution (30 ml., 20 per cent.). Extraction with ether, evaporation of the solvent, and distillation of the residual oil gave 6-chlorohexyldiethylamine, b.pt. 102·5° C./11 mm., n_D^{16} 1·4513 (19·8 g., 95 per cent.). Found: C, 63·1; H, 11·8 per cent. Calc. for $C_{10}H_{22}NCl$: C, 62·6; H, 11·6 per cent. Work[25] gives b.pt. 118 to 120° C./19 mm.

6-*Chlorohexylpiperidine* was prepared from 6-hydroxyhexylpiperidine (13·8 g.) by the above method, and was obtained as a colourless oil, b.pt. 131° C./12·5 mm., $n_D^{17·5}$ 1·4752 (10 g., 66 per cent.). 6-*Chlorohexylpiperidine hydrochloride* was obtained in the usual way as colourless plates, m.pt. 154·5 to 155° C. Found: C, 55·0; H, 9·6; N, 5·8 per cent. $C_{11}H_{22}NCl$ requires C, 55·0; H, 9·6; N, 5·8 per cent.

10-*Chlorodecyldiethylamine* was prepared from 10-hydroxydecyldiethylamine (15·1 g.) by the above method, and was obtained as a colourless oil, b.pt. 161° C./12 mm., n_D^{20} 1·4562 (14 g., 86 per cent.). Schinzel and Benoit[35] gave b.pt. 173 to 176° C./17 mm.

10-*Chlorodecylpiperidine* was prepared from 10-hydroxydecylpiperidine (14·2 g.) by the above method, and was obtained as a colourless oil, b.pt. 151 to 152° C./5 mm., n_D^{18} 1·4753 (14·5 g., 95 per cent.). Price, Guthrie, Herbrandson and Peel[36] give analytical figures for this compound, but no constants, except the hydrochloride m.pt. 135 to 136° C. Found: equiv. IV (by titration) 263, $C_{15}H_{30}NCl$ requires equiv. IV 260.

Bis-6-*diethylaminohexyl sulphide*. 6-Chlorohexyldiethylamine (19·8 g.) in ethanol (10 ml.) was slowly added to a hot solution of anhydrous sodium sulphide (5·5 g.) in water (6 ml.) and ethanol (10 ml.), and the mixture refluxed for 3·25 hours with continuous stirring. The residual liquor remaining after removing the bulk of the ethanol by distillation was poured into water (400 ml.) and extracted with ether. The ethereal

solution was dried (Na_2SO_4) and evaporated and the residual oil fractionally distilled. After a forerun of unchanged chlorohexyldiethylamine, *bis-6-diethylaminohexyl sulphide* was obtained as a pale straw coloured oil, b.pt. 208 to 209° C./5·5 mm., n_D^{19} 1·4757 (13 g., 72·5 per cent.). Found: C, 69·6; H, 12·3; N, 8·1 per cent. Equiv. (titration 172·5) $C_{20}H_{44}N_2S$ requires C, 69·7; H, 12·9; N, 8·1 per cent. Equiv. 172·3. *Dihydrochloride* (from ethanol), m.pt. 130·5 to 131·5° C. Found: C, 57·4, H, 11·2; N, 6·7 per cent. $C_{20}H_{46}N_2SCl_2$ requires C, 57·5; H, 11·1; N, 6·7 per cent.

Bis-6-(1′-piperidyl)-hexyl sulphide was prepared from 6-chlorohexylpiperidine (9·8 g.) by the above method, and was obtained as a yellow oil, b.pt. 230 to 231° C./3 mm., $n_D^{16·5}$ 1·5022 (5·7 g., 64 per cent.). *Dihydrochloride* (from ethanol-ether), m.pt. 226·5 to 227·5° C. Found: C, 59·5; H, 10·5; N, 6·3 per cent. $C_{22}H_{46}N_2SCl_2$ requires C, 59·8; H, 10·5; N, 6·3 per cent.

Bis-10-diethylaminodecyl sulphide was prepared from 10-chlorodecyldiethylamine (13·7 g.) by the above method, and was obtained as a yellow oil b.pt. 275° C./3·5 mm., $n_D^{15·5}$ 1·4775 (5 g., 40 per cent.). Found: Eqiv. (titration) 227. $C_{28}H_{60}N_2S$ requires Eqiv. 228. *Dihydrochloride* (from ethanol-ether), m.pt. 141 to 142° C. Found: C, 63·4; H, 11·4; N, 5·2 per cent. $C_{28}H_{62}N_2S$ requires C, 63·5; H, 11·8; N, 5·3 per cent.

Bis-10-(1′-piperidyl)-decyl sulphide was prepared from 10-chlorodecylpiperidine (14·4 g.) by the above method, and was obtained as a pale yellow, low-melting solid (9·7 g., 73 per cent.). Found: equiv. (titration) 186·5. $C_{30}H_{60}N_2S$ requires equiv. 184·4. *Dihydrochloride* (from ethanol-ether), m.pt. 204 to 204·5° C. Found: C, 64·4; H, 10·9; N, 5·1 per cent. $C_{30}H_{62}N_2SCl_2$ requires C, 65·0; H, 11·3; N, 5·1 per cent.

Bis-6-diethylaminohexyl sulphone. Potassium permanganate (3 per cent.) in acetic acid (50 per cent.) was slowly added (20 min.) to an ice cold solution of bis-6-diethylaminohexyl sulphide (1·8 g.) in acetic acid (50 per cent.; 3 ml.), until present in slight excess. After a further 20 minutes the solution was decolourised with sulphur dioxide, and evaporated to dryness under reduced pressure. Sodium carbonate solution was added to make alkaline and the solution again evaporated to dryness. The solid residue after continuous extraction with ether and distillation gave *bis-6-diethylaminohexyl sulphone* as a colourless liquid, b.pt. 220° C./3 mm., n_D^{20} 1·4743 (1·75 g., 90 per cent.). Found: C, 63·4; H, 11·6; N, 7·4 per cent. $C_{20}H_{44}O_2N_2S$ requires C, 63·8; H, 11·8; N, 7·4 per cent. *Dihydrochloride* (from ethanol-ether), m.pt. 139 to 140° C. Found: C, 53·1; H, 10·3; N, 6·2; Cl, 15·8 per cent. $C_{20}H_{46}ON_2SCl_2$ requires C, 53·4; H, 10·3; N, 6·2; Cl, 15·8 per cent.

Bis-6-(1′-piperidyl)-hexyl sulphone was prepared from bis-6-(1′-piperidyl)-hexyl sulphide (1·87 g.), and was obtained as colourless plates (from ether-petroleum), m.pt. 50·5 to 51° C. (1·75 g., 86 per cent.). Found: C, 65·9; H, 10·8, 11·3; N, 6·9 per cent. $C_{22}H_{44}O_2N_2S$ requires C, 65·9; H, 11·1; N, 7·0 per cent. *Dihydrochloride* (from ethanol-ether) m.pt. 191·5 to 192·5° C. Found: Cl, 14·96 per cent. $C_{22}H_{46}O_2N_2SCl_2$ requires Cl, 14·94 per cent.

Bis-10-*diethylaminodecyl sulphone* was prepared from bis-10-diethylaminodecyl sulphide (1·51 g.), and was obtained as a low melting solid (1·6 g., 97 per cent.), which gave a *dihydrochloride* (from ethanol), m.pt. 142·5° C. Found: C, 59·7; H, 10·8; N, 4·9; Cl, 12·7 per cent. $C_{28}H_{62}O_2N_2SCl_2$ requires C, 59·8; H, 11·1; N, 5·0; Cl, 12·6 per cent.

Bis-10-(1′-*piperidyl*)-*decyl sulphone* was prepared from bis-10-(1′-piperidyl)-decyl sulphide (2·2 g.), and was obtained as colourless flakes (from ether), m.pt. 74·5 to 75° C. (2·4 g. crude, 100 per cent.). Found: C, 70·1; H, 11·6; N, 5·4 per cent. $C_{30}H_{60}O_2N_2S$ requires C, 70·2; H, 11·8; N, 5·5 per cent. *Dihydrochloride* (from ether), m.pt. 182° C. Found: C, 61·3; H, 10·9; N, 4·5; Cl, 12·1 per cent. $C_{30}H_{62}O_2N_2SCl_2$ requires C, 61·5; H, 10·7; N, 4·8; Cl, 12·1 per cent.

Summary

1. A series of bisdialkylaminoalkyl sulphides and sulphones have been prepared for testing as antituberculosis agents.

2. The preliminary bacteriological examination reveals that none of the compounds is of the same order of activity as streptomycin or isoniazid. Compounds with long chains are more active than short chain compounds, whilst sulphides are more active than the corresponding sulphones.

3. The short chain unsaturated sulphone, bis-3-diethylamino-prop-1-enyl sulphone, shows a surprisingly high level of activity.

We wish to thank Dr. S. R. M. Bushby of the Wellcome Research Laboratories for testing the compounds described, and Mr. W. McCorkindale, Dr. A. C. Syme and Mr. W. Gardiner for the microanalyses.

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DISCUSSION

The paper was presented by MR. D. EDWARDS.

DR. R. F. TIMONEY (Dublin) suggested, with reference to the failure to prepare bis-3-diethylamino-2-hydroxypropyl sulphone by the route found to give bis-3-diethylamino-prop-1-enyl sulphone, that the authors might try to prepare the sultone, which was comparable to a lactone, and hydrolyse it.

DR. J. B. STENLAKE, in reply, thanked Dr. Timoney for his suggestion.

MAGNESIUM TRISILICATE—ANTACID PROPERTIES AND CRYSTAL STRUCTURE

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ACCORDING to Mutch[1], the particular value of magnesium trisilicate in the treatment of gastric hyperacidity and ulceration of the stomach and duodenum lies in its ability to give a quick primary neutralisation of the stomach acidity followed by a progressive secondary effect. Thus, ideally, a continuous antacid action should be exerted throughout the whole period of normal gastric digestion without the danger of the *p*H of the gastric contents being raised above the neutral point. This should be effected by the administration of a single dose of antacid. He defined medicinal magnesium trisilicate as a "chemically prepared substance with a composition represented by the formula $H_4Mg_2Si_3O_{10}$, and yielding a pure diffraction radiograph of sepiolite No. 1 or No. 2[2]." A further advantage is the strong adsorptive effect exerted in the stomach and duodenum by either the un-neutralised excess of magnesium trisilicate or by the hydrated colloidal silica produced during the interaction with gastric acid: $Mg_2Si_3O_8 \cdot 2H_2O + 4HCl = 2MgCl_2 + 3SiO_2 + 4H_2O$. The British Pharmacopœia of 1953 contains a monograph on Magnesium Trisilicate in which standards for composition and acid neutralisation are prescribed. It has been found, however, that various magnesium trisilicates of commerce, all of which complied with the B.P. monograph, gave very different antacid responses when tested by the *in vitro* procedure of Armstrong and Martin[3]. It is the purpose of this paper to draw attention to these differences in B.P. quality magnesium trisilicate, and also to the relationship which exists between the crystal structure of magnesium trisilicate and its antacid performance. This we feel extends the work of previous investigators[4,5,6].

For the investigation, six samples of magnesium trisilicate B.P. which showed marked difference in density were selected. The analytical data for these is given in Table I. These samples were examined by the technique of Armstrong and Martin[3] in which the requisite dose of antacid (1·5 g.) is added to 150 ml. of artificial gastric juice of *p*H 1·5, at 37° C., which is kept continuously stirred. The *p*H of the mixture is recorded at stipulated time intervals up to 10 minutes. After this time 20 ml. of the mixture, representing physiological loss from the stomach, is withdrawn and 20 ml. of artificial gastric juice added. The *p*H is again recorded at intervals up to the 10 minute "emptying time" and the withdrawal etc. is repeated until the *p*H readings indicate that all the antacid has been neutralised or is no longer effective. Under these conditions the antacid responses of the six products were markedly varied. These are represented graphically in Figure 1. A and B showed a slow initial effect, the maximum *p*H (4·5 and 5·0) being reached in 20 minutes after which

TABLE I

ANALYTICAL DATA FOR MAGNESIUM TRISILICATE SAMPLES TESTED

Sample	Per cent. MgO	Per cent. SiO_2	SiO_2/MgO ratio	Per cent. loss on ignition	ml. 0·05N HCl Neutralised B.P. 15 min.	1 hr.	2 hrs.	4 hrs.	24 hrs.	Apparent density
A	30·2	68·5	2·27	30·0	116	164	169	207	299	Light
B	31·3	66·1	2·15	20·7	121	170	191	235	312	Light
C	30·4	67·4	2·22	23·6	222	275	271	279	310	Heavy
D	30·5	67·2	2·20	27·6	207	268	283	289	322	Heavy
E	31·3	68·8	2·20	28·9	174	221	241	275	284	Medium
F	31·9	67·2	2·15	22·8	295	307	309	315	316	Light

time the acid neutralising effect was rapidly lost. C, D and E gave a rapid initial neutralisation, *p*H 6·75 to 7·0 being reached within 10 minutes the buffering effect being maintained for a further 60 minutes. The curves for C and E were practically superimposed and are represented as being identical for the sake of clarity. F exhibited the most powerful response, raising the *p*H to 6 to 7 within 4 minutes and maintaining the buffering effect for a period of 75 minutes.

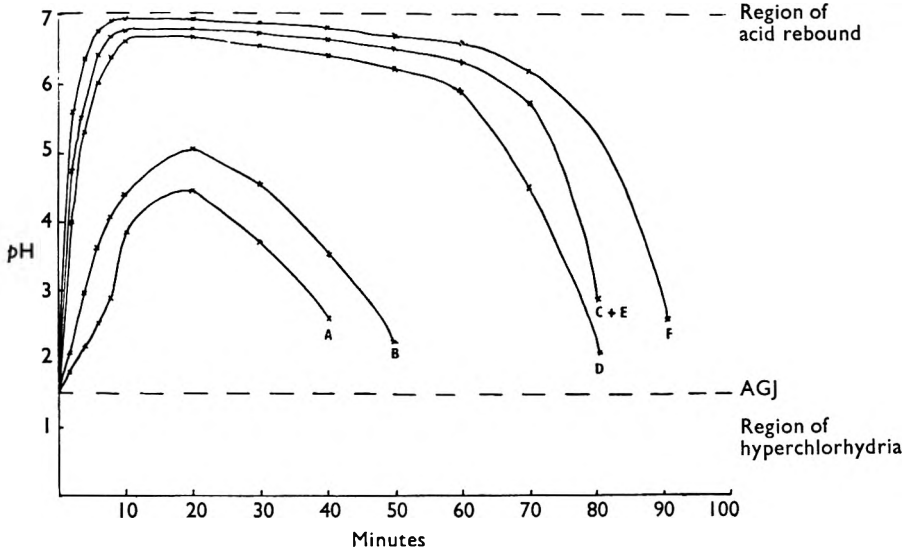


FIG. 1. Comparison of the antacid effect of samples of magnesium trisilicate. AGJ = Artificial gastric juice, *p*H 1·5, 37° C.

The B.P. acid absorption procedure was carried out on each powder, and in addition to the reading at 4 hours which is specified in the B.P. test the amount of acid neutralised after 15 minutes, 1 hour, 2 hours and 24 hours was determined. This data is also given in Table I and is shown graphically in Figure 2. Examination of the two sets of curves shows that there is but slight difference in the antacid performance as measured by the two methods.

These evaluations of the antacid properties of the various samples of magnesium trisilicate showed that A and B, although complying with the

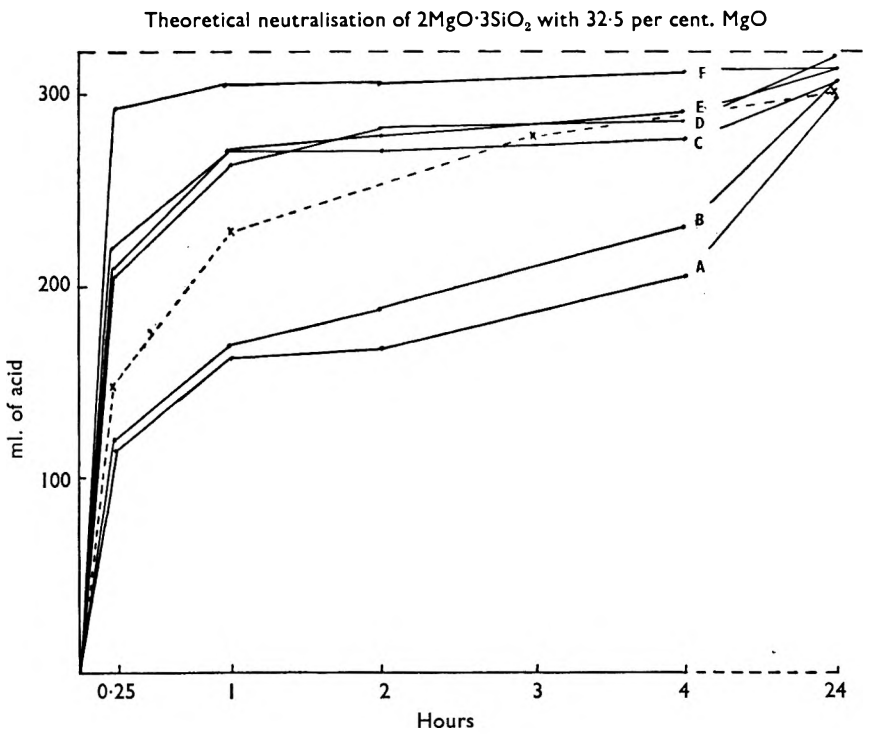


FIG. 2. Progressive neutralisation of 0·05N hydrochloric acid by magnesium trisilicate. × – – – × Curve transcribed from Mutch's graph (*Brit. med. J.*, 1936, **1**, 205). Letters correspond to samples.

B.P. requirements had, in our opinion, fallen short of Mutch's criteria. It seemed that some other more fundamental factor was concerned in the antacid behaviour of the materials. As the bulk densities of the powders encompassed a fairly wide range this property was first investigated in relation to antacid effect. Bulk density as a factor was soon ruled out as Table I shows that no strict correlation existed between the density type and the antacid performance. F is a light grade product giving a good antacid control, whereas A and B are both light grade products giving poor antacid control. Particle size was next investigated using the ordinary microscope. This technique was not very successful due to the great tendency for clumping and agglomeration to occur, and was abandoned.

Examination of the material by means of the electron microscope showed a striking difference in the external form of the particles. This is demonstrated in the micrographs. Figure 3 is of meerschaum, a naturally occurring magnesium silicate dihydrate, $2MgO,3SiO_2{\cdot}2H_2O$. This is a form of sepiolite which occurs in a highly crystallised state, and the fascicular crystalline form of the material is well demonstrated. Figure 4 is an electron micrograph of A and when this is compared with meerschaum and kaolin (Figure 10) the nature of the crystal imperfections can

FIG. 3. Meerschaum, × 12,500.

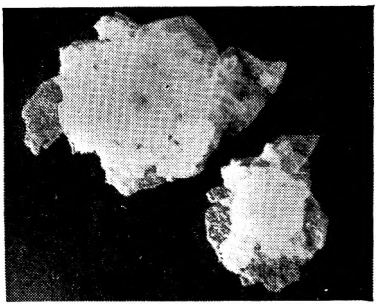


FIG. 4. Sample A, × 12,500.

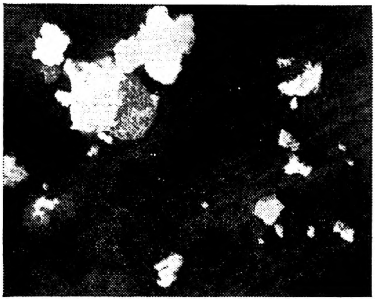


FIG. 5. Sample B, × 12,500.

FIG. 6. Sample C, × 12,500.

FIG. 7. Sample D, × 12,500.

FIG. 8. Sample E, × 12,500.

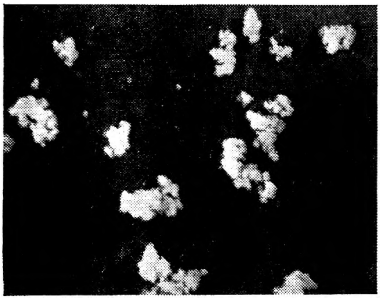


FIG. 9. Sample F, × 12,500.

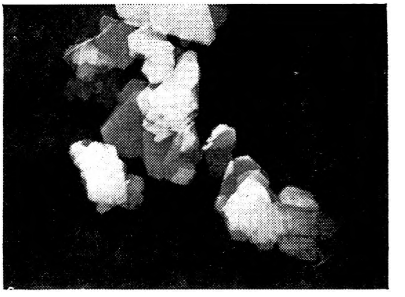
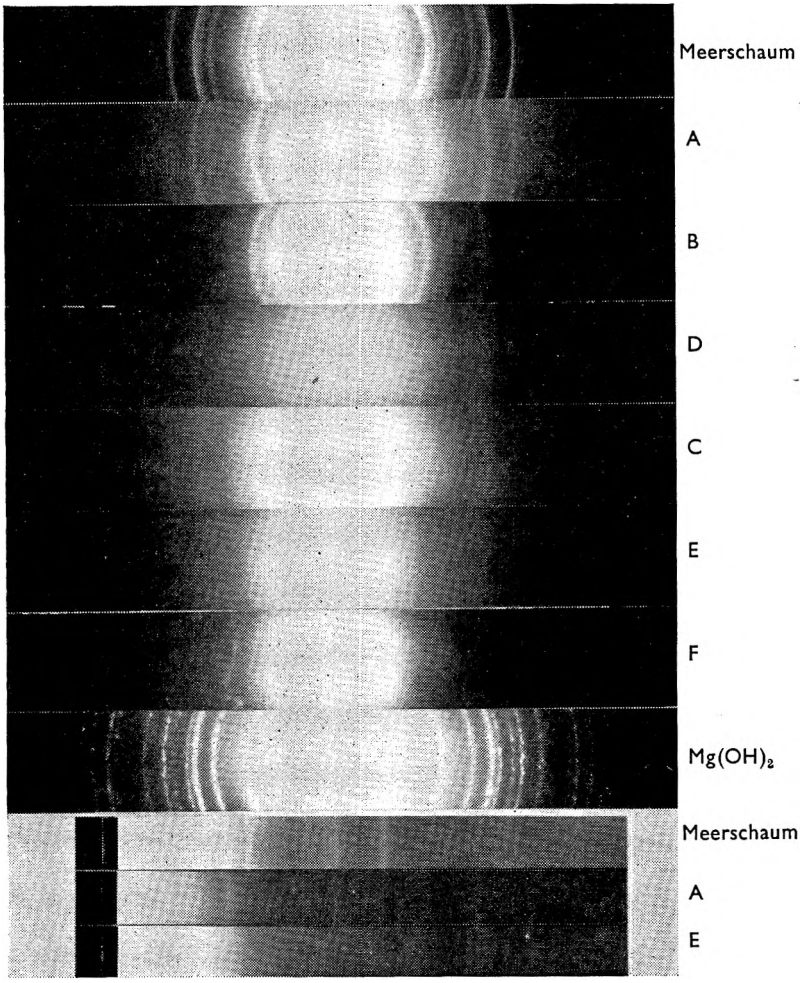


FIG. 10. Kaolin, × 12,500.

be appreciated. The distinctive sheets, evidenced by a comparative transparency and lack of shadow—typical of a layer-lattice silicate—are distorted and sometimes folded. B, Figure 5, resembles A and there is a gradation in the appearances of C, D, E and F which is well shown in Figures 6 to 9. These small particles do not seem to be homogeneous in



FIGS. 11 and 12.

structure, being rounded and irregularly shaped, and there is an absence of the "plate" formation seen in samples A and B.

The materials were then investigated from the viewpoint of crystal structure, electron and X-ray diffraction techniques being employed.

For the electron diffraction a thin layer of the powder was mounted on a collodion membrane and the patterns obtained by the use of 80 Kv. electrons. To ensure that the patterns recorded were truly representative

and not affected by any local non-homogeneity, several portions of the specimen were selected at random and the results compared. The electron diffraction patterns are shown in Figure 11. The materials were then heated to 900° C. for six hours and the residues examined by diffraction.

The X-ray diffraction studies were obtained by mounting the powder on a fine glass fibre using a water-based adhesive. Nickel-filtered copper radiation was used and long exposures were necessary. Three of the patterns are shown in Figure 12.

Diffraction patterns were also obtained for colloidal magnesium hydroxide and a mixture of this with silicic acid. These were quite different from the magnesium trisilicate patterns.

Discussion

Comparison of the electron diffraction patterns of the six artificial samples of magnesium trisilicate with that of meerschaum (sepiolite) a natural form of magnesium trisilicate shows that the latter is highly crystalline, whilst the artificial samples are all, more or less, disordered crystallographically. Of these A is most crystalline, F is much less crystalline, while the other samples occupy an intermediate position. This variation is related to antacid activity. A low antacid activity is shown by samples which exhibit a "plate-like" appearance under the electron microscope and give a clear diffraction pattern (i.e., are the more crystalline). On the other hand a high antacid activity is possessed by samples which are less regular in appearance when examined under the electron microscope and which give a more diffuse type of diffraction pattern (i.e., are less crystalline). Meerschaum had a very low antacid activity when examined by the extended B.P. neutralisation test.

After the samples were heated to 900° C., all the residues of the trisilicates, including that of meerschaum, were found to be inactive in the acid absorption tests. When these residues were examined by the diffraction techniques, the pattern of each was seen to be identical in clarity as well as in spacing of the lines, all having been transformed to enstatite $MgOSiO_2$. This may be regarded as further evidence, particularly in the case of the less crystalline samples, for the original existence of the sepiolite structure. The fact that removal of the combined water has such an effect suggests that differences in structure may be the result of variable degrees of hydration. It was found that the loss of weight on ignition of the dry material was such as to make the number of "water molecules" n in the formula $2MgO{\cdot}3SiO_2\, n\, H_2O$, integral in the case of the more crystalline sample, but not in the case of the less crystalline materials. The results of X-ray diffraction substantiate those of electron diffraction. By greatly increasing the exposure, the outer lines in the patterns of E and F could be developed and they were then seen to be considerably broadened and diffuse. Magnesium hydroxide gave a pattern as sharp as that of meerschaum, but could not be mistaken for it.

The interplanar spacings were calculated from measurements on the X-ray diffraction patterns and are shown in Table II. The measurements

TABLE II

X-RAY DIFFRACTION DATA FOR NATURAL AND "ARTIFICIAL" MAGNESIUM TRISILICATE ETC.

Substance	Interplanar spacing in Ångstrom Units							
Meerschaum	4·67*s*	3·81*m*	3·40*md*	2·64*s*	2·30*m*	1·53*m*	1·31*md*	
Sample A, etc.	4·67*s*		*d**	2·63*s*	*d**	1·54*m*	*d**	
Meerschaum after ignition	4·56*md*		3·24*s*	2·94*s*	2·55*s*			
Sample A, etc., after ignition	4·48*m*		3·22*s*	3·0*s*	2·62*s*			
Magnesium hydroxide ..	4·50*s*	2·26*s*	1·80*s*	1·58*m*	1·50*w*	1·38*w*	1·32*w*	1·19*w*

s = Strong. *m* = Medium. *w* = Weak. *d* = Diffuse. *d** = Too weak and diffuse for measurement.

on the patterns of all the artificial samples and on the meerschaum (a form of sepiolite) conform with the lattice openings for sipiolite as quoted by Mutch[1] and also given by Migeon[7], although in some cases the measurements could only be approximate. Only one anomaly exists, the line corresponding to 4·6 A, indicating that the artificial forms resemble more the meerschaum than the sample of sepiolite used as a standard by Mutch[1].

CONCLUSIONS

1. Magnesium trisilicates which conform to the present B.P. requirements can vary markedly in their physical characteristics as well as in their efficiency as antacids.

2. Investigation has shown that the less crystalline the sample of magnesium trisilicate the higher the antacid efficiency when measured *in vitro*.

3. It would appear that antacid activity of magnesium trisilicate is associated with the degree of distortion of the crystal structure, and that this distortion is possibly related to the amount of "combined water" in the crystal and the way in which this is included in the lattice. A magnesium trisilicate with a high degree of distortion of its crystalline structure gives a better antacid control *in vitro* than does one which is more crystalline.

Our acknowledgments and thanks are due to Professor L. F. Bates, F.R.S., for permission to use the X-ray diffraction apparatus at the University of Nottingham, and to Miss P. C. Cutts, Mr. F. Hobson, Mr. S. Clunie, Mr. B. F. Miller and Mr. D. A. Meads for technical assistance.

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DISCUSSION

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

DR. G. E. FOSTER (Dartford) said that there were several ways of making magnesium trisilicate. One was to add magnesium sulphate to a solution

of sodium silicate, silicic acid was first precipitated, subsequently magnesium hydroxide came down and combined with the acid. The composition of the magnesium trisilicate produced depended on the amount of alkali present in the sodium silicate. Another was to treat sodium silicate with acid, so precipitating silicic acid, and add the correct amount of magnesium hydroxide. It seemed the authors could tell the difference between magnesium trisilicate and mixtures of magnesium oxide and silicic acid. If such a mixture were left to mature so that the magnesium oxide had a chance to combine with the silicic acid, the X-ray pattern might change.

MR. N. BRUDNEY (London) said it seemed that the water content of magnesium trisilicate might have some influence on the antacid properties. Were the samples of magnesium trisilicate treated in any way before testing by the Armstrong and Martin technique? Had the technique been tried with a suspension of magnesium trisilicate, as that was often the way in which it was administered?

DR. F. HARTLEY (London) asked whether an attempt was being made to define what the crystallographic pattern of magnesium trisilicate should be? If not, had the authors any suggestions to make whereby the present B.P. specification could be improved to eliminate the less satisfactory samples?

MR. W. H. STEPHENSON, in reply, said that the investigation was an attempt to discover a brand of magnesium trisilicate which would be generally suitable for pharmaceutical purposes. The basic reaction mechanisms had not been investigated. He agreed that change of X-ray diffraction pattern of a mixture of magnesium oxide and silicic acid to that of a silicate type might occur, but he had no experimental evidence. The magnesium trisilicate had not been pretreated in any way. No suspensions had as yet been tried. With regard to a test which might be suitable for the B.P., if Mutch's magnesium trisilicate were taken as standard at least two points were required on the neutralisation curves. Probably the 15-minute and 3-hour times as postulated would be satisfactory. The higher the initial neutralisation the better, provided the lag neutralisation was maintained, and the *p*H of stomach contents kept up. For that reason samples E and F would be the type recommended. He would set standards of not less than 175 ml. of acid at 15 minutes rising to 300 ml. at the end of 3 hours. Such magnesium trisilicate should be satisfactory for all purposes.

STUDIES IN THE KEEPING QUALITIES OF UNCOATED TABLETS OF PROCAINE BENZYLPENICILLIN

By R. Levin

From the Pharmaceutical Laboratory, Research and Development Division, Distillers Company (Biochemicals) Limited, Speke

Received June 30, 1955

Uncoated tablets of the soluble salts of benzylpenicillin have been available in this country for several years. For much of this time it was believed that these preparations were stable for lengthy periods if stored under dry conditions. This assumption was shaken rather rudely by Bagnall[1] who claimed that an appreciable loss of potency may occur under these conditions within the anticipated life of the product, especially if they are stored in bulk containers from which samples were frequently withdrawn or in containers which did not provide an adequate barrier against moisture. In the light of these findings a comprehensive investigation of the keeping qualities of tablets of the soluble salts of benzylpenicillin was carried out by the Ministry of Health, using information supplied by the laboratories of several manufacturing houses. From the results of this investigation Davis[2] concluded that tablets of the soluble salts of benzylpenicillin could be considered to be stable for eighteen months provided that they had a low initial moisture content (i.e. not more than 1 per cent.) and were packed only in small bulk containers fitted with tight closures.

Procaine benzylpenicillin is relatively insoluble and might for this reason be expected to show greater resistance to decomposition due to the presence of moisture than do the soluble salts. The literature on the clinical efficacy of oral procaine benzylpenicillin shows it to compare favourably in all respects, with the soluble salts of penicillin[3–10].

The information now presented was obtained by preparing a batch of tablets to a simple formulation and subjecting them to various storage conditions for a period up to nine months. The keeping qualities were assessed by examining the important characteristics of the tablets, namely potency, disintegration, appearance and firmness, at frequent intervals. The moisture content was determined initially and on completion or near completion of each storage test.

Experimental

1. *Formulation* (250,000 units = 0·276 g.)

Granulation mixture		*Compression mixture*	
Procaine benzylpenicillin, 200 mesh powder (1000 units per mg.)	100·0 g.	Granulation mixture as above, dried	
Corn starch, 80 mesh powder	5·0 g.	Corn starch, 80 mesh powder	5·0 g.
Solution sodium carboxymethyl cellulose 1 per cent. w/v	26 ml.	Magnesium stearate, 80 mesh powder	0·5 g.

The granulation mixture was passed through a No. 12 sieve and dried at 37° C. for twelve hours. The dried granules were passed through a No. 16 sieve and mixed with the lubricating agents to form the compression mixture. This was compressed by means of a Manesty Type "E" tabletting machine fitted with 11/16 in. concave punches into tablets each containing 250,000 units of procaine benzylpenicillin.

2. *Storage*

The prepared tablets were divided into three batches A, B and C which were stored as described below, all being protected from light.

Batch A. The tablets were packed 20 each into 2 oz., screw-cap glass jars, fitted with metal caps complete with waxed card inserts. The jars were sealed with Sellotape and set aside at 4° C., 18° ± 5° C. (i.e., room temperature) and at 37° C.

Sufficient samples were prepared to enable one jar to be removed from each place of storage every month over the test period of nine months. Assays and disintegration tests were carried out at monthly intervals. Moisture determinations were made initially and after seven months.

Batch B. One hundred tablets were placed in a 16 oz. wide mouth, screw cap, glass jar, with an unlined loose fitting cap, and set aside at room temperature for nine months. Assays and moisture determinations were carried out at the end of the test period.

Batch C. Fifty tablets were placed in an open 2 oz. glass jar and exposed to the atmospheric conditions of the laboratory for four weeks during the month of May (temperature 18° ± 5° C.; relative humidity 50 to 80 per cent.). Assays and moisture determinations were carried out at the end of this period.

3. *Assessment of Keeping Qualities*

(i) *Determination of weight variation in the initial tablets.* The weight variation of the tablets was determined by the method of the British Pharmacopœia 1953.

Theoretical weight per tablet = 0·276 g.
Observed average weight per tablet = 0·2844 g.
Weight variation = ±1·86 per cent.

(ii) *Determination of the free moisture content.* The free moisture content was determined by crushing the tablets and sampling without delay; the powdered sample was dried under vacuum for 5 hours at 60° C. Determinations were duplicated and the average recorded. Results are shown in Table I.

(iii) *Determination of penicillin content.* The penicillin content of the tablets was determined by dissolving each tablet in 250 ml. of water and assaying the solution iodometrically by the method of Alicino[11]. The assay was repeated twice and the average recorded. The assay variation within each group of three readings was not greater than ±1 per cent.

After storage for six months samples removed from Batch A were

assayed in addition by the microbiological method using a cavity plate diffusion technique with *Staphylococcus aureus* as the test organism.

Results are shown in Table II.

(iv) *Test for disintegration.* Since the initial disintegration time was short, small changes would have been difficult to detect. The pharmacopœial method permits the presence of an appreciable air bubble, which by impact can speed the disintegration. A technique was therefore used which excluded the presence of an air bubble of significant proportion, thus disintegration took place more slowly.

TABLE I

MOISTURE CONTENT (PER CENT. W/W)

Initial moisture content	=	1·74
Batch A: After 7 months at 4° C.	=	2·13
After 7 months at 18° ± 5° C.	=	2·11
After 7 months at 37° C.	=	1·89
Batch B: After 9 months at 18° ± 5° C.	=	1·86
Batch C: After 4 weeks at 18° ± 5° C.	=	2·01

A tablet was dropped into a 1 in. test tube full of water at 37° C. The tube was stoppered, ensuring that no air bubble was present. The tube was inverted frequently so that the tablet passed to and fro but did not touch the glass. Disintegration was taken to be complete when the largest remaining granule was not greater than No. 8 mesh, as judged by eye. The test was repeated twice and the average of the three readings taken. These were usually quite close and in no case varied by more than ±10 per cent. from the average. After eight and nine months comparative readings were obtained using the official test method.

TABLE II

PENICILLIN CONTENT (UNITS PER TABLET)

Storage period (months)	No. of samples averaged	Storage temperature		
		4° C.	18° ± 5° C.	37° C.
Batch A: 0	6	252,000	252,000	252,000
1	3	247,000	241,000	246,000
2	3	245,000	242,000	245,000
3	3	236,000	241,000	243,000
4	3	238,000	239,000	236,000
5	3	241,000	247,000	241,000
6	3	241,000	244,000	239,000
6	3	238,000•	244,000•	246,000•
7	3	237,000	235,000	230,000
8	3	242,000	239,000	242,000
9	3	246,000	241,000	240,000
Batch B: 9	9	—	238,000	—
Batch C: 1	3	—	241,000	—

• Microbiological assay.

TABLE III

TIME FOR DISINTEGRATION IN WATER (SECONDS)

Average time for three samples

Storage period (months)	Storage temperature		
	4° C.	18° ± 5° C.	37° C.
1	80	90	120
2	76	103	130
3	80	70	139
4	85	105	155
5	83	85	145
6	130	128	197
7	112	134	160
8	60•	60•	94•
9	73	121	155
9	44•	70•	113•

• B.P. test method.

Results are shown in Table III.

(v) *Examination for appearance and firmness.* The appearance and firmness of the initial tablets

were assessed by eye and by fracture between fingers and thumb. These observations were repeated on all samples during and at the completion of their test. It was found that the tablets showed no change in appearance or firmness during or after their respective periods of test.

Discussion and Conclusions

This experiment shows that when stored at temperature of 4° C., 18° ± 5° C. and 37° C., the tablets showed a potency loss not greater than 4 per cent. after nine months. This loss is not increased by storing the tablets in a large insecurely sealed bulk container. The evidence appears to suggest that potency losses brought about by the presence of free moisture are due rather to residual moisture in the original tablets than to that taken up during subsequent storage. If this be so, a lower initial free moisture content might be expected to reduce or eliminate the small potency loss sustained by the test samples. A slight increase in the time for disintegration was noted with ageing, this being greater as the temperature of storage increases. Tablets stored for nine months at 37° C. disintegrated completely within two minutes. The free moisture content showed very small increases, the greatest taking place at the lowest storage temperature. Differences in degree between the moisture content increases appear to have been without significance. Procaine benzylpenicillin tablets have been stored in sealed jars, in a large loosely capped jar and in an open jar, in an effort to detect a tendency to deterioration either by potency loss or in other respects. Within the limitations of the test, namely time and condition of storage, it would appear that tablets of procaine benzylpenicillin are relatively stable in atmospheric conditions normal to this country, even where they are not packed in a tightly sealed container.

Summary

1. Tablets of procaine benzylpenicillin have been prepared to a simple formulation and subjected to various conditions of storage.

2. Data on the keeping qualities of the tablets are presented, as assessed by examining the important characteristics of the tablets, namely potency, disintegration, appearance and firmness.

The author wishes to thank Miss E. Watkin and Mr. E. Thompson for their help.

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DISCUSSION

The paper was presented by THE AUTHOR.

MR. A. R. ROGERS (Brighton) asked whether the authors had determined the free and combined moisture content by the Karl Fischer method. Had the combined moisture any effect on the keeping qualities of the tablets?

MR. T. C. DENSTON (London) suggested that the sentence in the first paragraph "This assumption was shaken rather rudely" might be reconsidered as, in his view, the assumption referred to had been completely confirmed. The material investigated by Bagnall had not, in fact, been stored under dry conditions.

MR. D. JACK (Harrow) asked why the author differentiated between the effect of initial moisture, and moisture taken up during storage. It was difficult to see that there would be any great difference between the effects of the two as there was nothing in the granulation to protect the procaine penicillin from moisture absorbed.

MR. G. SYKES (Nottingham) said that the paper appeared to omit any confirmation of the stability of the powder or granulation mixture. He reminded the author that there was an aqueous suspension of procaine penicillin on the market.

DR. R. A. WEBB (Hull) said he had noticed that tablets of penicillin had a mottled appearance after long storage. Had the author noticed this with other penicillin preparations, and was it a sign of deterioration?

MR. R. LEVIN, in reply, said that the Karl Fischer method had not been used. He was interested only in the free moisture content to see whether any potency losses might be related to the free moisture present. He was aware that the instability which developed in the samples examined by Bagnall occurred only because of incorrect conditions of storage, and agreed with Mr. Denston's comment. He found that development of slight mottling after twelve months did not indicate potency losses. He was aware that aqueous suspensions of procaine penicillin existed, but those products relied on the presence of buffering agents for their stability.

AN IMPROVED TEST FOR UNIFORMITY OF WEIGHT OF TABLETS

By K. L. Smith

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Received June 23, 1955

Introduction

The British Pharmacopœia 1953 includes a test for the uniformity of weight of tablets which requires, that when the tablets in a sample of 20 are weighed singly, not more than 2 deviate from the average weight by a percentage greater than that specified and no tablet deviates by more than double that percentage.

The procedure described in the B.P. may be preferred by those making occasional examinations, but those carrying out the test regularly must often consider that, when there is evidence that the batch is uniform, it should not be necessary to complete the 20 weighings and that, in the absence of a provision to take a further sample, the division between acceptance and rejection is too rigid.

The method of sequential analysis developed by Wald[1] will provide an alternative procedure which meets these criticisms and discriminates between satisfactory and unsatisfactory batches as efficiently as the official test. In this alternative procedure the average weight of 20 tablets is determined and the tablets weighed singly. After each weighing, reference is made to criteria for acceptance and rejection, which can be previously established. If either criterion is reached the examination is halted, if not, the weighings are continued.

Discrimination of the B.P. Test

The values in Table I have been obtained using the standard arithmetical methods which are described in the appendix to this paper. The Table illustrates the probability of accepting, by the official test, batches of tablets containing varying proportions of "defectives." Defectives are defined as tablets having weights which deviate from the average by the amount specified in the B.P. The Table also records the proportions of "double defectives" (tablets deviating by double the specified amount) and of "half defectives" (tablets deviating by half the specified amount) to be expected in these batches.

The Alternative Procedure

It follows from Table I that, if the alternative procedure is to have the discrimination of the official test, it should satisfy the requirements that when a batch of tablets contains 5 per cent. "defectives" there should be 92 chances in 100 of it being accepted and that when a batch contains 25 per cent. "defectives" there should be only 8 chances in 100 of this happening. Using these values in the formulæ given by Wald[1], which are reproduced in the appendix, the numbers of "defectives" critical for acceptance and rejection to be observed in any number of weighings have

been calculated. These are recorded in Table II where the entries involving fractions of tablets are necessarily omitted.

TABLE I

THE PROBABILITY OF ACCEPTING, BY THE OFFICIAL TEST, BATCHES OF TABLETS CONTAINING VARYING PROPORTIONS OF "DEFECTIVES" AND THE PROPORTIONS OF "HALF DEFECTIVES" AND "DOUBLE DEFECTIVES" EXPECTED TO BE PRESENT IN SUCH BATCHES

Proportion of			Probability of acceptance by official test
"Defectives"	"Half defectives"	"Double defectives"	
0·05	0·32	0·0001	0·92
0·10	0·40	0·001	0·67
0·15	0·47	0·004	0·39
0·20	0·52	0·0104	0·19
0·25	0·57	0·0214	0·08
0·30	0·60	0·022	0·03

Table II shows that with the alternative procedure a batch of tablets would be accepted at the eleventh weighing if no "defective" had been found, but that if 2 "defectives" were found in the first 5 weighings, it would be rejected. The batch would not be considered satisfactory if 2 "defectives" were found in 20 weighings, and the weighings would be continued unless 4 "defectives" had been observed in which case the batch would be rejected.

When the occurrence of "defectives" are being observed, no batch, however uniform, could be accepted with less than 11 weighings. High uniformity, however, also shows itself by the lower frequency with which "half defectives" occur. It is seen from Table I that the proportion of "half defectives" present in batches containing 5 per cent. and 25 per cent. "defectives," are 32 per cent. and 57 per cent. respectively. Using these values in the formulæ given by Wald[1] the critical acceptance and rejection numbers for "half defectives" have been calculated and are shown in Table III.

TABLE II

CRITERIA FOR ACCEPTANCE AND REJECTION BY THE IMPROVED PROCEDURE WHEN THE OCCURRENCE OF "DEFECTIVES" IS CONSIDERED

Number of "defectives" observed	Accept if number of tablets weighed is not less than:	Reject if number of tablets weighed is not greater than:
0	11	
1	19	1
2	26	5
3	34	13
4	42	20
5	50	28
6	58	36

The data in Table III shows that by observing the occurrence of "half defectives" not only could a batch of tablets be accepted with as few as 6 weighings, but that the interval between the numbers of weighings at which decisions can be made is smaller than that which obtains when "defectives" are being considered (Table II).

The improved procedure, counting the number of "half defectives," has been adopted for routine assays by a colleague and the information from the examination of 104 consecutive batches of 5 grain aspirin tablets, all of which were acceptable by the official test, is available. It was found that one batch demanded 24 weighings before it could be accepted. The remaining 103 batches were acceptable at or before the 13th weighing, and 96 of them at the 6th or 8th weighings. In the examination of one

other batch of tablets which did not pass the official test in that 3 "defectives" were observed in 20 weighings, it was noted that the first 5 tablets weighed were all "half defectives."

TABLE III

CRITERIA FOR THE ACCEPTANCE AND REJECTION BY THE IMPROVED PROCEDURE WHEN THE OCCURRENCE OF "HALF DEFECTIVES" IS CONSIDERED

Number of "half defectives" observed	Accept if number of tablets weighed is not less than:	Reject if number of tablets weighed is not greater than:
0	6	—
1	8	—
2	10	—
3	13	—
4	15	—
5	17	5
6	19	8
7	22	10
8	24	12
9	26	15
10	28	17
11	31	19
12	33	21
13	35	24
14	37	26
15	40	28

DISCUSSION

The method of sequential analysis has been used to provide an alternative procedure for testing the uniformity of weight of tablets. This alternative procedure discriminates between satisfactory and unsatisfactory batches as efficiently as the official test, using a sample of 20 tablets. It also meets the criticisms made against the official test in that it should not be necessary to complete the 20 weighings when there is evidence of uniformity, and that, in the absence of a provision to take a further sample, the division between acceptance and rejection is severe. The B.P. test has also been criticised by Dunnett and Crisafio[2] who suggest that the use of 20 tablets in the manner described may not be adequate. Methods suggested by them are that the standard deviation of 10 or 20 tablets could be used as a measure of uniformity or that a sample of 50, with provision to halt the examination at 20 tablets, should be taken. If it were permitted to estimate the standard deviation by the range in the tablet weights, the first method would not be laborious, but the criticism of the rigid division between acceptance and rejection would not be met. The objection to the second suggestion lies in the rigidity in the number of weighings demanded. The alternative procedure considered in this paper could be readily applied to give the discrimination of an examination where a sample of 50 was used and 5 "defectives" permitted.

The information obtained by using the alternative procedure in the examination of routine batches of tablets, which were acceptable by the official test, illustrates the saving in the number of weighings to be expected when the occurrence of "half defectives" is noted. Information like this does not prove that the alternative method discriminates as efficiently as the official test, but, on purely theoretical grounds it must, in the long run, do so.

APPENDIX ILLUSTRATING THE ARITHMETICAL METHODS USED TO ESTABLISH VALUES USED IN THE BODY OF THE PAPER

Estimation of the Proportion of Other Specified Percentage Defectives

It is generally accepted that the normal distribution is a satisfactory approximation to the distribution of the weights of tablets in a batch.

Therefore, if x is the value taken from a table of normal distribution[3] which corresponds to the proportion of "defectives," then $2x$ and $0{\cdot}5x$ are the values which correspond respectively to the proportions of "double defectives" and "half defectives" present in the batch.

Discrimination of the B.P. 1953 Test

When the proportion of "defectives" in a batch of tablets is p, the probabilities of obtaining 2, 1 and 0 "defectives" in a sample of 20 are represented by the last three terms of the binomial

$$(p + q)^{20} \text{ where } p + q = 1$$

The sum of these three terms, corrected for the probability that the "defectives" chosen are not "double defectives," will give the probability of acceptance by the official test. If the proportion of "double defectives" present is (p') this sum is equal to

$$190\, p^2 q^{18}\left(1 - \frac{p'}{p}\right)^2 + 20\, pq^{19}\left(1 - \frac{p'}{p}\right) + q^{20}$$

Calculation of the Acceptance and Rejection Numbers

When quality is measured by a proportion possessing a certain characteristic Wald[1] has shown that if the desired probability of rejection of the more acceptable quality (p_0) is α and the desired probability of acceptance of the less acceptable quality (p_1) is β, then the numbers possessing this characteristic to be observed in a sample of size m critical for acceptance and rejection are given by the formulæ

$$\text{Acceptance number} = \frac{m \log \dfrac{1 - p_0}{1 - p_1} + \log \dfrac{\beta}{1 - \alpha}}{\log \dfrac{p_1}{p_0} - \log \dfrac{1 - p_1}{1 - p_0}}$$

$$\text{Rejection number} = \frac{m \log \dfrac{1 - p_0}{1 - p_1} + \log \dfrac{1 - \beta}{\alpha}}{\log \dfrac{p_1}{p_0} - \log \dfrac{1 - p_1}{1 - p_0}}$$

SUMMARY

1. The method of sequential analysis is used to provide an alternative procedure to that described in the B.P. 1953 to test the uniformity of weight of tablets.

2. This alternative procedure allows uniform batches to be accepted with fewer weighings than the official test and removes the rigid division between acceptance and rejection in the borderline cases.

3. When the occurrence of tablets having weights which deviate from the average by half the amount specified in the B.P. is noted, the number of weighings needed in the alternative procedure is greatly reduced.

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DISCUSSION

The paper was presented by THE AUTHOR.

DR. F. HARTLEY (London) said that the virtues of the application of the sequential analysis technique had been seen in connection with the pyrogen test. An assumption which must be made in connection with the author's proposal was that there was normal distribution of possible errors and that assumption could not be made when taking an odd sample of 20 tablets. Mr. Smith had made the point—and had been criticised—that a sample of 20 was too small. He submitted that the task of the Pharmacopœia was to enable a clear decision for pass or failure to be reached on the average size of sample which could normally be expected to be taken by prescription or by a public analyst. A good deal hung on the sentence in the second paragraph "when there is evidence that the batch is uniform." In manufacturing additional control was possible and the method was a very helpful statistical tool which simplified the task of routine control of large numbers of batches of tablets. However, decisions had to be taken in borderline cases, and he did not think it advisable for the size of samples to be variable in the official specification.

DR. D. C. GARRATT (Nottingham) said that the method would give the same results with less work and save a great deal of time in large laboratories. Size of the sample was not really an important factor, if instead of taking the defective value, the half defective value was taken.

DR. G. E. FOSTER (Dartford) said he was worried because on modern tablet machines there were as many as twenty punches, and it was necessary to give every punch a chance to supply a tablet to be weighed. One punch might be maladjusted, and if one took only ten tablets and weighed them, tablets from that punch might be missed. Were any batches included in the paper which did not comply with the Pharmacopœial test?

MR. P. S. STROSS (London) said he wondered whether the point made by Dr. Garratt that the method would save time was really true. It was simpler to give the junior analyst tablets to weigh, and get him to write a report from which the senior analyst could decide at a glance whether the tablets complied with the Pharmacopœial requirements or not. If he understood the paper correctly the half defective method was questionable because it assumed normal distribution which would not be the case in a rotary machine with a faulty punch.

MR. E. W. RICHARD (Upminster) suggested that there might be three distinct checks during the life of a batch of tablets. First, at the manufacturing stage there was production control; secondly, when a batch had been completed a test was carried out by the control department and thirdly, there was the sample taken by the public analyst. At the manufacturing stage and possibly at the analytical control stage one might say

that the quantity of material available was unlimited. Many machines turned out more than 20 tablets per revolution and from that point of view alone he disliked the B.P. test. The wider information obtained by weighing a large number of tablets and the time and labour required might be reconciled in the near future, particularly in view of a new electronic device being developed which would enable tablets to be weighed very rapidly. Sequential analysis, although a very useful tool, could possibly then be supplemented at the manufacturing stage by weighing, which would give more information on the product being turned out.

DR. G. BROWNLEE (London) said that unless he had wrongly understood the arithmetic, all that the author was proposing was that better use should be made of the present information. He was not asking readers to assume anything that they did not already assume for the purposes of the B.P. test. The problem became complicated when the second issue was raised, namely, "What are you going to regard as the population that you are examining?" The only way to find out what was the population and how it was distributed was by weighing every tablet. In a process which was going on all day in which one punch might be contributing to skewness quite possibly the population was a hazard over a period of time and tests must be related to time. The B.P. test and the proposed test were both tests to detect abnormal distribution.

DR. R. E. STUCKEY (London) said that the Pharmacopœial test was of little practical value to a public analyst, and in manufacture far more samples than those provided for by the B.P. should be taken for proper control. He was therefore uncertain as to who should use the B.P. test.

MR. A. R. ROGERS (Brighton) agreed that there was a satisfactory approximation to normal weight distribution in good batches but not in unsatisfactory batches. Both the B.P. test and that suggested by the author assumed that the criterion based on normal distribution was adequate.

MR. K. L. SMITH, in reply, said that as to the question who should carry out the B.P. test, the best answer might well be no one. The manufacturer might wish to carry out a more severe one. The public analyst should also be wary of making a decision on a sample in which the pass criterion was not reached. It did not seem unreasonable to have such criteria that firm decisions might be made in the case of good or bad batches, and one of "not proven" in certain intermediate ones. It was of course true that the suggested test did no more and no less than the B.P. test, but it did it efficiently. He had been informed that when tablets made on a single punch machine failed the B.P. test, the distribution of the weights was mostly skew. The fact that the distribution was not always normal did not affect the calculation appropriate to the occurrence of defectives. Normal distribution had only been assumed to estimate the equivalent frequency of the occurrence of half defectives. The effect of the error would, he felt, be small. The suggestion was made that there might be an error in the arithmetic in view of the fact that by the B.P. test three defectives in 20 tablets failed the batch, whereas

in the suggested test four defectives were needed to do that. That was the logical result of a different mathematical approach and was balanced by the fact that one was not permitted to pass a batch when two defectives were observed in 20 tablets. He was not conversant with the vagaries of the odd punch in a multipunch machine but the argument that the taking of a fixed sample of 20 rather than sampling sequentially avoided this was naïve. The evidence of uniformity referred to was the evidence collected during manufacture, and not the evidence collected during the actual test. He had failed to emphasise that decisions with a small number of weighings would be possible only when the batch was uniform.

A TEST FOR THE MECHANICAL STRENGTH OF COMPRESSED TABLETS

By A. R. Webster and N. J. Van Abbé

From the Formulation Dept., Genatosan Ltd., Loughborough

Received May 18, 1955

Compressed tablets should reach the user free from mechanical damage; they should exhibit the correct dosage in each product and present an elegant appearance. Normal conditions of transport and storage give rise to diverse forms of damage, especially to fracture, crushing and abrasion. Liability to damage from mechanical stresses varies according to the tablet manufacturing process employed; different formulations of the same active ingredients may show varying responses under apparently identical conditions of stress.

A reliable laboratory test for the susceptibility of tablets to mechanical damage would be of value in the comparative assessment of formulation techniques and for the routine control of production batches; several authors[1,3–5], have described tests designed for this purpose These measured resistance to fracture, crushing, indentation, or to agitation and abrasion. Abrasion and fracture are the principal stresses met in the normal transport and storage of tablets and a shaking-test might be expected to evaluate susceptibility to damage in "field" conditions in a reliable manner, the response being accelerated by magnification of these stresses.

Many laboratories are known to conduct shaking-tests by agitating the tablets in a closed vessel. The tablets break down to form a fine powder which is permitted to accumulate in the vessel, thereby progressively decreasing the mechanical action exerted on the remaining larger fragments. This effect is unusual in field conditions, where the amount of fine powder in relation to the number of tablets is seldom sufficient for this "cushioning" to occur. It was, therefore, decided to improve the shaking-test by removing the "fines" as they were formed, and also to devise an apparatus and procedure that could easily be reproduced.

The Shaking-Test Apparatus (Fig. 1)

The vessel in which the tablets were agitated, consisted of a 6 in. length of standard 1 in. diameter borosilicate glass pipe-line. This was connected to an identical piece by means of a normal joint, but was separated from it by a 12-mesh 22-gauge wire screen to form a diaphragm between the tubes. The open end of the upper tube was closed with a rubber-bung; the lower tube had a cotton plug in its far end. Two such shaking-tube assemblies were fitted by means of felt-lined clamps to a hard-wood panel, which was attached to the baseboard by a pivot arm and by means of a suitable casting, to an eccentric on a heavy flywheel. The flywheel was belt-driven from a constant-speed electric motor. The baseboard was bolted in a vertical position, to a brick wall so that at "rest" the long axes of the

shaking-tubes were vertical. Because of the method of attachment of the shaking-panel, the shaking-tubes traversed an eccentric path so that the tablets were agitated in a random fashion. The reproducibility of the apparatus is dependent upon the principal dimensions and the speed of the motor. To remove adherent dust from the tablet-remnants after shaking, the remnants were allowed to roll freely down an inclined half-cylinder of 18-mesh wire gauze onto the scale-pan of a balance.

Basic test procedure. (*a*) The apparatus, as constructed, was found to give 260 to 270 strokes per minute in tests over a period of 18 months. This agitation was found to be satisfactory since the tablets were shaken vigorously, but did not strike the rubber-bung (the resilience of which might be variable). (*b*) A fixed number of tablets was employed in each determination; after weighing, they were transferred to a clean and dry shaking-tube (a similar load being applied to the other tube on each occasion). (*c*) The apparatus was started by a switch on the motor and allowed to run for a period of time measured by stop-watch. (*d*) The powder accumulated in the cotton-plugged end of the lower tube was allowed to fall to waste and the tablet remnants in the upper tube were rolled down the inclined sieve on to the scale-pan. The final weight was then recorded.

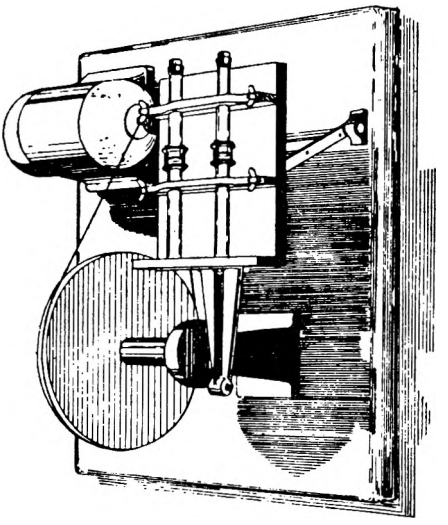

FIG. 1. Drawing of the apparatus for testing the mechanical strength of tablets. (Detailed drawings will be gladly supplied by the authors on request.)

Critical Examination of the Test

Effect of period of agitation on response. Two batches of a compound analgesic tablet were selected on the basis that Batch A consisted of firm tablets (by manual inspection) whilst Batch B was of poor to moderate mechanical strength. Mean initial weights of 20 tablets were, for Batch A, 10·67 g., and for Batch B, 10·61 g. Each point on the time-response curves (Fig. 2) represents the mean of 5 results for the loss in weight (in g.). From these results, it was decided to adopt a 2 minute period as the standard time of agitation for the following reasons. (*a*) The degree of agitation could be easily reproduced, (*b*) The losses in weight were readily measurable, (*c*) A clear distinction between the losses obtained with different batches of tablets was obtained, and (*d*) A reasonably short time-interval was desirable as a practical consideration.

Number of tablets per shaking-tube. It was necessary to determine the

effect of varying the number of tablets used in each test, and to decide upon the numbers to be used for different types of tablet. An indication of the spread of results obtained by varying the number of tablets per test, was derived from Table I; the results were most stable when 20 tablets were used in each test. A schedule to cover a wide range of average tablet weights was prepared, and is given in Table II.

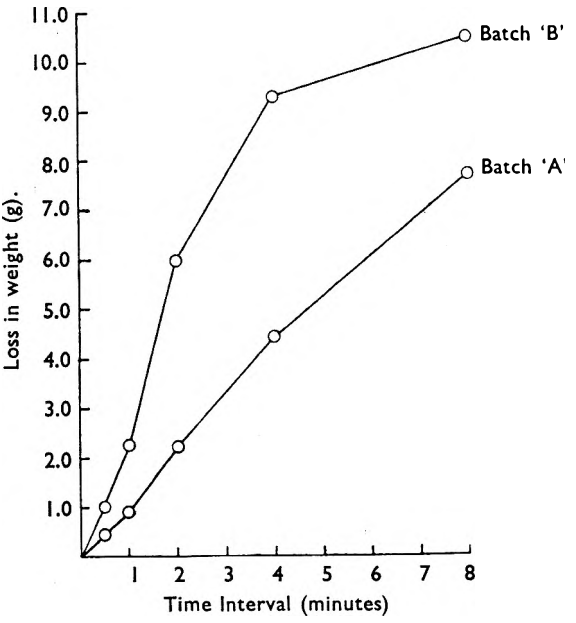

FIG. 2. Time response curves for an analgesic compound tablet. Each point represents the mean of 5 results.

Comparisons between samples in tests by one operator. The use of the test to discriminate between the mechanical strengths of different batches of tablets, was examined as shown in Tables III and IV. In Table III, two batches of tablets of the same formulation compressed on separate occasions were examined, 10 tests being conducted on each batch. The results showed a low standard deviation for each batch. Whether the difference between the 2 observed mean values was significant, was tested by a t-test: $t = \dfrac{\bar{x}_1 - \bar{x}_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$ where $\bar{x}_1$ and $\bar{x}_2$ were the mean values and ϵ_1 and ϵ_2 were the standard errors of the means. With 10 tests on each of batches X and Y, the value of t was 6·9, indicating a very high level of statistical significance for the difference between the means. For the first 4 tests on each batch, t is still 3·6 which is statistically significant. In Table IV, the results obtained by one operator on a single batch of tablets before and after a short period of storage are shown. In comparing the mean values obtained, the value of t was 3·5; this level of significance indicated that there was a true difference in mechanical strength between the samples which could be demonstrated by this method of testing. If the comparison was made for the first 4 readings on each sample, the value of t was 1·9; the difference between the mean values could not now be regarded as significant. The study of these results gave an indication of the lower limit of differences in mechanical strength that could be demonstrated in a reliable manner with only 4 readings.

TABLE I

RESULTS (LOSS IN WEIGHT IN G.) OBTAINED BY ONE OPERATOR ON A SINGLE DAY FOR ONE BATCH OF AN ANALGESIC COMPOUND TABLET, TO SHOW THE EFFECT OF THE NUMBER OF TABLETS PER SHAKING-TUBE ON THE NATURE AND REPRODUCIBILITY OF THE RESPONSE

(Mean tablet weight: 0·534 g.)

Test No.	No. of tablets per shaking-tube		
	10	20	30
1	1·77	1·96	2·29
2	2·08	1·88	1·94
3	1·77	1·95	2·40
4	1·43	1·98	2·25
5	2·07	1·82	2·34
Mean ($\bar{x}$)	1·82	1·92	2·24
Standard deviation (s)	0·27	0·07	0·18
Co-efficient of variation $\frac{(100s)}{x}$	14·8	3·7	8·0

Results obtained by different operators testing various types of tablet. The results given in Table V indicated on inspection, that there would not be any significant difference between the results obtained by different operators using the new test. It was also seen that the test was applicable to different types of tablet and that there was little variation within the readings obtained for each batch of tablets; this suggested that there would generally be sufficient uniformity within batches of tablets for the test to be carried out on a minimal number of observations.

TABLE II

RECOMMENDED NUMBER OF TABLETS PER SHAKING-TUBE, IN RELATION TO THE AVERAGE TABLET WEIGHT OF THE SAMPLE

Average tablet weight (g.)		No. of tablets per shaking-tube
Exceeding	Up to	
—	0·05	150
0·05	0·1	100
0·1	0·2	75
0·2	0·3	40
0·3	0·4	30
0·4	0·6	20
0·6	0·8	15
0·8	1·0	12
1·0	1·1	10
1·1	1·3	8
1·3	1·5	7

TABLE III

RESULTS OBTAINED BY ONE OPERATOR ON 27.3.53, USING 20 TABLETS PER TEST (OF AN ANALGESIC COMPOUND TABLET) ON TWO DIFFERENT BATCHES, COMPRESSED ON THE SAME PRESS

Batch X			Batch Y		
Initial weight (g.)	Final weight (g.)	Loss (g.)	Initial weight (g.)	Final weight (g.)	Loss (g.)
10·43	5·54	4·89	10·11	6·00	4·11
10·37	5·08	5·29	10·70	5·88	4·82
10·39	4·53	5·86	10·71	7·14	3·57
10·41	4·93	5·48	10·71	7·26	3·45
10·44	5·25	5·19	10·67	6·21	4·46
10·38	5·08	5·30	10·61	6·11	4·50
10·44	5·32	5·12	10·68	6·44	4·24
10·49	4·45	6·04	10·69	7·09	3·60
10·39	4·78	5·61	10·70	7·13	3·57
10·44	4·55	5·89	10·67	6·09	4·58
Mean		5·47			4·10
Standard deviation		0·37			0·50
Standard error of the mean		0·12			0·16

TABLE IV

RESULTS OBTAINED BY ONE OPERATOR ON TWO DIFFERENT OCCASIONS, USING 20 TABLETS PER TEST (OF AN ANALGESIC COMPOUND TABLET) IN THE EXAMINATION OF A SINGLE BATCH; DURING THE PERIOD OF STORAGE, THE TABLETS WERE STORED IN LABORATORY CONDITIONS OF TEMPERATURE AND RELATIVE HUMIDITY

A. (Tests conducted on 27.3.53)			B. (Tests conducted on 2.4.53)		
Initial weight (g.)	Final weight (g.)	Loss (g.)	Initial weight (g.)	Final weight (g.)	Loss (g.)
10·69	8·75	1·94	10·66	8·48	2·18
10·70	8·60	2·10	10·71	8·55	2·16
10·66	8·57	2·09	10·69	8·23	2·46
10·64	8·54	2·10	10·67	8·56	2·11
10·64	8·78	1·86	10·58	8·02	2·56
10·79	8·89	1·90	10·69	8·31	2·38
10·67	8·93	1·74	10·71	8·50	2·21
10·65	9·00	1·65	10·69	8·50	2·19
10·65	8·58	2·07			
10·67	8·35	2·32			
Mean		1·98	.. ..		2·28
Standard deviation ..		0·20	.. ..		0·16
Standard error of the mean		0·06	.. ..		0·06

TABLE V

RESULTS OBTAINED BY TWO DIFFERENT OPERATORS ON 3 BATCHES COMPRISING VARIOUS TYPES OF TABLET (ALL TESTS PERFORMED ON THE SAME DAY)

Product	Batch No.	No. of tablets per test	Operator A			Operator B		
			Initial weight (g.)	Final weight (g.)	Loss (g.)	Initial weight (g.)	Final weight (g.)	Loss (g.)
Tab. Codein Co. ..	320	15	9·78	8·52	1·26	9·81	8·50	1·31
			9·77	8·62	1·15	9·80	7·81	1·99
			9·80	7·92	1·88	9·74	7·91	1·83
			9·79	7·97	1·72	9·77	8·26	1·51
		Mean			1·50	.. .. ..		1·66
		Standard deviation			0·35	.. .. ..		0·30
		Standard error of the mean ..			0·18	.. .. ..		0·15
Tab. Dexamphet. Sulph. (5 mg. strength) ..	15	40	8·82	8·71	0·11	8·83	8·73	0·10
			8·83	8·72	0·11	8·84	8·74	0·10
			8·85	8·72	0·13	8·81	8·67	0·14
			8·81	8·67	0·14	8·86	8·73	0·13
		Mean			0·12	.. .. ..		0·12
		Standard deviation			<0·02	.. .. ..		<0·02
		Standard error of the mean ..			<0·01	.. .. ..		<0·01
Tab. Phenobarb. (½ gr. strength)	83	100	6·24	5·81	0·43	6·26	5·79	0·47
			6·26	5·77	0·49	6·26	5·80	0·46
			6·27	5·82	0·45	6·28	5·81	0·47
			6·26	5·79	0·47	6·25	5·80	0·45
		Mean			0·46	.. .. ..		0·46
		Standard deviation			0·02	.. .. ..		<0·01
		Standard error of the mean ..			0·01	.. .. ..		<0·01

DISCUSSION

The object of our experiments was to devise a reliable test for the susceptibility of compressed tablets to mechanical damage during transportation and storage, as this would help to prevent the issue of products that might otherwise reach the user in a damaged state. The reported test

seems suitable for the purpose and is relatively free from experimental error and sensitive to small differences between the mechanical strengths of various batches of tablets. The special feature of the test is that it removes fine powder, as it is formed, from the vicinity of the larger tablet remnants. The shaking vessel may be reproduced easily.

The test has been described as an assessment of "mechanical strength" in order to avoid confusion with factors such as "hardness" measured, for example, by indentation. Results were expressed in terms of "loss in weight" of a given number of tablets. The method of expression was preferred to a percentage calculation, as used in the "Friability Value" of Burlinson and Pickering[3] since this could yield an unduly favourable indication of the mechanical strength of overweight tablets.

A critical analysis of some results obtained in the test is reported. Similar results were recorded repeatedly over a period of 2 years, using various types of tablets in several hundred determinations. The test procedure was controlled by mechanical factors devoid of personal error and it was found that there was no significant variation in the agitation applied to the tablets in successive tests. The main source of error in the results for a given batch of tablets, was the variation between individual tablets in the samples taken.

Four readings were considered sufficient to yield a valid result on most occasions but the application of a *t*-test would be necessary to verify its significance in borderline cases.

A single mesh-size was chosen for the diaphragm of the shaking-vessel, since this allowed the apparatus to be used over the range from 6/32 to 20/32-in. tablet diameter, without alteration. No attempt was made to design the test as a means of comparison with a control tablet of standardised mechanical strength, since there was no likelihood that such a product could be prepared and stored satisfactorily.

The test was found to be most suitable for tablets prepared from materials of a crystalline, micro-crystalline or readily-pulverised amorphous nature. Results with coarsely fibrous and soft materials were unreliable. Readings obtained on those rare occasions on which the tablets became jammed together, were disregarded.

Results in the test have repeatedly demonstrated the following pattern. (*a*) Preparations involving the preliminary dry compression process appeared to show marked variation between the values of single observations, as well as high mean losses of weight. (e.g., Tab. Codein Co. see Table V). (*b*) Tablets prepared by moist granulation, gave moderate losses in weight in the test, but with small variations between observations, when a high proportion of starch was present. (e.g., Tab. Phenobarb.). (*c*) Tablets prepared by moist granulation and containing a high proportion of a sugar, showed the smallest losses in weight along with minimal variations between readings. (e.g., Tab. Dexamphet. Sulph.). These patterns agree with the general experience of the processes of tablet manufacture.

Some laboratories employ the Monsanto Hardness Test in which an individual tablet is crushed between a spring-loaded spindle and an anvil

(the test usually being carried out on each of 10 tablets from a batch). In comparing this method with our shaking-test, it is observed that, (*a*) the new test provides a closer assessment of susceptibility to the normal stresses encountered in field conditions, and (*b*) The Monsanto Test has the larger factor of personal error (in the positioning of the tablet, in setting the zero and in determination of the end-point). The Monsanto Test is useful, however, for checking the adjustment of a tablet press during operation and it may also give an estimate of variations in mechanical strength between individual tablets.

In the preparation of compressed tablets, it is desirable to adopt a compromise between optimal mechanical strength and disintegration time, as noted by Berry and Ridout[2]. Whilst there is a pharmacopœial method for the control of disintegration of tablets in water, it is noteworthy that the British Pharmacopœia has not yet adopted a procedure for the estimation of mechanical strength. It is suggested that the new test, perhaps modified by simplification of the apparatus and in the light of wider experience, could be the basis for an official procedure. Although the results quoted in this paper refer only to the comparison of batches of tablets of identical formula, it has been our experience that the test may be applied satisfactorily to compare different formulations provided that the active ingredient(s), average weight and diameter are standardised.

Summary

1. The need for a reproducible test for the mechanical strength of compressed tablets is considered.

2. A Shaking Test is described, which involves the separation from the larger tablet remnants of fine powder as it forms; the result is expressed in terms of the loss in weight of a specified number of tablets.

3. An apparatus is described and illustrated; the principal feature is an easily reproduced shaking vessel.

4. Consideration is given to the errors of the test and the significance of the results.

5. Comparisons are drawn between the Monsanto hardness test and the new shaking test.

6. It is suggested that the new test might form the basis for a Pharmacopœial procedure to standardise the mechanical strength of compressed tablets.

The authors wish to acknowledge the technical assistance of Mr. K. R. Bramley in carrying out much of the experimental work, the co-operation of Mr. R. Ganday, B.Sc., F.R.I.C., in whose analytical department the routine application of the test has been observed and the help of Mr. T. G. Desborough in preparing this paper.

APPENDIX*

FURTHER APPLICATION OF THE *t*-TEST TO TIME-RESPONSE PHENOMENA

(1) CODEINE COMPOUND TABLETS

Theoretical Tablet Weight 0·650 g.

Period of shaking (minutes) ..	2		3		4	
Batch	A	B	A	B	A	B
Individual readings for loss in weight in G.:						
1.	1·25	2·40	2·30	3·62	3·02	5·61
2.	1·42	2·48	2·60	3·77	3·27	6·28
3.	1·42	2·19	2·65	4·41	3·71	6·00
4.	1·41	2·78	2·48	3·77	3·69	5·79
Mean in g. ($\bar{x}$)	1·38	2·46	2·51	3·89	3·42	5·92
Standard deviation (s)	0·08	0·24	0·16	0·35	0·34	0·29
Coefficient of variation $\left(\frac{100\ s}{\bar{x}}\right)$	5·8	9·8	6·2	9·0	9·9	4·9
Standard error (ϵ)	0·04	0·12	0·08	0·18	0·17	0·15
$t\left(=\frac{\bar{x}_1-\bar{x}_2}{\sqrt{\epsilon_1^2+\epsilon_2^2}}\right)$	8·3		7·0		11·0	

(2) EPHEDRINE HYDROCHLORIDE TABLETS

Theoretical Tablet Weight 0·065 g.

Period of shaking (minutes) ..	2		3		4	
Batch	A	B	A	B	A	B
Individual readings for loss in weight in g.:						
1.	0·14	0·03	0·15	0·07	0·17	0·08
2.	0·16	0·03	0·15	0·06	0·23	0·09
3.	0·12	0·05	0·18	0·09	0·19	0·10
4.	0·21	0·04	0·14	0·08	0·24	0·10
Mean in g. ($\bar{x}$)	0·16	0·04	0·16	0·08	0·21	0·09
Standard deviation (s)	0·04	0·01	0·02	0·01	0·03	0·01
Coefficient of variation $\left(\frac{100\ s}{\bar{x}}\right)$	24·4	25·0	11·2	17·5	15·7	8·4
Standard error (ϵ)	0·02	0·01	0·01	0·01	0·02	0·01
$t\left(=\frac{\bar{x}_1-\bar{x}_2}{\sqrt{\epsilon_1^2+\epsilon_2^2}}\right)$	5·4		5·7		5·4	

(3) DEXAMPHETAMINE SULPHATE TABLETS

Theoretical Tablet Weight 0·225 g.

Period of shaking (minutes) ..	2		3		4	
Batch	A	B	A	B	A	B
Individual readings for loss in weight in g.:						
1.	0·24	0·26	0·53	0·15	0·67	1·26
2.	0·25	0·49	0·52	0·57	0·66	1·04
3.	0·33	0·17	0·49	0·47	0·64	1·10
4.	0·22	0·91	0·48	0·79	0·60	0·98
Mean in g. ($\bar{x}$)	0·26	0·46	0·51	0·50	0·64	1·09
Standard deviation (s)	0·05	0·33	0·02	0·27	0·03	0·12
Coefficient of variation $\left(\frac{100\ s}{\bar{x}}\right)$	19·2	71·6	4·7	54·0	4·9	11·2
Standard error (ϵ)	0·03	0·17	0·01	0·14	0·02	0·06
$t\left(=\frac{\bar{x}_1-\bar{x}_2}{\sqrt{\epsilon_1^2+\epsilon_2^2}}\right)$	1·2		0·07		7·4	

* Added after the paper was presented.

Discussion

The results quoted in this Appendix illustrate the time-response phenomena in batches, each consisting of tablets of relatively uniform mechanical strength, of widely differing theoretical tablet weight. In addition, the dexamphetamine sulphate tablets, Batch B is evidently composed of tablets of variable mechanical strength. The application of the *t*-test to a comparison of the mean losses in weight shows that the significance of the difference between the means can be seriously affected by the choice of an uneven batch.

When comparing uniform batches, it is seen that 4 readings at the 2-minute period of shaking, yield a result that (in comparisons of batches differing to a degree similar to those examined) is of acceptable significance. As the period increases, the coefficient of variation tends to fall; this presumably indicates that the more fragile tablets have been reduced to fragments passing No. 12 mesh during the first 2 minutes and that the later stage represents slower break-up of the more durable material.

If this is applied as a limit test for the mechanical strength of tablets, it is desirable to state:—

(*a*) An upper limit for the mean loss in weight.
(*b*) An upper limit for the coefficient of variation

all with reference to a 2-minute period of shaking and 4 readings.

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3. Burlinson and Pickering, *ibid.*, 1950, **2**, 630.
4. Smith and Frosch, U.S. Patent 2,041,869.
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DISCUSSION

The paper was presented by MR. N. J. VAN ABBÉ.

MR. E. W. RICHARD (Upminster) referred to the statement in the paper that "The object of our experiments was to devise a reliable test for the susceptibility of compressed tablets to mechanical damage during transportation and storage," and pointed out that tablets were generally sent out from manufacturers in fairly well filled and stuffed containers. During the test it would seem that the tablets were quite free to move up and down in the vessel. Did the authors consider that the conditions obtaining when tablets loosely held in a container were shaken, were comparable with those of a well packed container sent through the post?

DR. B. K. MARTIN (Slough) suggested that the term "friability value" adopted by Burlinson and Pickering was a more apt description of the degradation of the tablet than the author's term "mechanical strength." He was concerned to know by what factor the normal stresses which were encountered by the tablet in its every day life had been magnified in the author's test.

MR. D. STEPHENSON (Dartford) referred to the suggestion that the test should be included in the Pharmacopœia and said that the patient was already adequately protected by the present tests and no advantage would be gained by including a test for hardness.

MR. N. J. VAN ABBÉ, in reply, said that difficulty had been encountered in correlating the small amount of normal damage with the great amount of damage in an accelerated test. The effect of dropping a bottle of tablets was different from the abrasion effect. He was unable to state to what extent normal conditions of usage were magnified in his test but it was valuable for comparing different batches of the same tablets.

THE HYDROLYSIS OF ASPIRIN IN PHARMACEUTICAL PREPARATIONS. A LIMIT TEST FOR FREE SALICYLIC ACID

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Aspirin hydrolyses readily in the presence of water, therefore its pharmaceutical preparations may contain free salicylic acid unless produced and stored in an environment free from moisture.

Since salicylic acid has irritant properties it is desirable to limit the hydrolysis of aspirin before presentation to the consumer, and this is reflected by the B.P. Limit Tests for free salicylic acid in Aspirin and Tablets of Aspirin.

Difficulties met in applying the B.P. colorimetric test prompted an investigation of the kinetics of aspirin hydrolysis and of conditions affecting the formation and stability of the ferric-salicylate complex. A number of factors such as time, temperature, *p*H, concentration, ionic strength and the presence of certain ions, are all influential in determining the accuracy of the colorimetric test. A method of general applicability is described, with provision for a preliminary isolation of the salicylic acid if interference from citrate, phosphate or sulphate is expected. A simplified modification of the proposed general method is also described.

In the course of the present investigation, proprietary tablets and powders containing aspirin, were examined. Some of the tablets also contained codeine, phenacetin and caffeine and were of similar composition to those described in the Pharmacopœia.

Kinetics of Aspirin Hydrolysis

Hydrolysis of apirin proceeds by an acid-base catalysis mechanism[1–6]. Edwards[6] showed that if six simultaneous reactions involving dissociated and undissociated aspirin, water, hydrogen ions, and hydroxyl ions be assumed then a quantitative account could be given.

The rate of hydrolysis can be expressed by the equation

$$\frac{dm}{dt} = CKe^{-kt} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where m is the amount hydrolysed in a time t, C is the initial concentration of aspirin, and K the net velocity constant.

The rate of hydrolysis is dependent on *p*H, since an acid-base catalysis mechanism is involved. In certain *p*H ranges only one or two of the six reactions predominate. Thus, in strongly acid solutions the main reaction is $H_3CCOO{\cdot}C_6H_4{\cdot}COOH + H_3O^+ \rightarrow HO{\cdot}C_6H_4{\cdot}COOH + CH_3{\cdot}COOH + H^+$ In strongly alkaline solution $H_3CCOO{\cdot}C_6H_4{\cdot}COO^- + OH^- \rightarrow HO{\cdot}C_6H_4{\cdot}COO^- + CH_3COO^-$ is the principal reaction. At about *p*H 7 the reaction is primarily $H_3CCOO{\cdot}C_6H_4{\cdot}COO^- + H_2O \rightarrow HO{\cdot}C_6H_4{\cdot}COOH + CH_3{\cdot}COO^-$; and in the neighbourhood of *p*H 2·5 three reactions take

place at similar velocities. All these reactions are dependent on temperature. The variation of the net velocity constant K with *p*H and temperature is seen in Figure 1. Under conditions of hydrolysis where K is small the reaction approximates to a zero order reaction, and is of constant rate for the first few hours. If equation (1) is converted to parts per million per hour the initial rate is given by the expression

$$7{\cdot}667 \times 10^5 \text{Kp.p.m./hr.} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Thus for a solution containing 4 g/l. of aspirin at 25° C. the rate becomes 1850 p.p.m./hr.

Factors Affecting the Ferric-Salicylate Test

Time. Time is an important factor in a quantitative test for free salicylic acid in aspirin. Errors due to this cause are minimised in the limit test described in the B.P. monograph on Aspirin, but are less controllable in the case of the test on Aspirin Tablets where a preliminary filtration

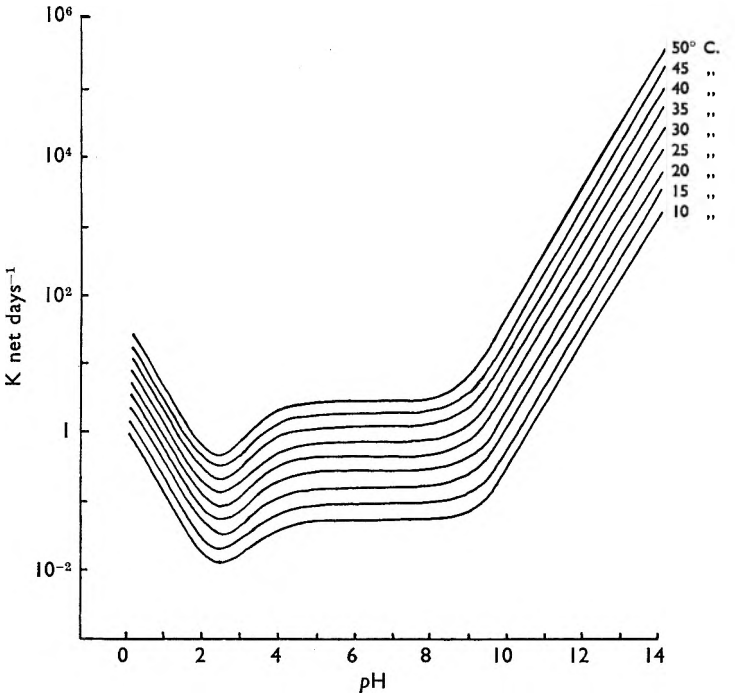

Fig. 1. The effect of variation of *p*H and temperature on the net velocity constant of aspirin hydrolysis.

is required. A larger error may arise with compound tablets of aspirin, because of the variable time taken to arrive at the point of colour matching.

Temperature. Edwards[6] described the influence of temperature on the rate of hydrolysis of aspirin and the relation is shown in Figure 1.

*p*H *Ionic Species and Ionic Strength*

Figure 1 shows the need to make quantitative tests at a constant *p*H. In estimating free salicylic acid, an additional factor was encountered in the sensitivity of the ferric-salicylate colour to changes in *p*H. Also the intensity of the ferric-salicylate complex was found to be influenced by the concentration of certain ions.

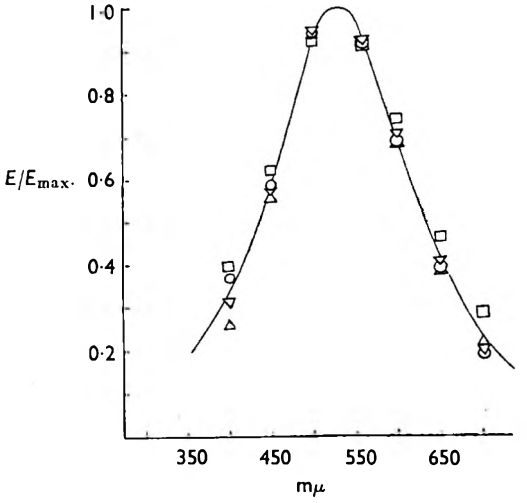

FIG. 2. Absorption spectrum of the ferric-salicylate complex in solutions of different *p*H and ionic strength (I).

Comparison cell solution. 2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + appropriate acid and base all made up to 50 ml.

Test cell solution. Ditto + 600 μg. salicylic acid. *p*H and I adjusted by addition of the correct proportions of appropriate acid and base.

○ Potassium chloride + hydrochloric acid. *p*H = 2·75, I = 0·1.

△ Ammonium hydroxide + sulphuric acid. *p*H = 2·75, I = 0·1.

▽ Ammonium hydroxide + sulphuric acid. *p*H = 2·75, I = 0·03.

□ Ammonium hydroxide + sulphuric acid. *p*H = 2·0, I = 0·1.

Spectrophotometric measurements. Some of the results of absorption measurements of the ferric-salicylate complex expressed as $E/E_{max.}$, are shown in Figure 2. Maximum absorption at 530 mμ was in good agreement with previous workers[7–10] and the extinction coefficient at this wavelength (E 530) was used in all subsequent measurements.

Choice of buffer solution is complicated by the formation of iron-ion complexes, or insoluble compounds with many of the conventional buffer systems. The variation of $E_{4\,cm.}$ 530 with *p*H in a number of these systems for a constant concentration of salicylic acid and ferric alum is shown in Figure 3. All the test solutions in this series of experiments were prepared with a constant calculated ionic strength (I) of 0·1 ($I = \frac{1}{2}\Sigma cz^2$ where c and z are respectively the molar concentration and charge of each ion present.)

Although ferric hydroxide is precipitated at or above a *p*H of approximately 3·0, some buffers, notably those containing acetates, inhibit precipitation; thus it is possible to see a maximum in the E 530 — *p*H curves in Figure 3.

Figure 4 shows the buffering effect of the various systems under the experimental conditions likely to be met in estimations of free salicylic acid in aspirin. The variation of E 530 with salicylate concentration, shown in Figure 3 is greatest in the region *p*H 2·75 to 3·75. For measurements

in this region the acetic acid—ammonium chloroacetate or glycine-acetic acid buffer systems appear the most suitable. The low buffer capacity of the nitric acid system in this region should be noted. The solution obtained in the B.P. method is essentially similar, though less concentrated. The *p*H of this solution may vary between 2·5 and 3·5 depending on the accidental presence of traces of acid or base, consequently errors of up to 20 per cent. can be expected.

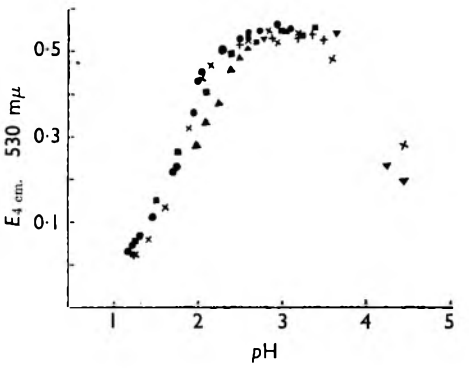

FIG. 3. The change in absorption of the ferric-salicylate complex with variation of *p*H in different buffer solutions of ionic strength 0·1.

Comparison cell (4·0 *cm.*) *solution.* 2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + buffer all made up to 50 ml.

Test cell (4·0 *cm.*) *solution.* Ditto + 600 μg. salicylic acid.

● Nitric acid–potassium hydroxide.
× Hydrochloric acid–potassium acetate.
■ Hydrochloric acid–glycine.
▲ Hydrochloric acid–ammonium monochloroacetate.
▼ Acetic acid–ammonium acetate.
\+ Acetic acid–ammonium monochloroacetate.

Addition of acids, bases, salts, and other materials were made to solutions containing fixed amounts of salicylic acid, ferric alum, and buffer to test the stability of the monochloroacetate system. The *p*H and $E_{4\ cm.}$ 530 values were then measured and are shown in Table II. The system appears to buffer sufficiently to deal with a number of possible constituents of aspirin preparations, as well as the slight contamination of acids and bases which might be accidentally encountered in the laboratory.

The influence of the ionic strength and species was assessed, and the change in $E_{4\ cm.}$ 530 per unit ionic strength of solution, at constant *p*H, is given in Table I. The effects of sulphate and phosphate ions are notable.

Despite the wide variation in *p*H, ionic strength and species in experiments, little change was observed in the wavelength of the absorption maximum, or the approximately symmetrical form of the absorption band, as illustrated in Figure 2. This confirms the assumption that it is the concentration and not the composition of the ferric-salicylate complex which changes with environment.

Solubility

The B.P. Test specifies an amount of aspirin equivalent to 4 g. per litre of 4 per cent. ethanol. The solubility of aspirin in this solvent has been determined spectrophotometrically[6] and the results are reproduced in Table III. They indicate that the current B.P. limit tests for salicylic acid are suspect at temperatures below 21°C.

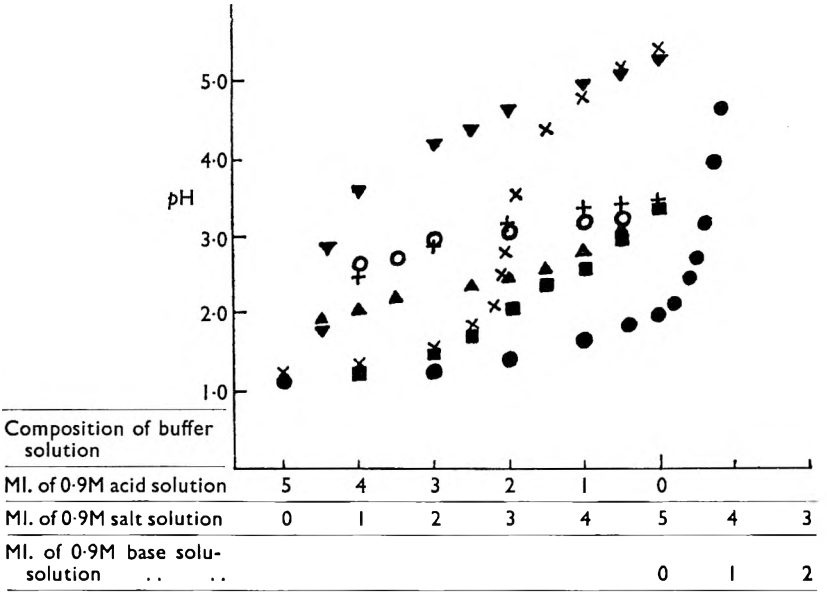

FIG. 4. The buffering effect of various buffer systems in the presence of the ferric-salicylate complex.

Solution. 2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + 5 ml. 0·9M buffer solution + 600 μg. salicylic acid all made up to 50 ml.

- ● Nitric acid—potassium hydroxide.
- × Hydrochloric acid—potassium acetate.
- ■ Hydrochloric acid—glycine.
- ▲ Hydrochloric acid—ammonium monochloroacetate.
- ▼ Acetic acid—ammonium acetate.
- \+ Acetic acid—ammonium monochloroacetate.
- ○ Acetic acid—glycine.

Pharmaceutical Materials Commonly Associated with Aspirin

Materials commonly occurring with aspirin in pharmaceutical preparations have been examined for possible interference in the formation of the ferric-salicylate complex. Phenacetin, codeine phosphate, caffeine and quinine sulphate were added separately to solutions containing a fixed concentration of ferric alum and varying concentrations of salicylic acid. The values of E 530 obtained were compared with those from control solutions and are shown in Table IV. The materials were added in such a way as to simulate the concentrations expected in the examination of a compound preparation.

With the exception of the codeine phosphate, and possibly quinine sulphate, the results showed, within the limits of experimental error, that no interference occurs.

DISCUSSION

A number of factors affect the accuracy of estimations of free salicylic acid in aspirin.

The kinetics of the hydrolysis of aspirin shows the necessity to correct for the degree of hydrolysis occurring in the course of a test for free salicylic acid. Consequently, it is essential to fix the temperature and *p*H of the test solution.

The experimental evidence on the formation and stability of the ferric-salicylate complex emphasises the importance of controlling the *p*H and the concentration of various substances in the test solution. Interference of certain ions, particularly phosphate and sulphate is noted. The inhibiting effect of citrate is, of course, well known[11,12]. Neither phenacetin, codeine nor quinine interfere, but phosphate or sulphate ions do.

A spectrophotometric method, designed to take into account these various factors, is described below and a special procedure is recommended where phosphates, sulphates or citrates would be expected to interfere.

TABLE I

CHANGE IN ABSORPTION AT 530 mμ OF THE FERRIC-SALICYLATE COMPLEX WITH VARIATION IN CALCULATED IONIC STRENGTH (I)*

System examined	Change in absorption at 530 mμ ($E_{4\ cm.\ 530}$) per unit ionic strength of solution at constant *p*H	
	*p*H 2·75	*p*H 2·0
$NH^+ - NO_3^-$	+0·35	+0·55
$NH_4^+ - SO_4^{2-}$	+1·50	+3·3†
NH_4^+ — Acetate	+0·25	
$K^+ - NO_3^-$	+0·20	+0·65
$K^+ - SO_4^{2-}$	+1·85	+3·8†
$K^+ - PO_4^{3-}$	+5·20†	
$K^+ - Cl^-$	+0·20	
K^+ — Acetate	+0·25	+0·90
Mg^{2-} — Acetate	+0·30	
Glycine — Cl^-	+0·25	
Glycine — Acetate	+0·30	
$Al^{3+} - NH_4^+ - Cl^-$	+0·9†	
$Ca^{2+} - NH_4^+ - NO_3^-$	−0·3†	

* *Comparison cell* (4 *cm.*) *solution*:—2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + appropriate acid and base all made up to 50 ml.

Test cell (4 *cm.*) *solution*:—Ditto + 600 μg. salicylic acid.

*p*H + I adjusted by addition of the correct proportions of the appropriate acid and base.

† Very approximate due to non-linearty of $E_{4cm.\ 530}$ — I relationship.

THE PROPOSED PROCEDURE FOR ESTIMATING SALICYLIC ACID IN ASPIRIN PREPARATIONS

TABLE II

THE EFFECT OF VARIOUS ADDITIONS TO BUFFERED FERRIC ALUM–SALICYLATE SOLUTIONS*

Addition	*p*H	$E_{4\ cm.\ 530}$
No additions	3·0	0·540
1 ml. 0·9M Monochloroacetic acid	2·75	0·528
0·5 ml. 0·9M Hydrochloric acid	2·50	0·495
1·0 ml. 0·9M Acetic acid	3·0	0·540
0·5 ml. 0·9M Ammonium acetate	4·0	0·570
1·0 ml. 0·9M Glycine	3·3	0·564
0·5 ml. 0·9M Potassium acetate	3·7	0·572
1·0 ml. 0·3M Calcium nitrate	3·0	0·547
1·0 ml. 0·9M Potassium nitrate	3·0	0·552
1·0 ml. 0·2M Potassium hydroxide	3·3	0·589
1·0 ml. 0·3M Ammonium sulphate	3·1	0·510
1·0 ml. 0·9M Ammonium nitrate	3·1	0·544
1·0 ml. 0·3M Magnesium acetate	3·9	0·576
1·0 ml. 0·3M Aluminium chloride	3·1	0·521
0·5 ml. 0·9M Nitric acid	2·6	0·487
1·0 ml. 0·2M Ammonium hydroxide	3·3	0·556

* *Comparison cell* (4 *cm.*) *solution*. 0·032M ammonium chloroacetate and 0·008M acetic acid. 0·0008 per cent. ferric ammonium sulphate. 4·0 per cent. ethanol.

Test cell (4 *cm.*) *solution*. Ditto + 12 μg./ml. salicylic acid (equivalent to 3000 p.p.m. on the basis of the B.P. test).

The procedure may be subdivided into (*a*) one of general applicability for aspirin or preparations containing aspirin in the absence of interfering materials, and (*b*) a modification of (*a*) for use when citrates, sulphates, or phosphates are present in the preparation.

Procedure (*a*)

Reagents. 0·2 per cent. Ferric ammonium sulphate. Buffer

solution 0·08M acetic acid and 0·32M ammonium monochloroacetate. Absolute ethanol.

Preparation of ferric ammonium sulphate solution. To 0·2 g. of ferric ammonium sulphate crystals are added 6 ml. of 10 per cent. nitric acid and about an equal amount of water. This is boiled until dissolved (about ½ minute). After cooling, the solution is made up to 100 ml. in a graduated flask.

TABLE III

THE SOLUBILITY OF ASPIRIN IN 4 PER CENT. ETHANOL

Temperature °C.	Solubility of aspirin g./litre
10	2·65
15	3·26
20	3·93
20·4	4·00
25	4·76
30	5·72

Preparation of buffer solution. After dissolving the appropriate quantities of ammonium monochloroacetic acid and acetic acid (A.R. grades) the *p*H should be adjusted by addition of either acetic acid or ammonia, so that a solution of *p*H 2·95 ± 0·05 is obtained on mixing 5 ml. of buffer solution, 5 ml. of ferric alum solution, 2 ml. of ethanol and making up to 50 ml.

TABLE IV

THE INFLUENCE OF PHARMACEUTICAL MATERIALS ON THE FERRIC-SALICYLATE COMPLEX

Salicylic acid		Absorption of test solution $E_{4\ cm.}$ 530				
µg./ml.	Equivalent in B.P. test	No addition	+ Phenacetin	+ Caffeine	+ Quinine sulphate	+ Codeine phosphate
2·0	500	0·105	0·106	0·104	0·100	0·100
6·0	1500	0·284	0·280	0·289	0·286	0·277
12·0	3000	0·545	0·545	0·555	0·535	0·530
20·0	5000	0·895	—	—	—	0·840

METHOD

The powdered sample, 0·2 g., is weighed and dissolved (or partially dissolved in the case of certain preparations) in 2 ml. of ethanol in a 50 ml. graduated flask. The flask is placed in a thermostat at 25° C., and about 35 ml. of distilled water at 25° C. is added. Simultaneously the time (T_0) is noted. As soon as possible afterwards, 5 ml. of buffer solution and 5 ml. of ferric ammonium sulphate solution is added, and the volume of the solution made up to the mark. The whole is then well shaken to ensure adequate mixing and solution of any precipitated aspirin. After about 10 minutes slightly more sample than is required to fill a 4 cm. cell is then removed with a pipette and, if necessary, filtered as quickly as possible through a No. 1 Whatman paper into the absorption cell. The time (T_1) is noted when the extinction coefficient measurement at 530 mµ is made, and a comparison cell containing an identical solution without the aspirin is used. Speed of filtration is important, particularly if the laboratory temperature differs greatly from 25° C. In order to hasten filtration, sintered glass filter sticks or plugs of cotton wool in 0·5 cm. polythene tubes attached to pipettes, with or without the aid of a filter pump, have been used to advantage.

At least 3 samples should be withdrawn at intervals of not less than

10 minutes. A value for the extinction coefficient at zero time (T_0) is then obtained by extrapolation.

The salicylic acid concentration in μg./50 ml. is then read from the salicylic acid $E_{4\ cm.}$ 530 mμ calibration curve, the data for which is given in Table V. If the aspirin content of the preparation is known, the result is readily converted to p.p.m. of aspirin.

TABLE V

RELATIONSHIP BETWEEN SALICYLIC ACID CONCENTRATION AND EXTINCTION COEFFICIENT AT 530 mμ*

Salicylic acid μg./50 ml.	$E_{4\ cm.}$ 530
200	0·188
400	0·358
600	0·545
800	0·720
1000	0·895
1200	1·078

* *Comparison cell* (4 *cm.*) *solution:*—2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + 5 ml. buffer solution all made up to 50 ml.
Test cell (4 *cm.*) *solution:*—Ditto + salicylic acid.

Corrections for Irrelevant Absorption

In order to correct for irrelevant absorption, measurements can be made using a similar solution and control solution but omitting the ferric alum solution. If the correction is significant it is advisable to make extinction measurements over a period of time so that an appropriate correction can be made for each of the readings at T_0, T_1, T_2, T_3. The correction for aspirin is very small and inside the normal experimental error of the extinction coefficient determination.

It is essential that the absorption cells themselves be matched, or that their absorption differences at 530 mμ be known.

Procedure (b)

Reagents. Benzene A.R. Tested for absence of reaction with ferric alum solution and dried over anhydrous sodium sulphate. 0·2 per cent. ferric ammonium sulphate solution. Absolute ethanol. Buffer solution 0·08M acetic acid and 0·32M ammonium monochloroacetate.

Method

0·2 g. of dry powdered sample is placed in a clean dry 100 ml. separating funnel plugged just above the tap with a small piece of cotton wool. The sample is extracted with four 10 ml. portions of benzene, the benzene extracts being collected in another dry separating funnel. The bulked benzene extracts are then extracted with 5 ml. of a solution containing 5 ml. 0·2 per cent. ferric ammonium sulphate solution and 5 ml. of buffer solution made up to 45 ml. with distilled water. The aqueous layer is run off into a 50 ml. graduated flask containing 2 ml. of absolute ethanol. Extractions are repeated until there is no further pink colouration in the aqueous layer.

When the salicylic acid has been completely extracted from the benzene, the rest of the buffered ferric alum solution, if any, is poured into the graduated flask and the solution is made up to the mark and shaken so that adequate mixing is ensured. If necessary, traces of benzene in this solution can be removed by filtration through a wetted filter paper, the first portion of the filtrate being discarded. A further portion is collected

and the absorption of this solution is measured in a 4 cm. cell at 530 mμ, and the free salicylic acid in the sample is calculated as before.

Comments on the Proposed Procedure

A simplification of procedure (*a*) may be expedient in certain routine work. Once the hydrolysis rate at a fixed temperature and given weight of sample has been determined, only one extinction coefficient reading need be taken at a known time.

No hydrolysis correction need, in most cases, be made when procedure (*b*) is used, provided the time for extraction and colour measurement is relatively small (about 10 minutes). This is because the partition of aspirin between benzene and ferric ammonium sulphate solution is such that the final aspirin concentration in the ferric alum solution can be neglected.

The limits of reproducibility of procedure (*a*) are within plus or minus 10 per cent., or plus or minus 50 parts per million for samples of free salicylic acid content of less than 200 parts per million.

In the case of procedure (*b*) control experiments showed that the recovery of salicylic acid was never less than 95 per cent.

Examination of Aspirin Preparations

A number of proprietary compound aspirin preparations were examined for free salicylic acid using the proposed procedure (*a*). The results were compared with those obtained using the B.P. method (Table VI). To obtain a proper comparison a range of salicylic acid standards (intervals equivalent to 100 p.p.m.) was used in the case of the B.P. method.

In both instances it was necessary to filter the solution before estimation of the extinction coefficient or a comparison of colour could be made. With the B.P. method the solutions sometimes developed a turbidity after filtration due to crystallisation or coagulation of a component. With 1 sample (No. 13) the colour developed was so far removed in hue from the standard that comparison was impossible in the case of the B.P. method. The solution of the particular sample was markedly alkaline and the error in this instance could be attributed to a *p*H effect.

The difficulties with turbidity in test solutions in the B.P. method were not experienced with the suggested procedure, probably because the monochloroacetic acid buffer hastens and completes coagulation of colloidal matter before filtration. Crystallisation was eliminated by virtue of the constant raised temperature used (25° C.).

The use of a simple colorimetric method is unsatisfactory with many compound aspirin preparations. If an adverse significance is to be attached to the presence of small quantities of salicylic acid in such preparations it is obviously desirable to have a presumptive standard, which must necessarily depend upon a reliable method of estimation. With this purpose in view it is suggested that attention might be given to establishing a limit for salicylic acid in the compound aspirin preparations in the Pharmacopœia, and that the procedure proposed in the present communication might serve as a method for consideration.

TABLE VI

EXAMINATION OF COMPOUND ASPIRIN PREPARATIONS FOR SALICYLIC ACID USING THE PROPOSED PROCEDURE (1) AND B.P. METHOD (2)

Sample No.	Active ingredients	Form	Free salicylic acid content		
			μg./g. of preparation	p.p.m. of aspirin in preparation	
				1	2
1	Aspirin	Tablet	750	850	500
2	Aspirin Phenacetin Caffeine	Tablet	1000	700	1100 Filtration lengthy
3	Aspirin Phenacetin Caffeine	Powder	425	460	300 Slightly turbid
4	Aspirin Phenacetin	Powder	200	360	300 Slightly turbid
5	Aspirin Phenacetin Caffeine	Powder	1100	2200	1800 Turbid
6	Aspirin Phenacetin Caffeine	Tablet	475	1100	800
7	Aspirin Phenacetin Caffeine	Tablet	1150	2330	2200 Filtration lengthy
8	Aspirin Phenacetin Codeine phosphate	Tablet	400	1000	400 Turbid
9	Aspirin Phenacetin Codeine phosphate	Tablet	700	1740	700 Turbid
10	Aspirin Phenacetin Caffeine Codeine phosphate	Tablet	3900	8950	2300 Turbid
11	Aspirin Phenacetin Caffeine Quinine sulphate	Tablet	1170	1940	900 Fluorescence due to quinine
12	Cacium Aspirin	Powder	3800	4500	3000
13	Aspirin Sodium bicarbonate	Powder	4200	35,000	Unobtainable. Solution was orange-brown in colour
14	Aspirin Phenacetin Caffeine	Tablet	400	830	400 Slightly turbid
15	Aspirin Citric acid Calcium carbonate	Tablet	1270*	2560*	Unobtainable. No colour owing to presence of citrates
16	Aspirin Citric acid Sodium bicarbonate	Powder	570*	7200*	Unobtainable. No colour owing to presence of citrates

* Procedure (*b*) used.

SUMMARY

1. The kinetics of the hydrolysis of aspirin and the conditions for formation and stability of the ferric-salicylate complex have been studied.

2. The resulting information is discussed in relation to the B.P. method for assay of free salicylic acid in aspirin preparations.

3. A spectrophotometric method based on the experimental data is described.

4. Results of assays, using both methods, on currently available preparations are compared.

The authors thank Miss J. Ashwin for some of the experimental work.

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DISCUSSION

The paper was presented by MR. D. N. GORE.

DR. D. C. GARRATT (Nottingham) said that as far as he knew there was no pharmacological evidence that the amount of free salicylic acid in aspirin or aspirin tablets complying with the limit test of the Pharmacopœia was of any significance. While not disparaging the work of the authors he appealed to analysts to use a sense of proportion with regard to limit tests. In the case of some sulphate and chloride limit tests the effect of other ions present might introduce errors of the order of 50 per cent., but the tests in his opinion were nevertheless sufficient.

He asked whether the optical density of the colour developed in the test was not very low and would it not be better if the test were modified so that a greater depth of colour was obtained.

DR. W. MITCHELL (London) suggested that in the Pharmacopœial test, it was usual to end up by trying to match entirely different colours! If the author had succeeded in eliminating this difficulty it would be very valuable.

DR. G. E. FOSTER (Dartford) suggested that in the determination of the free salicylic acid in compound tablets of aspirin numerous factors were involved. For instance, while the solutions were being prepared for the test, the aspirin might hydrolyse. In that connection it would be interesting to know whether there was evidence of any increase in free salicylic acid while extraction was in progress. Again, was there any information concerning the time required for the ferric salicylate to give maximum colour? It was common knowledge that in compound tablets

of aspirin there was more free salicylic acid present than in simple aspirin tablets when calculated on the basis of the aspirin actually present. In his experience a reasonable limit for the compound tablets would be about three times the amount permitted in the B.P. aspirin tablet.

MR. H. E. BROOKES (Nottingham) said it was refreshing to find that the authors had included in the paper the mechanism of the reaction. For a calculation of the hydrolysis of aspirin in equation 2, if K were taken for 35° C. the rate was approximately 7 to 8 per cent. per hour, presumably at the *p*H of the gut. The authors stated "since salicylic acid has irritant properties," but surely aspirin must have irritant properties if that were the rate of hydrolysis in the gut? Did the authors consider that it was really necessary to prescribe a narrow limit for salicylic acid and to use a rather more complicated method? Any considerable decomposition would be readily detectable by the odour of acetic acid in the tablets.

MR. C. A. JOHNSON (Nottingham) said he had found difficulty in completing an extraction within ten minutes, which was the suggested time, and the solution he obtained increased in optical density quite rapidly.

MR. R. L. STEPHENS (Brighton) said that care should be taken not to set such a low limit for the salicylic acid content of tablets that they could not be kept for a reasonable time under normal conditions of storage.

DR. J. G. DARE (Leeds) said that in fixing limits for any drug consideration must be given to the upper limits as determined by the adverse pharmacological effects and the lower limits determined by manufacturing economics. There was little evidence at present that aspirin tablets could not be made with an amount of free salicylic acid below the present limits, and he did not support any suggestion that the limits should be amended.

The CHAIRMAN of the Session (Dr. H. Davis) pointed out that while it was very easy for analysts, pharmacists, pharmacologists and doctors to maintain a sense of proportion with regard to the amount of free salicylic acid, if some persons could produce tablets of a lower limit than that of the Pharmacopœia, they had the finest advertisement they could possibly have.

DR. D. C. GARRATT (Nottingham) said he did not intend to suggest that there should be greater tolerance in the permissible amount of free salicylic acid but that a sense of proportion should be maintained.

MR. GORE prefaced Mr. Rapson's reply by expressing the view that two separate issues were involved in setting standards for free salicylic acid in aspirin or in compounded preparations containing aspirin. The physiological significance of traces of salicylic acid was a first consideration, and secondly there was the accuracy of the analytical procedure. The present contribution was primarily concerned with the latter, and suggested a method which was more adequate for stringent standards than the simple colorimetric procedure described in the B.P. monograph on aspirin and tablets of aspirin.

MR. RAPSON, in reply, agreed that the pharmacological evidence for the

deleterious effects of free salicylic acid is very slender. The colour developed in the test is weak. It could be increased by raising the concentration, which would require on solubility grounds an alteration in the alcohol content of the solvent. However, to depart too greatly from the B.P. test was felt undesirable at this early stage. He agreed with the comment on the difficulties of matching the colours in the B.P. test. He said there is evidence for hydrolysis during the extraction procedure B. Calculation indicated that errors due to hydrolysis are much less than one-tenth of those obtaining in procedure A. and was in agreement with experiments. He explained that errors can arise, if for example, finely divided aspirin passed through the cotton wool plug and in some cases it has been found necessary to use a filter paper. The experimental details in the paper are not precise enough in this respect. The 10 minutes allowed for extraction time may not be enough. The time taken for the ferric salicylate colour to develop is very short—less than one second. He said that the calculation of 7 to 8 per cent. per hour hydrolysis in the stomach—perhaps a little low due to the presence of enzymes—was very pertinent in connection with a limit for free salicylic acid. In conclusion, he agreed that a due sense of proportion must be maintained in setting the limits for salicylic acid content.

THE PHARMACOGNOSY OF THE ASPIDOSPERMA BARKS OF BRITISH GUIANA

PART II. THE MICROSCOPY OF THE BARK OF *Aspidosperma excelsum* BENTH.

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IN a previous communication[1] the macroscopical characters of the bark of *Aspidosperma excelsum* Benth. have been described, and illustrated by means of photographs showing the outer and inner surfaces of a typical specimen. The present communication deals with the detailed histology of this bark as seen in sections, macerations and powder. The material used consisted of three samples of bark previously designated 4A, 4B and 4C collected in British Guiana in 1949, 1950 and 1954 respectively. Line drawings to illustrate the diagnostic characters of outer and inner surfaces of this bark and of tissue distribution as seen in smoothed transverse section are given in Figure 1, A, B and C.

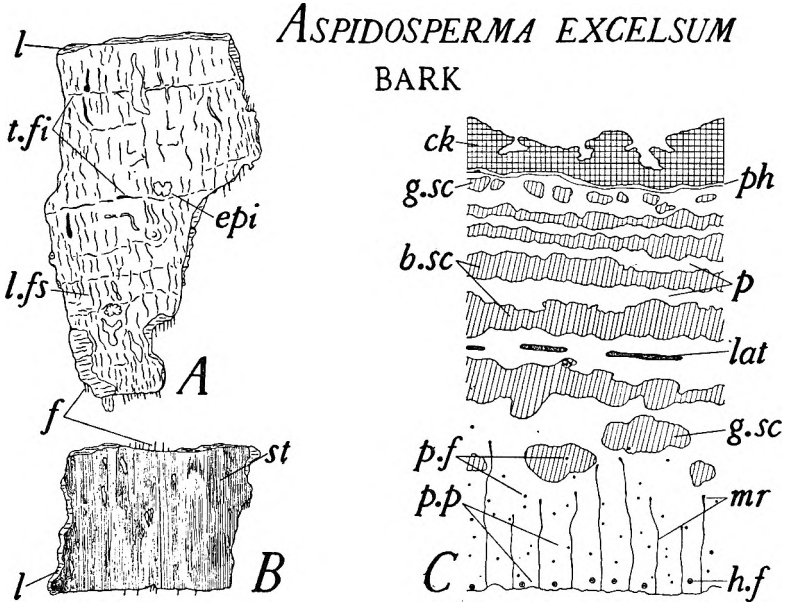

FIG. 1. *Aspidosperma excelsum* bark, macroscopical characters and T.S.:—*A*, outer surface × ¼; *B*, inner surface × ¼; *C*, smoothed T.S. × 15; *b.sc*, band of sclereids; *ck*, cork; *epi*, epiphyte; *f*, fibre; *g.sc*, group of sclereids; *h.f*, phloem fibre with large lumen; *l*, lamination; *lat*, latex canal; *l.fs*, longitudinal furrow; *mr*, medullary ray, *p*, cortical parenchyma; *p.f*, phloem fibre with narrow lumen; *ph*, phellogen; *p.p*, phloem parenchyma; *st*, longitudinal striation; *t.fi*, transverse fissure.

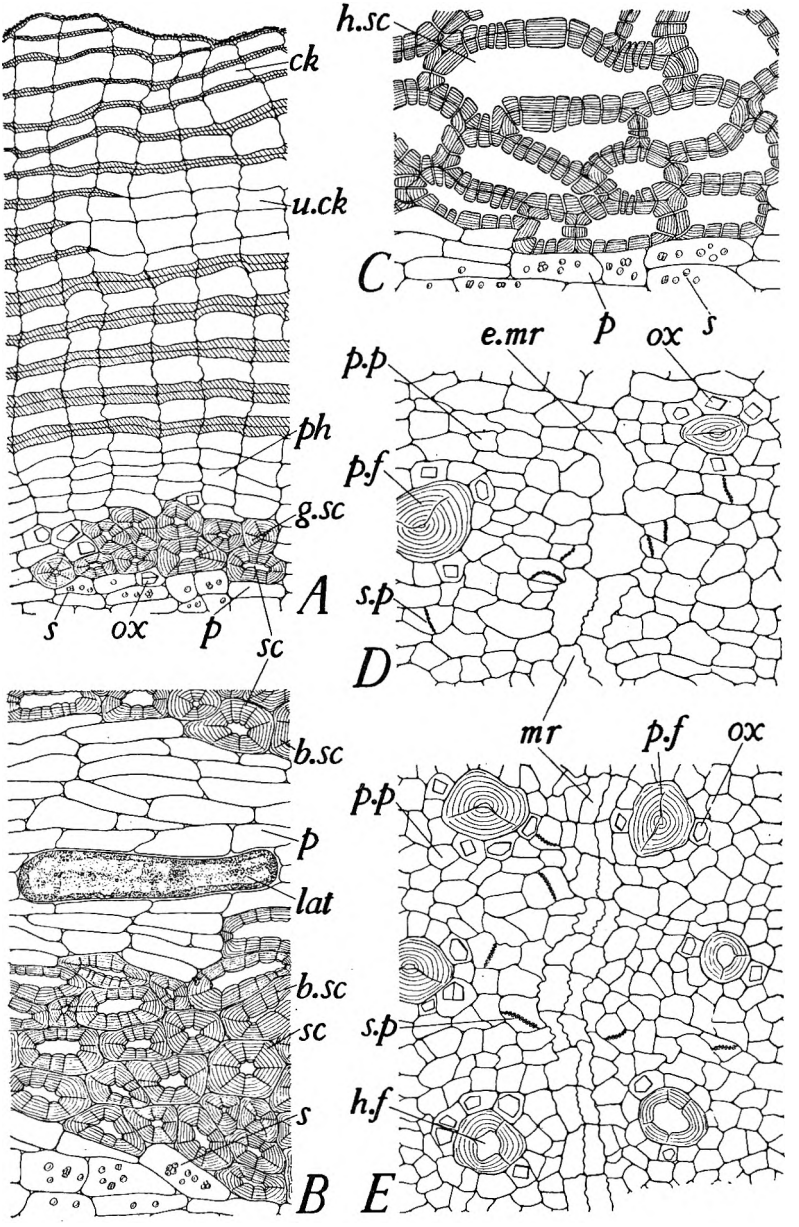

FIG. 2. *Aspidosperma excelsum* bark in T.S.:—*A*, cork, phellogen and phelloderm; *B*, cortex; *C*, inner cortex; *D*, outer phloem; *E*, inner phloem; all × 200; *b.sc*, band of sclereids; *ck*, lignified cork with thickened tangential walls; *e.mr*, end of the medullary ray; *g.sc*, group of sclereids; *h.f*, phloem fibre with large lumen; *h.sc*, sclereid with large lumen; *lat*, latex canal; *mr*, medullary ray; *ox*, crystal of calcium oxalate; *p*, cortical parenchyma; *ph*, phellogen; *p.f*, phloem fibre with narrow lumen; *p.p*, phloem parenchyma; *s*, starch; *sc*, sclereid with narrow lumen; *s.p*, sieve plate; *u.ck*, unlignified or slightly lignified cork.

Detailed Histology of the bark of A. excelsum (Figs. 2 and 3)

Cork consisting of some eight to sixteen layers of tangentially elongated brick-shaped cells of two types, arranged in groups to form a discontinuously stratified tissue, the greater number of cells with inner and outer tangential walls heavily thickened and lignified (Fig. 2, A, *ck*, and Fig. 3, A, *ck*); other cells with thin walls which are unlignified or only slightly lignified (Fig. 2, A, *u.ck*, and Fig. 3, A, *u.ck*); cells polygonal in surface view; R = 12 to **22** to 30 μ, T = 15 to **35** to 55 μ, H = 18 to **24** to 30 μ. Phellogen (Fig. 2, A, *ph*, and Fig. 3, A, *ph*) of one to three layers of thin-walled, tangentially-elongated cells; R = 6 to **10** to 15 μ, T = 22 to **28** to 36 μ, H = 14 to **22** to 28 μ. Phelloderm (Fig. 2, A, and Fig. 3, A), a well-marked tissue of sclerenchymatous cells arranged in small groups (Fig. 2, A, *g.sc*, and Fig. 3, A, *g.sc*), together with slightly tangentially-elongated parenchymatous cells, some of which contain a single prism of calcium oxalate; the sclerenchyma consisting of large isodiametric cells 22 to 60 μ in diameter, with small lumen, well-marked simple or branched pits traversing the thick, stratified and lignified walls. The cortex consists of groups of sclereids arranged in five to seven tangential, more or less continuous bands (Fig. 2, A, *b.sc*, and Fig. 3, A, *b.sc*) and separated by bands of cortical parenchyma; sclereids of the outer bands and parenchymatous cells are similar to those present in the phelloderm. Much tangentially-elongated latex canals, R and H = 36 to **54** to 86 μ, T = 560 μ to 2150 μ (Fig. 2, B, *lat*, and Fig. 3, B, *lat*), occurring in one or more of the bands of cortical parenchyma, which lie between the sclereid bands; latex, which is granular in appearance, is stained yellow with iodine solution. Towards the inner region of the cortex, the bands of sclerenchyma consist of thick-walled sclereids as described above, together with sclereids possessing a very large lumen, very well defined simple pits and thick, stratified and lignified walls, R = 25 to **36** to 54 μ, T = 54 to **85** to 144 μ, H = 18 to **36** to 90 μ (Fig. 2, C, *h.sc*, and Fig. 3, C, *h.sc*). No defined endodermis or pericycle are distinguishable but in this region discontinuous groups of thick-walled sclereids occur, resembling those of the phelloderm but also containing, at times, a small number of axially elongated, thick-walled fibres which are identical with those occurring throughout the phloem and to be described below.

Phloem, which is up to 40 per cent. of the thickness of the bark, consists of sieve tissue, phloem parenchyma, phloem fibres and medullary rays. Sieve tubes may be distinguished with oblique, compound sieve plates on the end walls (Fig. 2, D and E, *s.p*, and Fig. 3, D, *s.p*). Phloem parenchyma with few intercellular spaces (Fig. 2, D and E, *p.p*, and Fig. 3, D, *p.p*), of thin-walled cells R = 18 to **22** to 36 μ, T = 25 to **34** to 40 μ, H = 44 to **85** to 134 μ; having compound pits on the vertical walls and containing starch granules; prisms of calcium oxalate occur singly in a few of these cells (Fig. 3, D, *c.ox*). Phloem fibres, always isolated and surrounded by thin-walled parenchymatous cells each containing a prismatic crystal of calcium oxalate (Fig. 2, D and E, *ox*, and Fig. 3, D, *ox*), of two types; the greater number are scattered throughout the phloem

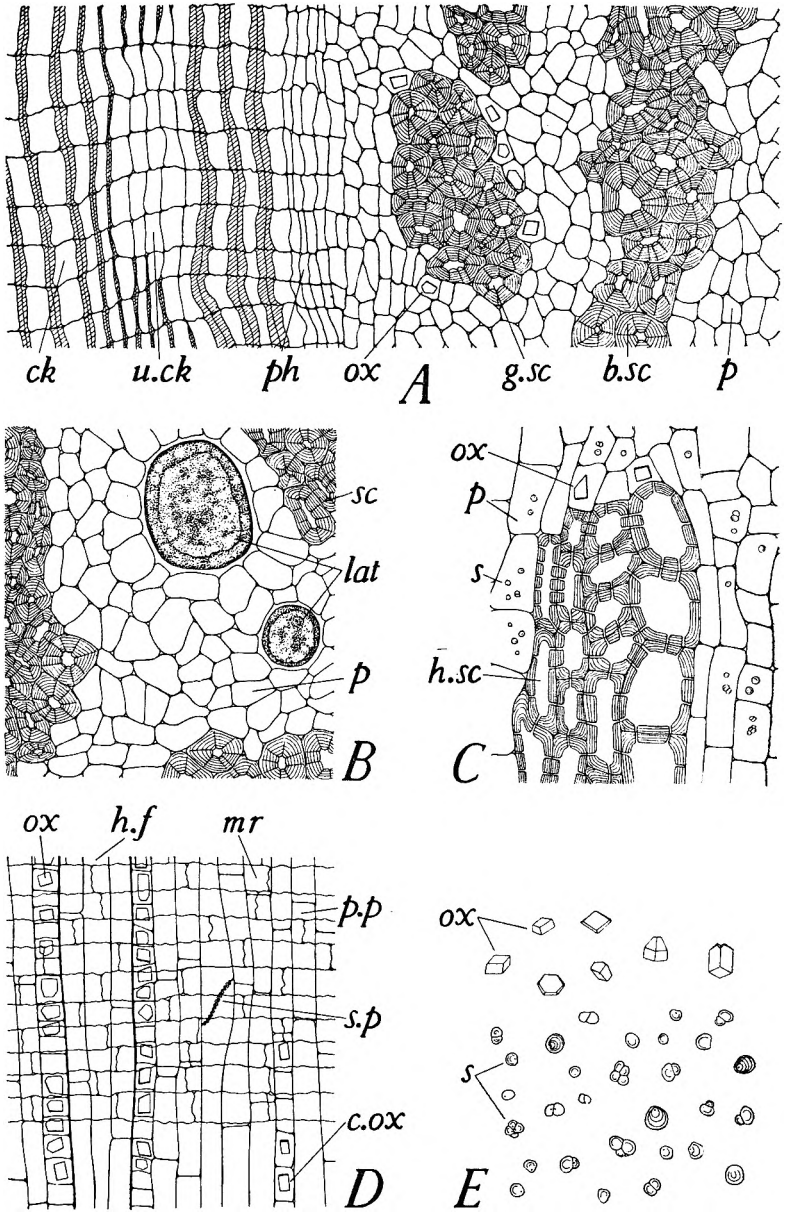

FIG. 3. *Aspidosperma excelsum* bark in L.S. and powder:—*A*, cork, phellogen, phelloderm and outer cortex; *B*, cortex; *C*, inner cortex; *D*, phloem; *E*, calcium oxalate crystals and starch granules, as seen in powder; all × 200; *b.sc*, band of sclereids; *ck*, lignified cork with thickened tangential walls; *c.ox*, phloem parenchyma cells containing prisms of calcium oxalate; *g.sc*, group of sclereids; *h f*, phloem fibre with large lumen; *h.sc*, sclereid with large lumen; *lat*, latex canal; *mr*, medullary ray; *ox*, crystal of calcium oxalate; *p*, cortical parenchyma; *ph*, phellogen; *p.p*, phloem parenchyma; *s*, starch; *sc*, sclereid with narrow lumen; *s.p*, sieve plate; *u.ck*, unlignified or slightly lignified cork.

(Fig. 2, D and E, *p.f*) and are spindle-shaped with bluntly pointed ends, R and T = 36 to **50** to 62 μ, H to 1550 to **2210** to 2800 μ, walls thick, lignified, stratified, traversed by a few simple pits along which splitting may have occurred, lumen very small; a smaller number of fibres occurring towards the innermost region of the phloem are somewhat thicker, R and T = 40 to **60** to 80 μ, the walls are somewhat thinner and the lumen is large, 18 to **30** to 45 μ in diameter; these fibres resemble those described above in all other characters (Fig. 2, E, *h.f*, and Fig. 3, D, *h.f*). Medullary ray (Fig. 2, D and E, *mr*, and Fig. 3, D, *mr*), straight, two cells wide but becoming one cell wide towards the periphery of the phloem (Fig. 2, D, *e.mr*), 15 to 25 cells in height; the cells R = 25 to **40** to 55 μ, T = 15 to **25** to 36 μ, H = 12 to **18** to 22 μ, very wavy in outline and containing starch granules.

Starch abundant in the cortical and phloem parenchyma, simple or 2- to 4-compound; individual granules with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ in diameter (Figs. 2 and 3, *s*). Calcium oxalate, as square, rectangular or obliquely rectangular prisms or small cubes of various sizes, measuring up to 25 μ, associated with the groups of sclereids and as a sheath around both types of fibres; there is no relation between the shapes of the crystals and the region of bark in which they occur (Figs. 2 and 3, *ox*).

Isolation of individual cells by maceration (Fig. 4, A and B)

In order to isolate the latex canal cells, transverse sections of about 100 μ in thickness were cut and the exact position of the latex canal located under a high power of magnification: the two bands of sclereids, in between which the latex canal lies, were then separated with the help of two fine dissection needles, leaving the whole latex canal together with the surrounding band of cortical parenchyma. The parenchyma was removed as far as possible with the help of two fine needles and the remainder dissolved out by the action of 80 per cent. sulphuric acid. The latex canal (Fig. 4, A and B, *lat*) is a long tube without any indication of transverse walls; its contents appear granular and are stained yellow with iodine solution. The phloem fibres, which have been described above (Fig. 4, A and B, *p.f* and *h.f*) were isolated by maceration with Schultze's maceration fluid.

Powder. Greyish dark-brown in colour: cork cells polygonal in surface view and reddish-brown in colour, the greater number with thick lignified inner and outer tangential walls and some cells with thin unlignified or slightly lignified walls (Fig. 4, C, *u.ck* and *ck*): sclereids of various shapes and sizes, with thick, stratified, lignified walls; of two types, the greater number with very narrow lumen, having simple or branched pits, others with large lumen and well-defined simple pits (Fig. 4, D, *sc and h.sc*): phloem fibres usually somewhat broken, of two types, the greater number with very narrow lumen, fewer cells with large lumen; both types of fibres with thick, stratified lignified walls, traversed by a few simple pits, and surrounded by a sheath of thin-walled parenchymatous cells, each containing a single prismatic crystal of calcium

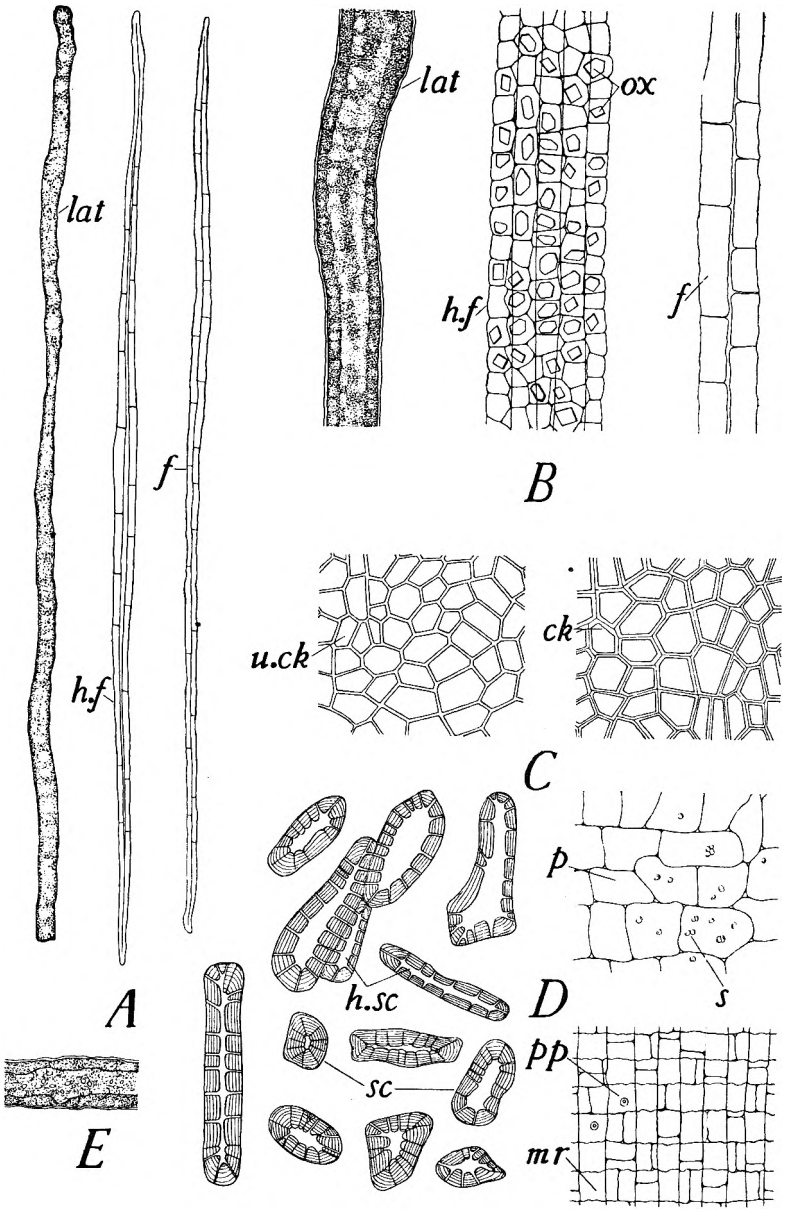

FIG. 4. *Aspidosperma excelsum* bark, macerate and powder:—*A*, latex canal and fibres, isolated by maceration; *B*, *C*, *D* and *E*, various components as seen in powder; *A* × 50, *B* to *E* × 200; *ck*, lignified cork; *f*, phloem fibre with narrow lumen; *h.f*, phloem fibre with large lumen; *h.sc*, sclereid with large lumen; *lat* and *E*, latex canal; *mr*, medullary ray; *ox*, crystal of calcium oxalate; *p*, cortical parenchyma; *p.p*, phloem parenchyma; *s*, starch; *sc*, sclereid with narrow lumen; *u.ck*, unlignified or slightly lignified cork.

oxalate (Fig. 4, B, *p.f*, and *h.f*): phloem parenchyma (Fig. 4, D, *p.p*), with thin pitted walls, may be associated with cells of the medullary rays (Fig. 4, D, *mr*), the cells of which are very wavy in outline and contain starch granules: cortical parenchyma of thin-walled cells which are tangentially elongated (Fig. 4, D, *p*): starch abundant, simple or 2- to 4-compound, individual grains with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ in diameter (Fig. 3, E, *s*): calcium oxalate prisms of various shapes and sizes and up to 25 μ in maximum length (Fig. 3, E, *ox*), as described previously.

Discussion

The diagnostic characters of the bark of *Aspidosperma excelsum* are summarised.

1. Cork cells, reddish-brown, tangentially-elongated, of two types; the greater number of cells with thick, lignified outer and inner tangential walls; the smaller number of cells with thin unlignified or very slightly lignified walls, arranged in small groups amongst the thick-walled cells to form an interruptedly stratified tissue.
2. Phelloderm, mainly of thick-walled sclereids arranged in groups.
3. Latex canals of the cortex, very much tangentially elongated.
4. Sclereids arranged in from 5 to 7 more or less continuous tangential bands in the wide cortex; cells isodiametric or slightly tangentially-elongated, of various shapes and sizes, having thick stratified lignified walls; of two types; the greater number with very narrow lumen and with simple or branched pits, others with large lumen and well defined simple pits.
5. Phloem fibres, with thick stratified lignified walls, traversed by a few simple pits and surrounded by a calcium oxalate crystal sheath; of two types, the greater number, which are scattered throughout the phloem, with very small lumen, others, which are present toward the innermost region of phloem, with very large lumen.
6. Phloem parenchyma, with thin walls and compound pits.
7. Narrow medullary rays, the cells with thin, very wavy walls and containing starch granules.
8. Starch in all parenchymatous tissue, simple or 2- to 4-compound, individual grains with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ in diameter.
9. Calcium oxalate of square, rectangular and obliquely rectangular prisms or as small cubes of various sizes, measuring up to 25 μ in maximum length, associated with fibres, sclereid groups or bands, and in the phloem parenchyma.

The macroscopical and microscopical characters of the bark of *Aspidosperma quebracho-blanco* Schlecht., a drug formerly included in the U.S.P. and B.P.C., and described in the U.S. Dispensatory 1943[2], have been the subject of papers by Schlechtendal[3], Holmes[4], and Short[5]. In a previous communication, by two of the present authors, on "The Pharmacognosy of the Aspidosperms Barks of British Guiana"[1], the macroscopical and microscopical characters of *Aspidosperma ulei* Mgf.

have been described. These two barks, along with that of *Aspidosperma excelsum* Benth., possess many characters in common. Thus they all occur in thick, curved or channelled pieces, the abundant cork being furrowed and fissured externally and bearing epiphytic lichens, the inner surfaces are longitudinally striated; the odour is indistinct but the taste of each is bitter and aromatic. Histologically, each of the three barks possesses a sclerotic phelloderm and abundant isodiametric sclereids, 20 to 60 μ in diameter with thick walls and small lumen, arranged in masses in the cortex. The phloem contains sieve tubes with compound sieve plates upon the oblique end walls; the medullary rays are narrow; the scattered phloem fibres are mainly of large spindle-shaped cells with thick walls, traversed by simple or compound pits and with small lumen; each fibre is surrounded by a parenchymatous sheath of cells containing prismatic crystals of calcium oxalate. Similar calcium oxalate crystals are associated with the sclereid groups. Starch granules, simple and 4 to 7 to 15 μ in diameter or 2- to 4-compound are found in each of the three barks.

Additional characters common to the barks of both *A. excelsum* and *A. quebracho-blanco* are, the presence of one type of isodiametric sclereid in the phelloderm, arranged in groups (the second type, of tangentially elongated, large sclereids found in *A. ulei*, being absent), and the absence of fibres from the cortex and the presence in the phloem of two types of fibres possessing either narrow or wide lumen. (*A. ulei* bark has two types of fibre, each with narrow lumen and thick walls, the first type, which are few in number, unlignified or only slightly lignified and up to 1000 μ in length, are associated with the sclereidal groups of the inner cortex, those of the second type, lignified and up to 5370 μ in length, are scattered throughout the phloem either isolated or in groups of 2).

A character common to the barks of both *A. excelsum* and *A. ulei* is the presence of latex-containing canals in the cortex; these are the more tangentially elongated but of smaller diameter in vertical section in *A. excelsum* and for this reason are readily found in powders of that bark, whilst in the powdered bark of *A. ulei* the latex canals are much broken and are difficult to distinguish. No latex canals occur in the bark of *A. quebracho-blanco.*

The characters, described and illustrated above, which distinguish the bark of *A. excelsum* from those of *A. ulei* and *A. quebracho-blanco* are the reddish-brown colour of the outer surface; the fracture, which is short and laminated in the cortical region due to the arrangement of the cortical sclereids in tangential bands; cork cells not collapsed, of two types, the larger number with thick lignified tangential walls; presence of a second type of somewhat tangentially elongated cortical sclereids with large lumen; also a narrow phloem 40 per cent. of the bark in thickness free from stone cells and with straight medullary rays one to two cells in width; calcium oxalate crystals are the smallest, being up to 25 μ in length. In contrast the bark of *A. ulei* has a dark reddish-brown outer surface, is hard to break; cork cells are much collapsed, of one type only with thin walls; cortical sclereids with small lumen are arranged in

small groups which are relatively few in number; the phloem is 60 per cent. of the bark in thickness with a few small groups of sclereids in the outer region and with wavy medullary rays one to three cells in width; calcium oxalate up to 35 μ in length. Also in contrast the bark of *A. quebracho-blano* is very dark brown in colour with shortly fibrous fracture; cork cells not collapsed, thin-walled; cortical sclereids with small lumen, arranged in large groups which are very numerous; phloem wide, 80 per cent. of the bark in thickness, with abundant groups of sclereids throughout, often traversing the very wavy medullary rays which are up to four cells in width, calcium oxalate up to 30 μ in length.

Summary

1. The histology of the bark of *Aspidosperma excelsum* has been described and illustrated.

2. The diagnostic characters by which this bark may be identified and distinguished from those of *A. ulei* and *A. quebracho-blanco* are tabulated and discussed.

3. The dimensions of cork, phellogen, sclereids, cortical parenchyma, latex canals, phloem parenchyma, fibres, starch and calcium oxalate crystals are recorded.

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THE PHARMACOGNOSY OF THE ASPIDOSPERMA BARKS OF BRITISH GUIANA

PART III. THE MICROSCOPY OF THE BARK OF *Aspidosperma album* VAHL.

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IN a previous communication[1] the macroscopical characters of the bark of *Aspidosperma album* Vahl. have been described, and illustrated by means of photographs showing the outer and inner surfaces of a typical specimen. The present communication deals with the detailed histology of this bark as seen in sections, macerations and powder. The material used consisted of three samples of bark previously designated 2A, 2B and 2C collected in British Guiana in 1949, 1950 and 1954 respectively. Line drawings to illustrate the diagnostic characters of outer and inner surfaces of this bark and of tissue distribution as seen in smoothed transverse section are given in Figure 1, A, B and C.

Detailed Histology of the Bark of A. album (Figs. 1, 2, 3 and 4)

Cork consisting of some fifteen to thirty layers of rectangular to somewhat tangentially elongated, thin-walled, unlignified or very slightly lignified cells, polygonal in surface view (Fig. 1, D, ck, and Fig. 3, A, ck); R = 8 to **16** to 24 μ, T and H = 12 to **18** to 24 μ. Phellogen (Fig. 1, D, ph, and Fig. 3, A, ph) of one to two layers of thin-walled, rectangular to somewhat tangentially elongated cells; R = 8 to **14** to 20 μ, and T and H = 12 to **18** to 24 μ. Phelloderm (Fig. 1, D, phe, and Fig. 3, A, phe), a well-marked tissue of sclerenchymatous cells, R = 24 to **32** to 40 μ, T = 24 to **35** to 44 μ, H = 16 to **28** to 40 μ; arranged in a more or less continuous band of some four to eight layers of rectangular or slightly isodiametric cells, with small lumen, well-marked simple or branched pits traversing the thick, stratified and lignified walls; together with slightly tangentially-elongated thin-walled parenchymatous cells, R = 27 to **32** to 35 μ, T = 27 to **34** to 40 μ, H = 20 to **28** to 40 μ, some of which contain a single prism of calcium oxalate (Fig. 1, D, pa, and Fig. 3, A, pa). The cortex consists of thin-walled parenchyma together with abundant sclereids, these latter are arranged in a continuous band in the outer cortex (Fig. 1, E, b.sc, and Fig. 3, B, b.sc), and occur as small groups in the inner cortex (Fig. 2, A, g.sc); individual sclereids, R = 40 to **55** to 70 μ, T = 70 to **100** to 145 μ, H = 46 to **66** to 86 μ, isodiametric or tangentially elongated, of various shapes and sizes, with very narrow lumen, or occasionally with somewhat larger lumen, well-marked simple or branched pits traverse the thick, stratified and lignified walls; cortical parenchyma with large intercellular spaces, individual cells R = 32 to **50** to 70 μ, T = 32 to **55** to 70 μ and H = 32 to **44** to 55 μ, thin-walled, very much

Aspidosperma album

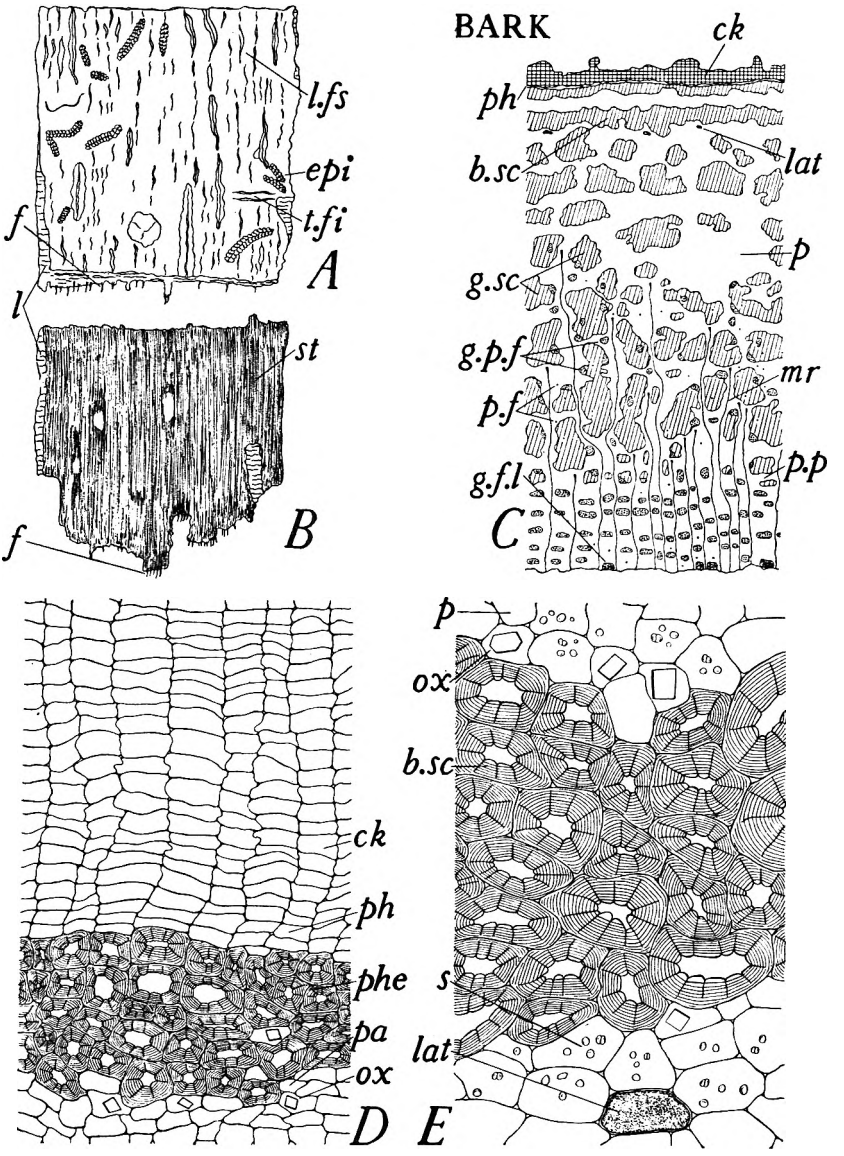

Fig. 1. *Aspidosperma album* bark, macroscopical characters and T.S.:—A, outer surface x ¼; B, inner surface x ¼; C, smoothed T.S. x 10; D, cork, phellogen and phelloderm; E, cortex; D and E, x 200; b.sc, band of sclereids; ck, cork; epi, epiphyte; f, fibre; g.f.l, group of phloem fibres with large lumen; g.p.f, group of phloem fibres with narrow lumen; g.sc, group of sclereids; l, lamination; lat, latex canal; l.fs, longitudinal furrow; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; pa, cortical parenchyma found associated with phellodermic sclereids; ph, phellogen; phe, phellodermic sclereids; p.f., isolated phloem fibre with narrow lumen; p.p, phloem parenchyma; s, starch; st, longitudinal striation; t.fi, transverse fissure.

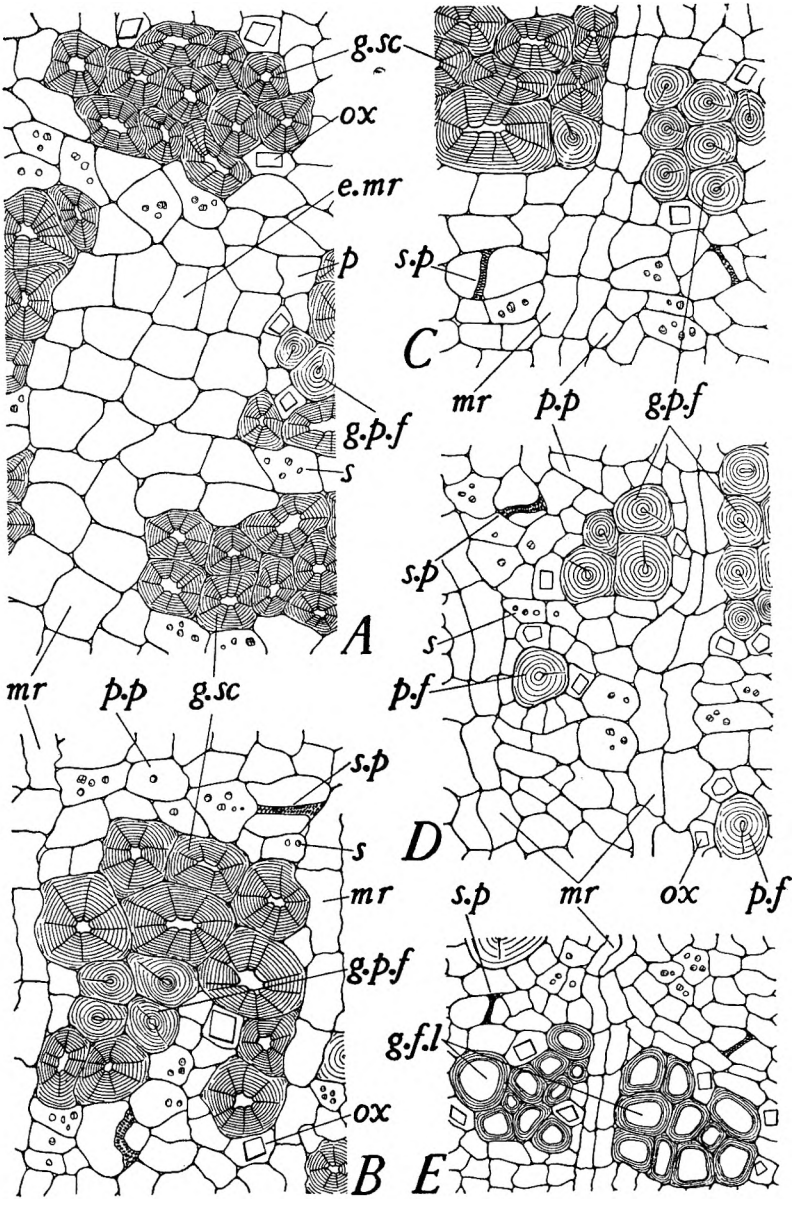

Fig. 2. *Aspidosperma album* bark in T.S.:—A, inner cortex; B, outer phloem; C, phloem; D, inner phloem; E, innermost phloem; all x 200; e.mr, end of medullary ray; g.f.l, group of phloem fibres with large lumen; g.p.f, group of phloem fibres with narrow lumen; g.sc, group of sclereids; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; p.f, isolated phloem fibre with narrow lumen; p.p, phloem parenchyma; s, starch; s.p, sieve plate.

tangentially elongated and containing starch granules (Fig. 1, E, p, Fig. 2, A, p, and Fig. 3, A, B and C, p). Much longitudinally-elongated latex canals, R and T = 32 to **44** to 55 μ, H = 98 to 300 μ, lying parallel to the vertical axis or very slightly obliquely thereto, are found associated with the inner boundary of the cortical band of sclereids and also in the cortical parenchyma (Fig. 1, E, lat, and Fig. 3, C, lat); the latex, which is very finely granular in appearance, is stained yellow with iodine solution. No defined endodermis or pericycle are distinguishable, but in this region discontinuous groups of thick-walled sclereids occur, resembling those of the cortex, but also containing, at times, axially elongated, thick-walled fibres, either singly or, more frequently, in groups of two to six fibres, which are identical with those occurring throughout the phloem and to be described below.

Phloem, which is up to 70 per cent. of the thickness of the bark, consists of sieve tissue, phloem parenchyma, phloem fibres, medullary rays and sclereids, and can be subdivided into four regions. In the outermost region groups of stone cells are present, some being associated with groups of fibres; in the second region, the groups of fibres are more or less surrounded by the sclereid groups; the third region is characterised by the presence of fibres either isolated or in groups and by the absence of sclereids; in the innermost region of phloem, fibres, both isolated and in groups, are found together with a few groups of a second type of fibre with very large lumen. Sieve tubes may be distinguished with oblique, compound sieve plates on the end walls (Fig. 2, B, C, D and E, s.p, and Fig. 4, A, s.p). Phloem parenchyma with large intercellular spaces, of thin-walled cells, having compound pits on the vertical walls and containing starch granules, R = 16 to **24** to 32 μ, T = 32 to **42** to 55 μ, and H = 48 to **70** to 90 μ (Figs. 2, 3 and 4, p.p). Phloem fibres, R and T = 25 to **32** to 40 μ, H = 800 to **1750** to 2600 μ, of two types; the greater number, either isolated (Fig. 2, p.f) or in groups (Figs. 2, 3 and 4, g.p.f) scattered throughout the phloem, are spindle shaped, with bluntly pointed ends, walls thick, lignified, stratified, traversed by a few simple pits, along which splitting may have occurred, lumen very narrow; crystal sheath surrounding the isolated phloem fibres and groups of fibres, except when these are embedded within groups of sclereids (Figs. 2, 3 and 4). A smaller number of fibres, occurring in groups towards the innermost region of the phloem, differ from those described above in the presence of walls which are somewhat thinner, the large lumen being 8 to **20** to 32 μ in diameter, crystal sheath surrounding some groups of these fibres but not always present. Medullary rays (Fig. 2, A, B, C, D and E, mr, and Fig. 3, E, mr) very wavy, due to displacement by groups of sclereids and fibres, one to two cells in width but becoming up to five cells wide (Fig. 2, A, e.mr) towards the periphery of the phloem, 15 to 20 cells in height, the cells, R = 40 to **62** to 86 μ, T = 20 to **25** to 32 μ, and H = 16 to **28** to 35 μ, straight to somewhat wavy in outline and containing starch granules.

Starch abundant in the cortical and phloem parenchyma, simple or 2- to 4- compound; individual granules with eccentric hilum, spherical, ovoid or plano-convex and up to 28 μ in diameter (Figs. 1, 2, 3 and 4, s).

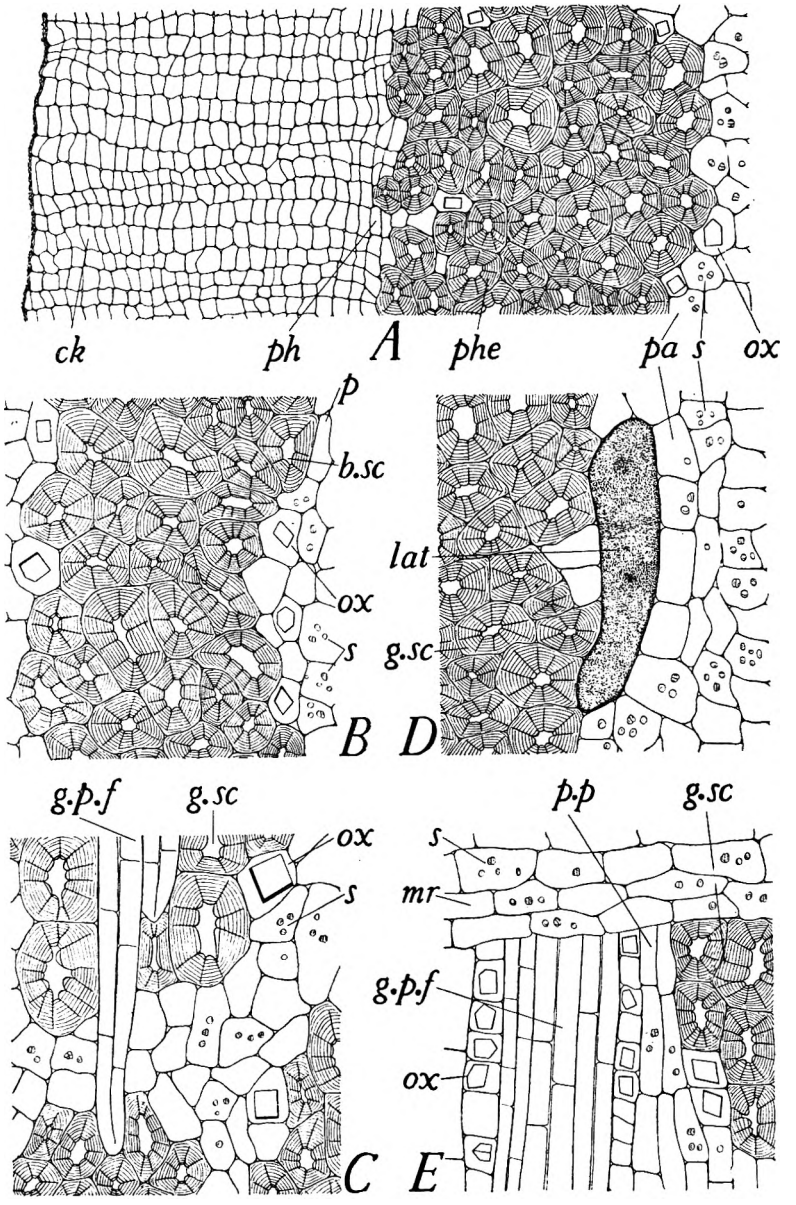

Fig. 3. *Aspidosperma album* bark in L.S.:—A, cork, phellogen, phelloderm and outer cortex; B, outer cortex; C, outer phloem; D, cortex; E, inner phloem; all x 200; b.sc, band of sclereids; ck, cork; g.p.f, group of phloem fibres with narrow lumen; g.sc, group of sclereids; lat, latex canal; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; pa, cortical parenchyma found associated with phellodermic sclereids; ph, phellogen; phe, phellodermic sclereids; p.p, phloem parenchyma; s, starch.

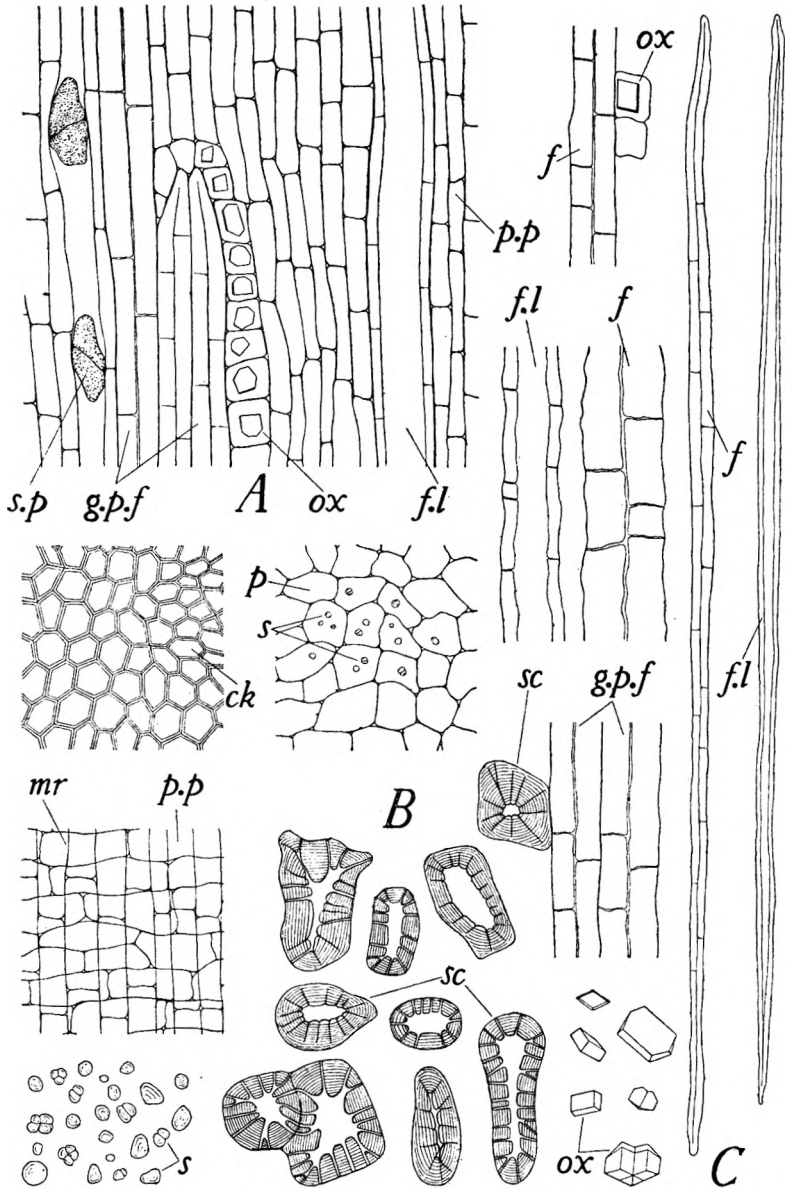

Fig. 4. *Aspidosperma album* bark in L.S., powder and macerate:—A, innermost phloem x 200; B, various components as seen in powder x 200; C, fibres, isolated by maceration x 50; ck, cork; f, phloem fibres with narrow lumen; f.l, phloem fibre with large lumen; g.p.f, group of phloem fibres with narrow lumen; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; p.p, phloem parenchyma; s, starch; sc, sclereid; s.p, sieve plate.

Calcium oxalate, as square, rectangular or obliquely rectangular prisms or small cubes of various sizes, measuring up to 32 μ, associated with the groups of sclereids and as a sheath around both types of fibres; there is no relationship between the shapes of the crystals and the region of the bark in which they occur (Figs. 1, 2, 3 and 4, ox).

Powder. Fawn in colour: cork cells polygonal in surface view and slightly reddish-brown in colour (Fig. 4, B, ck): sclereids of various shapes and sizes, with thick, stratified walls, either with narrow lumen or with a large lumen, having simple or branched pits (Fig. 4, B, sc): phloem fibres usually somewhat broken, isolated or in groups of two to three fibres, of two types, the greater number with very narrow lumen and frequently surrounded by a sheath of thin-walled parenchymatous cells, each containing a single prismatic crystal of calcium oxalate, a number of fibres are associated with sclereids, fewer fibres with large lumen either with or without prism-crystal sheath; both types of fibres with thick, stratified lignified walls, traversed by a few simple pits (Fig. 4, B, f, f.l and g.p.f): phloem parenchyma (Fig. 4, B, p.p) with thin walls, may be associated with cells of the medullary rays (Fig. 4, B, mr), the cells of which are somewhat wavy in outline and contain starch granules: cortical parenchyma of thin-walled, tangentially elongated cells containing starch granules (Fig. 4, B, p): starch abundant, simple or 2- to 4- compound, individual grains with eccentric hilum, spherical, ovoid or plano-convex and up to 28 μ in diameter (Fig. 4, B, s): calcium oxalate prisms of various shapes and sizes and up to 32 μ in maximum length (Fig. 4, B, ox), as described previously.

Discussion

The diagnostic characters of the bark of *Aspidosperma album* are:—

1. Cork cells, pale reddish-brown and rectangular to somewhat tangentially elongated, thin-walled, unlignified or very slightly lignified.

2. Phelloderm mainly sclerotic, of an irregular band of sclereids three to eight layers in radial thickness.

3. Latex canals of the cortex, very much longitudinally elongated.

4. Sclereids arranged in one, more or less continuous tangential band in the outer cortex, and in groups in the inner cortex and outer phloem, having thick, stratified walls, with simple or branched pits and with narrow or large lumen.

5. Phloem fibres, isolated or in groups of two to fifteen fibres, with thick stratified lignified walls traversed by a few simple pits and with or without surrounding crystal sheath; of two types, the greater number, which are scattered throughout the phloem, with very small lumen, others, which are present towards the innermost region of phloem, always in groups, with very large lumen.

6. Phloem parenchyma, with thin walls and compound pits.

7. Medullary rays, narrow, wavy, the cells with thin, somewhat wavy walls and containing starch granules.

8. Starch in all parenchymatous tissue, simple or 2- to 4- compound,

individual grains with eccentric hilum, spherical, ovoid or plano-convex and up to 28 μ in diameter.

9. Calcium oxalate as square, rectangular and obliquely rectangular prisms or as small cubes of various sizes, measuring up to 32 μ in maximum length, associated with fibres, sclereid groups or bands, and cortical and phloem parenchyma.

In previous communications[1,2], the macroscopical and microscopical characters of the barks of *Aspidosperma ulei* Mgf. and *Aspidosperma excelsum* Benth. have been described and compared with those of the bark of *Aspidosperma quebracho-blanco* Schlecht. The bark of *Aspidosperma album* Vahl. possesses many characters in common with these three barks. Thus it occurs in thick, curved or channelled pieces, the abundant cork being furrowed and fissured externally and bearing epiphytic lichens or liverworts, the inner surface is longitudinally striated; the odour is indistinct but the taste is bitter and aromatic. Histologically, each of the four barks possesses a sclerotic phelloderm and abundant isodiametric sclereids, 20 to 60 μ in diameter with thick walls and small lumen, arranged in masses in the cortex. The phloem contains sieve tubes with compound sieve plates upon the oblique end walls; the medullary rays are narrow; the scattered phloem fibres are mainly of large spindle-shaped cells with thick walls, traversed by simple or compound pits and with small lumen; each fibre is surrounded by a parenchymatous sheath of cells containing prismatic crystals of calcium oxalate. Similar calcium oxalate crystals are associated with the sclereid groups. Starch granules, simple or 2- to 4- compound and up to 28 μ in diameter, are found in each of the four barks.

A character common to the three barks *A. album*, *A. excelsum* and *A. ulei* is the presence of latex-containing canals in the cortex; these are much longitudinally elongated, running slightly obliquely in *A. album*, more tangentially elongated in *A. excelsum*, and slightly tangentially elongated or somewhat isodiametric in *A. ulei*, these latex canals can only be seen in the powders of the bark of *A. excelsum*. No latex canals occur in the bark of *A. quebracho-blanco*.

The barks of *A. album* and *A. quebracho-blanco* agree in the presence of one type of cork cells which are non-lignified, thin walled and are not collapsed; also in the presence of abundant groups of sclereids in all parts of the phloem except the innermost region. These two barks and that of *A. excelsum* are free from fibres in the cortex but possess phloem fibres of two distinct types; *A. album* differs however in that the narrow-cavitied fibres are usually arranged in groups whilst those with wide cavities always occur in groups. The bark of *A. album* may also be distinguished by the isodiametric sclereids of the phelloderm arranged in a continuous band and by the intermingling of two types of cortical sclereids in one continuous band and in scattered groups.

Summary

1. The histology of the bark of *Aspidosperma album* has been described and illustrated.

2. The diagnostic characters by which this bark may be identified and distinguished from those of *A. ulei*, *A. excelsum* and *A. quebracho-blanco* are tabulated and discussed.

3. The dimensions of cork, phellogen, sclereids, cortical parenchyma, latex canals, phloem parenchyma, fibres, starch and calcium oxalate crystals are recorded.

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DISCUSSION

The papers were presented together by J. D. KULKARNI.

MR. G. R. A. SHORT (London) congratulated Mr. Kulkarni on his excellent drawings. He heard today that these barks had active principles with a rauwolfia action. Dr. Rowson, commenting recently on a paper that he (Mr. Short) had written some years ago on aspidosperma, had told him that he and the other authors today agreed with what he had then said. The paper was written in 1926, and was the outcome of a little controversy with the late Mr. E. M. Holmes on the nomenclature of *Aspidosperma quebracho-blanco*. Mr. Holmes had a sample sent to him from Central America which had a rose-pink inner bark. He considered that this was not *Aspidosperma quebracho-blanco*, because it was not white, but *A. quebracho-colorado*; but this was not correct, because that name was given to a bark in another family. He had endeavoured to explain this in his paper, but felt sure that he had not convinced Mr. Holmes. He wondered whether any further species were to be examined. He understood that there were 65 species of aspidosperma. Could the authors give any explanation of the curious colours of the inner bark of *A. quebracho-blanco*? Those he had seen varied from cream through rose and red, and some samples were clove brown. Was this clove brown due to tannin?

DR. F. FISH (Glasgow) dealing with the first paper commented on the variation in size of the latex canals illustrated. He thought it rare for a drug to contain isolated fibres with a prismatic crystal sheath and if this was unique, as he thought, it was worth special mention as a diagnostic character. The phloem and medullary ray parenchyma contained starch granules, although this was not shown in the paper. Nor was there any indication of the striations on the starch granules. The maximum size of starch granules from the three species thus far examined was quoted as 15 mμ. In the second paper, with four species, the whole lot were grouped with a maximum of 28 mμ. If there was such a difference between the *A. album* and the other three barks, it might have been used as a distinguishing feature. He also asked whether it was necessary to submit the two papers separately. It might have been better, as had been done in the delivery that morning, to put them together, because there was some repetition in the summary of the second.

MR. KULKARNI, in reply, said that the colours of the barks varied considerably. They had examined Mr. Short's sample of 1926 and found it to be exactly the same as those which they had received in 1949, 1950 and 1954. There were 52 species in this genus, and they had worked on only six of them. *A. quebracho-blanco* was well known; *A. ulei* had been described in Part I; *A. excelsum* and *A. album* had been described in the present papers, and *A. megalocarpon* and *A. oblongum* were being investigated. The sample collected by Mr. Holmes was in the Society's museum, and he had examined it. After cutting a few transverse sections, he found that it was a different bark, because it had striated cork and did not compare at all with either Mr. Short's sample or their own recent samples collected from British Guiana.

DR. ROWSON, also replying to the discussion, said that at present they could not offer any explanation of the wide variation in colour of the inner bark of *A. quebracho-blanco*. He agreed with Dr. Fish that they should have described the starch striations and he accepted his criticism of the references to the size of the granules. He would add one comment on the drawings. It would be realised that the latex canals did not run perfectly vertically in one specimen or perfectly horizontally in the other. Some had been isolated and true transverse sections shown.

STUDIES IN THE GENUS DIGITALIS

PART III. THE EXTRACTION AND EVALUATION OF *Digitalis purpurea* LEAF

BY J. M. ROWSON

From the Museum of the Pharmaceutical Society of Great Britain

Received July 8, 1955

A RECOMMENDED process for the estimation of powdered digitalis leaf or of digitoxin by means of 3:5-dinitrobenzoic acid has been described in two previous papers of this series[1,2]. This present communication reports more detailed investigations of the precise conditions under which the dinitrobenzoic acid process will yield accurate and reproducible results: the preparation of leaf extracts by different periods of maceration in 70 per cent. ethanol has also been studied and the results obtained when powdered leaf samples of *Digitalis purpurea* were examined by biological methods and by the chemical process of estimation are compared.

EXPERIMENTAL

The recommended process for chemical estimation, using the 25 mg. level of Pb for decolorisation, has been employed throughout the present work[1]. Biological estimations were carried out by Dr. F. J. Dyer, using the intravenous guinea-pig method already described[2].

Sodium Hydroxide Concentration

A purple colour is produced when a mixture of 3:5-dinitrobenzoic acid and either digitoxin or decolorised tincture of digitalis, in the presence of dilute ethanol, is rendered alkaline with N sodium hydroxide; 1 ml. of alkali in 10 ml. of reaction mixture being employed. The theoretical equivalent of the dinitrobenzoic acid used is 1 ml. of 0·19N sodium hydroxide, thus a large excess of alkali is present throughout the reaction. To investigate the influence of this excess of alkali on the course of the reaction, a series of experiments was made in which 1 ml. quantities of sodium hydroxide solutions varying in strength from 0·6 to 3N were employed for the estimation of fixed quantities of both digitoxin and a decolorised tincture of digitalis. Results are expressed in Table I. The colour densities of a fixed concentration of glycosides are seen to increase considerably as the concentration of alkali in the reaction mixture is raised and a corresponding acceleration in the development of the maximum colour is observed. The change in apparent k (1 mg.) value for digitoxin with variation in alkali concentration was linear over the range 0·8 to 2N; below 0·8N the rate of change was still more pronounced, but between 2 and 3N it was less accentuated. Similar results were found for the decolorised tincture although the change at 0·6N and the increase from 2 to 2·5N were both less pronounced than for digitoxin. The time required for maximum colour development in both series of tests was inconveniently long at low concentrations of alkali but at concentrations

greater than 2N this time was too short to allow for accurate spectrophotometric measurement. Within the range of alkali concentration 0·8 to 1·8N there is little obvious advantage at any particular level and the previously recommended concentration of N sodium hydroxide is confirmed as suitable, but this reagent must be adjusted to strength with analytical accuracy.

TABLE I

3 : 5-DINITROBENZOIC ACID REACTION
VARIATION IN CONCENTRATION OF ADDED NaOH SOLUTION (1 ml.)

NaOH solution	Digitoxin		Leaf tincture (decolorised)	
	k (1 mg.)	Time of maximum colour development	k (1 ml.)	Time of maximum colour development
3·0N	1·071	2 minutes		
2·5N			0·456	3 minutes
2·0N	1·040	6 ,,	0·452	3 ,,
1·8N			0·450	5 ,,
1·4N			0·420	6 ,,
1·3N	0·870	9 ,,		
1·2N	0·832	9 ,,	0·375	7 ,,
1·1N	0·821	12 ,,		
1·0N	0·775	12 ,,	0·360	8 ,,
0·9N	0·743	16 ,,		
0·8N	0·718	17 ,,	0·330	8 ,,
0·6N	0·446	22 ,,	0·234	13 ,,

3 : 5-*Dinitrobenzoic Acid Reagent*

An ethanolic solution of this reagent, mixed with a dilute ethanolic solution of the glycosides, is immediately rendered alkaline in the recommended process for estimation. Some preliminary experiments have been made to investigate the possible influence upon the colour intensity of increase in time of interaction between the dinitrobenzoic acid and the glycosides before rendering alkaline. Using fixed quantities of both digitoxin and decolorised tincture this period of reaction was varied between half a minute and 1 hour, after which sodium hydroxide was added and maximum colour determined by the normal process of estimation. No differences in results were found and it was concluded that sodium hydroxide may conveniently be added immediately after shaking together the dinitrobenzoic acid reagent and the glycosides. It has also been shown that the ethanolic solution of dinitrobenzoic acid is stable for periods up to 7 days if stored in the dark.

TABLE II

VARIATION IN CONCENTRATION OF 3 : 5-DINITROBENZOIC ACID

Dinitrobenzoic acid		Digitoxin k (1 mg.)
2 Per cent. reagent, ml.	Concentration in reaction mixture	
0·5	0·1 per cent.	0·395
1·0	0·2 ,, ,,	0·519
1·5	0·3 ,, ,,	0·629
2·0	0·4 ,, ,,	0·770
2·5	0·5 ,, ,,	0·870
3·0	0·6 ,, ,,	0·878
5·0	1·0 ,, ,,	0·934

No chemical explanation for the colour produced in this reaction has as yet been proved and hence the chemical equivalence between digitoxin and dinitrobenzoic acid is not known, although from the disparity in amounts taken in the reaction mixture it is probable that a very large excess of dinitrobenzoic acid is present. The influence of varying the

concentration of dinitrobenzoic acid used in the colorimetric estimation of the same weight of digitoxin has been investigated by employing different quantities of a 2 per cent. solution of this reagent in ethanol, additional ethanol being added to give 9 ml. of reaction mixture before making alkaline. The same amount of sodium hydroxide has been used in each reaction and no attempt was made to maintain the same excess of alkali in each estimation. Apparent k (1 mg.) values are set out in Table II. The results show that there is a linear increase in colour density for the same weight of digitoxin, with increase in dinitrobenzoic acid content of reaction mixture over the range 0·1 to 0·5 per cent. (0·5 to 2·5 ml. of 2 per cent. reagent solution); the increase is less marked for higher levels of dinitrobenzoic acid concentration. Variation in the accuracy of preparing this reagent solution would thus be somewhat less marked at such higher concentrations but at any concentration investigated in this work it is essential that the solution be prepared by accurate weighing. Under these conditions the previously used level of 0·4 per cent. dinitrobenzoic acid in the reaction mixture (i.e., 2 ml. of 2 per cent. solution or 5 ml. of 0·8 per cent. solution) has been considered to be satisfactory.

TABLE III

TEMPERATURE OF 3:5-DINITROBENZOIC ACID REACTION

Temperature	Time of maximum colour development	Digitoxin k (1 mg.)	Decolorised tincture k (1 ml.)
5° C.	14 minutes	1·030	0·462
10°	14 "	0·919	0·449
15°	10 "	0·845	0·399
20°	7 "	0·778	0·355
25°	5 "	0·736	0·316
30°	2 "	0·580	0·282

Temperature of Reaction

In order to investigate the influence of temperature upon maximum colour density and time of its development in the recommended process of estimation, a temperature-controlled room was employed and the reaction was carried out at temperatures ranging from 5° to 30° C., using both digitoxin and decolorised tincture. The spectrophotometer and all reagents were allowed to stand for one hour at each temperature before carrying out the estimation. Results are given in Table III. It was found that the colour density for the same weight of glycoside decreased in linear proportion with increase in temperature throughout the temperature range. The development of maximum colour was much speeded up at higher temperatures but colour density values, measured at a fixed time interval of 5 minutes after making alkaline, were found to vary for reactions proceeding at different temperatures. The present results, taken at the time of maximum development, are considered to be the correct indication of the behaviour of the reaction at different temperatures. Because of the rapid colour development at 30° C. the k (1 mg.) value for digitoxin is low in relation to the behaviour of the reaction at lower temperatures.

Leaf Extraction

Earlier work has been based upon 1 in 10 tinctures prepared from powdered digitalis leaf using 70 per cent. ethanol and macerating with gentle agitation for a period of 48 hours[2]. A preliminary investigation, using the dinitrobenzoic acid method of estimation, was made to examine the efficacy of 70 per cent. ethanol as an extraction solvent when the periods of maceration were varied. In each experiment 1 in 10 tinctures were prepared by maceration with gentle agitation, employing a powdered leaf sample containing 11·1 I.U./g. of activity, and using the mechanical device previously described[3]. This consists of a suitably mounted cycle wheel carrying wooden racks on which 60 ml. bottles may be clamped, driven through a chain of gears by a low-speed electric motor fitted with a variable resistance. Macerations were carried out for periods of 72, 48, 42, 36, 30, 24, 18, 12 and 6 hours, also for 5, 4, 3 2, and 1 hour; finally, periods of maceration of 50 minutes, 40 minutes, 30 minutes, 20 minutes and 10 minutes were examined. Estimations of these tinctures showed no significant differences between those prepared by 40 minutes and by 72-hour macerations and concordance of duplicates was always obtained.

TABLE IV

EXTRACTION OF LEAF SAMPLES
3 : 5-DINITROBENZOIC ACID ESTIMATION

Activity compared with Standard Preparation of powdered digitalis

Leaf sample	Equivalent I.U./g.		Leaf sample	Equivalent I.U./g.	
	1-hr. maceration	48-hr. maceration		1-hr. maceration	48-hr. maceration
L.S.	13·2	13·8	33	12·2	12·5
2	13·3	13·3	101	10·3	10·3
5	14·3	15·6	102	10·0	10·1
7	13·5	13·2	103	9·0	9·0
8	12·4	12·4	104	9·7	9·8
26	10·3	11·3	140	11·1	11·0

Values for 30 minute, 20 minute and 10 minute macerations were somewhat more variable between duplicates but their averages showed the presence of 1·02 I.U./ml. of activity. A tincture prepared by maceration for one hour without agitation was shown to contain 1·07 I.U./ml. of activity, which is only slightly below the mean value for all estimations upon tinctures prepared by agitation.

The above results were considered to indicate that a 1-hour period of maceration was as efficient as the 48-hour period prescribed by the British Pharmacopœia for the preparation of Tincture of Digitalis. This was more fully investigated by preparing tinctures from 12 different leaf samples by both the 1-hour and the 48-hour maceration processes and these were estimated chemically, the results being given in Table IV and in Figure 1. Further confirmation was established by means of both chemical and biological methods of estimation applied to 1-hour and 48-hour tinctures prepared from 6 further leaf samples. The results are given in Table V and in Figure 2.

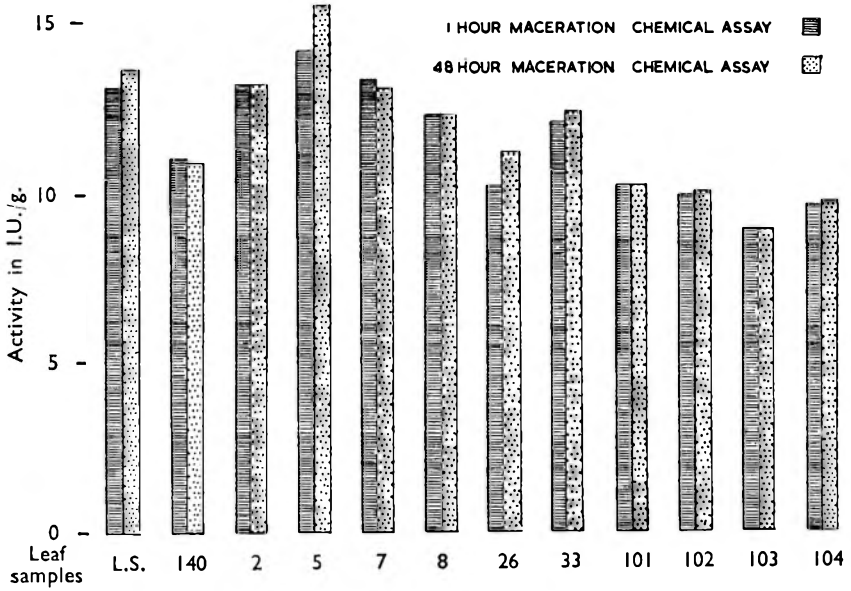

FIG. 1. Leaf extraction with 70 per cent. ethanol. 1 hour and 48 hour maceration.

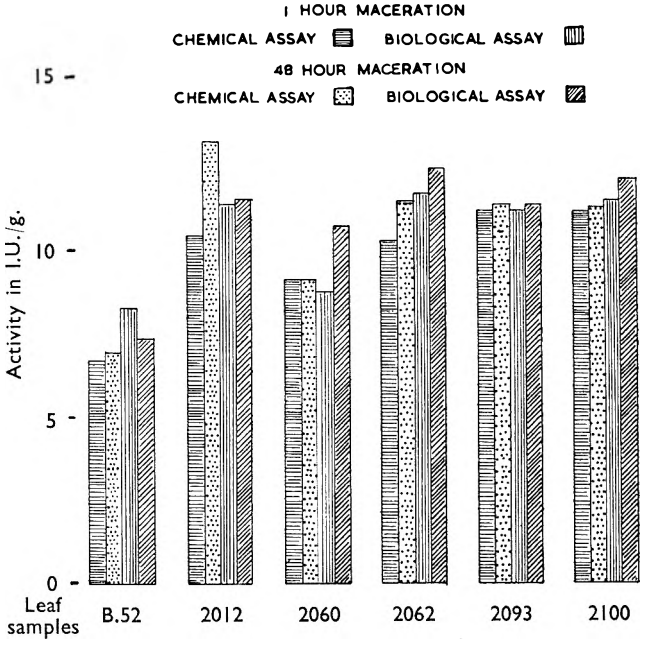

FIG. 2. Leaf extraction with 70 per cent. ethanol. 1 hour and 48 hour maceration.

TABLE V

EXTRACTION OF LEAF SAMPLES
1-hour and 48-hour maceration
Activity by chemical and biological estimations

Method of estimation	Chemical, equivalent I.U./g.		Biological, I.U./g.	
Period of maceration	1 hour	48 hour	1 hour	48 hour
Leaf B.52	6·7	6·9	8·2	7·3
2012	10·4	13·2	11·3	11·5
2060	9·1	9·1	8·7	10·7
2062	10·2	11·4	11·6	12·4
2093	11·1	11·3	11·1	11·3
2100	11·1	11·2	11·4	12·0

Application of Method

Using the 1-hour maceration process for leaf extraction, a number of samples have been investigated by the chemical method outlined in this paper and by the intravenous guinea-pig method in order to determine the comparability of results obtained by the two processes of evaluation. The results are given in Table VI. The leaf samples in column 1 were cultivated on four separate sites and were collected, dried and stored under controlled conditions. Leaf samples in column 4 were commercial specimens: the tincture recorded in this column was prepared from a leaf sample grown, collected and dried under controlled conditions and the purpose of that experiment was to show the behaviour of the tincture after standing for a period of 5 days from the date of its preparation.

TABLE VI

LEAF ESTIMATIONS
(1-hour maceration)
CHEMICAL AND BIOLOGICAL METHODS

Leaf sample	Method of estimation		Leaf sample	Method of estimation	
	Chemical, equivalent I.U./g.	Biological, I.U./g.		Chemical, equivalent I.U./g.	Biological, I.U./g.
A.2026	14·3	13·9	MS.31	15·9	14·6
A.2100	8·3	8·6	,, 32	14·9	15·4
B.2005	10·6	12·2	,, 33	15·1	14·1
B.2063	8·0	9·4	,, 41	16·9	15·4
M.2064	14·7	16·2	,, 42	15·1	14·5
M.2150	9·4	10·7	Tincture (fresh)	1·38	1·19
S.2005	10·0	12·9	,, (5 days)	1·35	1·12
S.2150	14·8	16·7			

DISCUSSION

The present detailed investigation of the behaviour of the dinitrobenzoic acid reaction for the estimation of digitoxin or of decolorised tinctures of digitalis has shown that the method is efficient and accurate when the conditions of its application are fully controlled. The concentration of the dinitrobenzoic acid reagent influences the colour density produced by a given weight of glycoside and in consequence the reagent must be accurately prepared. If stored in the dark the reagent may be

used for 7 days after its preparation. Despite its presence in great excess, the concentration of sodium hydroxide in the reaction mixture also influences the intensity of colour produced by the same weight of glycosides, other conditions in the reaction being the same, and hence the amount of alkali employed must be carefully controlled. Moreover, the rate of reaction is increased at higher temperatures but the intensity of maximum colour produced is decreased with increase of temperature. It is recommended that a temperature of 20° C. be employed for all estimations of digitoxin or of decolorised tincture of digitalis by the dinitrobenzoic acid process and that the exact concentrations and quantities of reagents be those previously described[1].

The extraction studies of powdered leaf using 10 volumes of 70 per cent. ethanol as solvent have demonstrated that a 1-hour period of maceration with gentle agitation is sufficient to extract the total glycosides. This efficiency of extraction has been shown by both chemical and biological methods of estimation upon a considerable number of leaf samples and it is recommended that the 1-hour maceration process be employed for future assay work.

The figures presented in Table VI are a further confirmation of the fact that the dinitrobenzoic acid method of estimation yields results which are similar to those given by the biological method of estimation when applied to leaves that have been cultivated, collected and dried under controlled conditions, also when applied to a further number of commercial samples.

The examination of a tincture immediately after preparation by 1-hour maceration and again after standing in the laboratory at normal temperature for a period of 5 days has shown that there is little variation in tincture potency during this period. The detailed investigation of changes in potency in digitalis tincture will be reported in a later paper. This present work demonstrates, however, a method which may be employed to ensure the availability of Tincture of Digitalis of standard potency for dispensing purposes. Small quantities of this tincture may be prepared extemporaneously in the pharmacy from standardised powdered digitalis leaf using the 1-hour maceration process, and this material will retain its potency at least for a period of 5 days after preparation.

Summary and Conclusions

1. The 3 : 5-dinitrobenzoic acid process for the estimation of digitoxin or of decolorised tinctures of digitalis should be carried out at a controlled temperature; 20° C. is convenient.

2. The reagents employed in the reaction should be standardised analytically.

3. Powdered digitalis leaf is completely extracted by maceration with gentle agitation for a period of 1 hour using 10 volumes of 70 per cent. ethanol.

4. A parallelism between the results obtained by both the 3 : 5-dinitrobenzoic acid and the biological methods of estimation has been

demonstrated, using 14 leaf samples of *Digitalis purpurea* cultivated and dried under controlled conditions and also using 5 commercial leaf samples.

5. Tincture of digitalis prepared by 1-hour maceration has been shown to retain its potency for at least 5 days and it is recommended that such a preparation be made extemporaneously from standardised leaf powder when tincture of digitalis is prescribed.

The author wishes to acknowledge his deep indebtedness to Dr. F. J. Dyer for the biological assay work reported in this paper. His thanks are also due to the Agricultural Research Council for a grant towards the erection of a greenhouse-drying shed employed in the processing of a number of the leaf samples investigated.

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STUDIES IN THE GENUS DIGITALIS

PART IV. THE INFLUENCE OF FERTILISERS AND OF LIME ON *D. purpurea*

BY J. M. ROWSON

From the Museum of the Pharmaceutical Society of Great Britain

Received June 23, 1955

THE influence of fertilisers upon the growing plants of *Digitalis purpurea* has been investigated by a number of workers and a number of contradictory results have been reported. The larger number of these experiments have been field trials, directed to an increase in the weight of leaf produced by unit field area: fewer experiments have been concerned with the influence of fertilisers upon the therapeutic potency of the leaf produced, as indicated by biological assay or by glycosidal content. Sand culture or water culture investigations of this species are very few.

An unfavourable influence upon weight of crop by stable manure has been shown by Boshart[1], whereas Dauphinée[2] found cow manure to be the best fertiliser for increasing leaf yield. Ammonium sulphate was considered to be very useful by Boshart, but Dauphinée did not find it outstandingly valuable, although it was better than no fertiliser at all in its influence upon crop yields: Mascré[3] showed that an optimum level of treatment with ammonium sulphate existed, which should not be exceeded. Koch and Butler[4] found that, on a clay loam, the best results were obtained by using a mixed fertiliser containing sodium nitrate as the only source of nitrogen and they considered this salt to be the most essential single fertiliser. The same workers found calcium acid phosphate to be the most important single fertiliser for increased leaf yield when the plants were grown in sand. Mascré considered potassium to exert the most effect upon leaf yield, and phosphates also gave good increases; Boshart agreed that both potassium and phosphate exerted a favourable influence upon leaf yield but he considered that influence to be less marked.

Dauphinée, using the cat and guinea-pig methods of assay, concluded that plants treated with cow manure were of the highest quality; whilst Boshart found that stable manure had an unfavourable influence upon activity of the leaves. Parisis[5] showed that the most active galenicals were obtained from *D. purpurea* plants grown in soils treated with a mixed fertiliser containing nitrate, phosphate, calcium and magnesium ions but Dauphinée has reported that superphosphates appeared to depress leaf activity. It is unsafe to expect a parallelism in behaviour to fertilisers between co-generic species but in passing it may be noted that Court and Allemann[6] found a decrease in activity in leaves of *D. lanata* when plants were treated with stable manure, whereas artificial fertilisers, especially those containing nitrogen, produced an increased activity in leaves of this species.

Digitalis purpurea occurs naturally on acid soils[7], it is regarded as a marked calcifuge[8] and is seldom found in the wild state on limestone or

chalky soils. Siegfried[9] commented on this fact and found that the species could nevertheless be cultivated on calcareous soils. Boshart grew these plants near Munich in a soil naturally containing 0·5 to 1·0 per cent. of chalk, it was necessary to raise the plants in a seedbed and then transplant, after which they grew well. The complete fertiliser used by Koch and Butler for optimum crop yields on clay loam contained over 50 per cent. of chalk and was applied at a level of 1000 lb. chalk per acre ($3\frac{1}{3}$ oz. per square yard). James[10], reporting on drug cultivation in America, has stated that the lime-deficient soils of New England are dressed with lime before digitalis cultivation, which results in an increased crop yield. Sanna[11] found that this species would tolerate soils containing 5 to 6 per cent. of calcite but even such a small proportion of calcium resulted in a decrease in glycosidal content of the leaves. Court and Allemann state that *D. lanata* occurs naturally in Switzerland on lime-containing dolomitic soil but that when such plants were cultivated on light, humus-containing soils, poor in lime, the crop yield and activity were much higher. Duquénois[12] planted out seedlings of *D. purpurea* into infertile calcareous garden soil, *p*H 7·2 to 7·3, in Strasbourg and 2nd year leaves were collected for assay by the guinea-pig method. Control plants were taken from their natural sites on the eastern slopes of the Vosges and the potencies of both wild and experimental materials were found to be almost identical in 1948 and again in 1950, although the crop yield from the calcareous soil was low.

Such a conflict of views upon the influence of artificial fertilisers and of lime upon leaf yield and the limited number of results dealing with their influence upon leaf potency have led to the present investigation.

Experimental

All cultivation experiments have been carried out in the Museum experimental grounds, Birdsgrove House, Mayfield. The soil was a light sandy loam in good heart which had been previously used for potato production. Strains of seeds of *Digitalis purpurea*, used in other selection and breeding experiments, have been employed; seedlings were raised in a heated greenhouse, were hardened off and were then planted out onto the prepared beds. In the fertiliser and the lime experiments 9 test plants formed a single block and adjacent blocks were separated by guard rows of plants which were not examined at any stage. Leaves were harvested from 1st year plants in late September in the late afternoon of a sunny day; for each plant any leaves on the outside of the rosette which were damaged or darkened in colour were rejected, also juvenile leaves and buds in the centre of each crown were not collected. The bulked leaves collected from plants in a block were immediately transferred to the drying shed and were rapidly dried in a forced draught at 55° C., after weighing they were powdered, returned to the drying shed for a further 12 hours and then sealed in screw-capped bottles containing silica gel[13]. Samples were estimated by means of the dinitrobenzoic acid method[14,15], which has been shown to yield results parallel to those given by the guinea-pig biological assay method when applied to leaf samples grown and

processed under similar conditions. Results have been expressed in terms of International Units of activity by comparison with the Standard Preparation of digitalis; moisture contents of samples varied only between 4 and 5 per cent. and these have not been considered in calculating results.

Fertiliser Trials

In the three years 1951, 1952, 1953, replicate factorial experiments using randomised blocks[16] were set up to investigate the influence of nitrogenous, phosphatic and potassium (NPK) fertiliser treatments. The fertilisers employed and their levels of treatment are set out in Table I. In each instance the appropriate amounts of fertiliser were worked into the blocks several days before planting out the seedlings.

TABLE I

ARTIFICIAL FERTILISERS (OZ. PER SQUARE YARD)

	1951	1952			1953	
		Low	Medium	High	High	Double High
N (sulphate of ammonia) ..	2	3	4	6	6	12
P (superphosphate)	1½	2	3½	5	12	24
K (sulphate of potassium) ..	¾	1	2	3	6	12

The 1951 experiment was carried out in triplicate at the one level of fertiliser treatment, which was that of normal horticultural practice. In 1952 duplicate experiments were carried out at three fertiliser levels, the "low" level being a little higher than that employed in 1951; the "high" level N content was double that of "low" level whilst P and K treatments were proportionately more increased. The levels of treatment selected

TABLE II

NITROGENOUS FERTILISERS 1954 CROP (OZ. PER SQUARE YARD)

Fertiliser	Base dressing	Top dressing		Total
		(*a*)	(*b*)	
Sulphate of ammonia ..	1	1	1	3
Nitrate of soda	1	2	1	4
Dried blood	2	2	1	5
Hoof and horn meal.. ..	3	2	0	5*

for 1953 experiments were excessively high, with proportionately greater increases in the amounts of P and K; the randomised blocks were laid out in duplicate: also further duplicate blocks were prepared in which lime at the rate of 4 oz. per square yard had been previously worked into the soil to a depth of 9 inches. The design of experiment using 5 replications of the randomised block system was again employed in 1954 but was directed to an investigation of the behaviour of four different nitrogenous fertilisers. Amounts of fertilisers containing the same equivalent nitrogen content were taken and were applied partially as a base dressing before planting out and partially as top dressings during the growth of the plants. Treatments employed were those shown in Table II.

TABLE III

ARTIFICIAL FERTILISERS

Weight of dry leaf per plant (g.)

	1951	1952			1953			
		Low	Medium	High	High	High + lime	Double High	Double High + lime
Control	71	5	5	4	48	47	67	46
N	55	5	6	5	53	47	56	39
P	64	8	8	9	43	49	71	46
K	74	5	6	5	41	57	66	46
NP	75	6	4	6	60	71	77	53
NK	61	5	8	8	44	54	46	44
PK	87	5	7	9	43	41	59	47
NPK	62	7	7	9	44	51	37	48

TABLE IV

NITROGENOUS FERTILISERS 1954

Weight of dry leaf per plant (g.)

Fertiliser	Replicate blocks					Average
	1	2	3	4	5	
Control	29	27	19	22	18	23
Sulphate of ammonia	16	11	24	21	21	19
Nitrate of soda	10	11	15	14	10	12
Dried blood	53	23	31	26	15	30
Hoof and horn meal	29	27	29	28	24	27

TABLE V

ARTIFICIAL FERTILISERS

Activity of leaf (I.U./g.)

	1951				1952			1953			
	1	2	3	Average	Low	Medium	High	High	High + lime	Double High	Double High + lime
Control	16·0	14·7	17·3	16·0	11·2	10·4	10·6	11·7	10·9	11·9	11·2
N	15·2	18·2	17·1	16·8	10·6	11·1	10·5	13·1	13·5	12·2	12·0
P	15·9	14·3	16·4	15·5	11·3	10·8	11·4	10·7	13·3	12·3	10·6
K	15·0	16·4	17·0	16·1	10·6	10·5	10·8	11·9	12·4	11·2	11·0
NP	19·3	15·5	15·9	16·9	11·4	9·7	10·1	11·7	13·5	13·2	11·6
NK	15·3	15·5	15·2	15·3	10·4	10·5	9·7	13·0	11·9	12·8	11·6
PK	15·6	18·3	16·8	16·9	10·7	10·2	11·2	12·7	11·5	12·4	12·1
NPK	18·0	16·4	15·2	16·5	11·7	10·5	12·2	12·3	11·1	11·8	11·4

TABLE VI

NITROGENOUS FERTILISERS 1954

Activity of leaf (I.U./g.)

Fertiliser	Replicate blocks					Average
	1	2	3	4	5	
Control	11·8	9·6	10·4	8·9	9·1	10·0
Sulphate of ammonia	9·8	9·1	10·0	7·9	8·6	9·1
Nitrate of soda	9·6	10·7	9·9	8·4	8·0	9·3
Dried blood	10·5	10·0	10·6	9·2	9·6	10·0
Hoof and horn meal	10·8	9·3	10·1	9·2	10·4	10·0

Digitalis purpurea strain B.26 was employed in 1951 and 1952, strain B.55 was used in 1953 and B.42 in 1954. The activities, in terms of International Units per g., of leaves taken from similar plants of these strains grown for other experiments in the same years were B.26, 1951, 15·6, 1952, 10·3; B.42, 1954, 10·4. Values for weight of dry leaf per plant, and for activity of leaf are given in Tables III to VI for all experiments and controls.

Liming Trials

Experiments were laid down in both 1951 and 1952 to show the effect of various quantities of lime on both leaf yield and activity, using randomised blocks. In 1951, lime levels from 2 oz. to 64 oz. per square yard were employed, the hydrated lime being lightly raked into the surface of the soil before the seedlings were transplanted. In 1952, levels of lime from $\frac{1}{4}$ lb. to 10 lb. per square yard were used, and these were worked into the soil by lightly forking, thus ensuring a somewhat deeper penetration of the lime. In 1953 similar levels of lime to those used in 1952 were employed but the lime was deeply dug into each plot and duplicate experiments were carried out. In 1954 three levels of lime at 3 lb., 6 lb. and 9 lb. to the square yard were used, and this was forked into the top six inches of soil, three replicate experiments being carried out.

The application of lime at different soil depths was investigated since the plants of *D. purpurea* possess a dense, shallow, fibrous root system mainly situated in the top 4 to 6 inches of soil, and seldom penetrating deeper.

At the time of harvesting the 1st year plants, *p*H readings of soil at 3, 6 and 9 inch depths were taken and these are recorded in Table VIII; the results for all experiments giving weight of dry leaf per plant and activity expressed in International Units per g., are given in Table VII. Strains of plants employed were B.155 in 1951, B.62 in 1952, B.55 in 1953 and B.22 in 1954.

TABLE VII

HYDRATED LIME TREATMENTS

Soil *p*H at a depth of 3 inches. Weight of dry leaf per plant (g.). Activity of leaf (I.U./g.)

Lime per sq. yd.	1951			1952			1953			1954		
	*p*H	Leaf	Activity	*p*H	Leaf	Activity	*p*H	Leaf	Activity	*p*H	Leaf	Activity
Control	6·5	40	12·0	6·7	19	11·1	6·2	33	10·0	6·2	6	8·7
2 oz.	6·5	51	11·4									
4 "	6·8	52	11·4	6·7	32	12·7						
8 "	6·5	40	11·4	7·5	35	14·5	6·5	35	9·8			
12 "	7·0	43	12·9									
1 lb.	7·0	53	12·9	7·5	30	14·4	6·5	39	9·9			
1½ "	6·8	44	12·0									
2 "				7·0	24	12·8	6·7	38	9·5			
2½ "	6·8	40	12·3									
3 "				7·5	24	14·2	6·5	18	9·7	7·2	4	9·3
4 "	7·5	38	12·6									
5 "				7·5	12	12·5	6·2	27	9·2			
6 "										6·7	3	10·2
7 "				7·5	25	15·9	7·2	34	10·6			
9 "										7·4	3	9·0
10 "				7·5	27	14·1	7·1	35	10·8			

TABLE VIII

SOIL *p*H BY DIFFERENT METHODS OF LIMING

	1951	1952	1953	1954
Weight of lime (sq. yd.)	2 oz.–4 lb.	¼–10 lb.	½–10 lb.	3–9 lb.
Method of liming	light raking	light forking	deep digging	forking (6 in.)
*p*H Soil surface	6·7–7·5	7·0–7·5	—	—
3 inches below surface	6·5–7·5	6·7–7·5	6·5–7·2	6·7–7·4
6 " " "	6·3–7·0	7·2–7·5	6·5–6·7	6·6–7·6
9 " " "	6·5–6·8	6·8–7·4	6·0–6·7	6·0–6·6
*p*H Control	6·5	6·7	6·2	6·2

DISCUSSION

The level of artificial fertilisers for the 1951 trials was relatively low and the influence upon weight of leaf produced per plant was not marked; the only suggested stimulus to growth occurred when treatments of PK were applied (Table III). The plants harvested in 1952 were small, due to late planting out and to unfavourable weather conditions and it is probably unsafe to draw any firm conclusions as to the influence of artificial fertilisers upon weight of leaf per plant from these materials. There is however a suggestion that P at each of the three levels employed had stimulated growth and that NK, PK and NPK at "medium" and "high" levels of fertiliser had resulted in increases in plant weight.

The relatively poor increases in weight of leaf obtained in the first two years of these trials, despite increasingly heavy fertiliser treatments, suggested that the low *p*H of the soil in the experimental gardens or the lack of calcium were probably limiting factors, and hence added calcium was employed in 1953, a rapid release of ammonia being anticipated. The only significant increases in leaf weight per plant were found with NP treatment either with or without added lime. In general terms the first three years of factorial experiments with artificial fertilisers have not produced any striking increases in crop weight, even though uneconomically high levels of artificial fertilisers have been applied at times, whilst the application of ammonium sulphate alone has at times resulted in a small decrease in weight of leaf compared with the controls.

The 1954 trials reported in Table IV have shown that, for equal nitrogen content and applied at economic levels, the two organic fertilisers were significantly more effective than were the two inorganic nitrogenous fertilisers. Thus dried blood appeared, under the conditions of experiment, to produce a marked increase in weight of dry leaf, and hoof and horn meal produced a somewhat smaller but nevertheless definite increase: sulphate of ammonia failed to produce any increase in weight of dry leaf and in fact some marked decreases in weight occurred; nitrate of soda was still more marked in producing lower weights of dry leaf per plant than in the controls. These results are thus in direct contradiction to those of Koch and Butler[4] although the type of loam employed in our experiments differed markedly from the clays employed by those workers.

The influence of artificial fertilisers upon activity of leaf is set out in Tables V and VI. In 1951 the relatively low levels of fertilisers showed

no pronounced augmentation in activity although some slight increases were found using N, NP, PK and NPK. In the 1952 experiments N at "medium" level, P at "high" level, and NPK at both "low" and "high" levels produced some small increases in activity. In 1953 some increases in activity were found with N at "high" and "high + lime" levels, with P at "high + lime" level, with NP at "high + lime" and "double high" levels, with NK at "high" level and with PK at "high" and "double high + lime" levels.

None of the increases in activity were consistent and marked although "high + lime" and "high" fertiliser alone were the most marked. Of the eight different randomised block treatments recorded in Table V, four increases were found for N treatment, three for P in the presence of N, and three for P in the presence of K. It may thus be concluded that N, P and K have some influence upon activity of leaf in *Digitalis purpurea*, especially at medium to high levels of treatment. This augmentation is, however, not marked and does not appear to be of great economic value.

Although the organic nitrogenous fertilisers produced greater increases in leaf yield than the inorganic fertilisers employed in 1954, the difference in activities of such leaf groups was not so marked. Certainly the activities of leaf samples from both sulphate of ammonia and nitrate of soda treatments were somewhat lower than those of control or organic-fertiliser-treated samples but the differences shown in Table VI are too small to be significant.

Treatments with hydrated lime were most effective when lightly dug into the top 6 inches of soil and under such conditions the soil *p*H values at depths of 3 and 6 inches were changed from 6·2 to 6·7 to as high as 7·5 (Table VIII). Deep digging-in of the lime was much less useful in influencing the *p*H of the upper levels of the soil in which the digitalis roots are located. It will be noted that this latter method of lime application was used in 1953 and the results for leaf yield and leaf activity, as recorded in Table VII for that year, do not parallel those obtained in the experiments carried out in other years. No evidence can be produced in this work to distinguish between the influence of calcium and of hydroxyl ions in the lime treatments, and both may well be significant.

The present investigation confirms the conclusions of other workers that seedling plants of *D. purpurea* will grow satisfactorily when planted out into a soil made distinctly alkaline by the presence of lime. Such plants grow as vigorously as the controls when lime treatments up to 10 lb. per square yard have been applied, with no loss in leaf yield; rather, with lime treatments up to 1 lb. per square yard, a definite increase in leaf yield per plant has been recorded and is shown in Table VII. The activity of these lime-treated plants has never been depressed as compared with the controls and thus the work of Duquénois[12] is confirmed. Conversely, there is distinct evidence of increases in glycosidal content in these plants and, in 1952, plants grown in soil treated with 7 lb. of lime per square yard were found to contain 15·9 International Units of activity whilst the controls showed 11·1 Units of activity.

Summary and Conclusions

The influence of fertilisers upon the 1st year leaves of *Digitalis purpurea* plants grown in light sandy garden loam, *p*H 6·2 to 6·7, are as follows:

1. Sulphate of ammonia in low, medium or high levels of treatment does not increase leaf yield: at medium and high levels it may fractionally increase glycosidal content of the leaves. This is most marked in the presence of lime.

2. Nitrate of soda decreases leaf yield but exerts no influence upon glycosidal content of the leaves.

3. Dried blood and, to a less marked extent, hoof and horn meal increase leaf yield but do not influence glycosidal content of the leaves.

4. Phosphatic fertiliser alone and in association with potassium sulphate or with sulphate of ammonia may produce a small increase in leaf yield and in glycosidal content of the leaves.

5. Hydrated lime in quantities up to 1 lb. per square yard, with change of soil *p*H up to 7·5, increases leaf yield, and quantities up to 10 lb. per square yard are tolerated by the plants without decrease in leaf yield. Under similar conditions glycosidal content of leaves is never depressed and generally an increase has been recorded, a maximum augmentation to 143 per cent. being obtained with a treatment of 7 lb. of lime per square yard.

6. The remarkable constancy of glycosidal content of leaves under all treatments excepting that with lime is most significant.

7. Normal horticultural NPK fertilisers containing organic nitrogen together with hydrated lime are recommended for optimum plant growth.

These investigations are being continued.

The author's thanks are due to Miss F. L. C. Blackwall and Miss K. M. Walsh for cultivation experiments, also to the Agricultural Research Council for a grant towards the provision of a greenhouse-drying shed employed in this investigation.

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DISCUSSION

The papers were presented together by THE AUTHOR.

DR. F. FISH (Glasgow) said that the author seemed to be recommending the use of extemporaneously made tinctures. He disagreed with the use of the tincture, although if a doctor prescribed it, it must be dispensed. A patient could not be trusted to measure out 15 drops of a tincture and dilute it with water as required, though he could be trusted to take tablets as directed. The work on fertilisers needed to be continued much longer before a clear picture would be obtained. It appeared that in one year the fertiliser gave a better leaf yield than lime, while in another year the same fertiliser gave a poor yield. He thought that the number of plants used—nine—was too small.

MR. G. R. A. SHORT (London) agreed with Dr. Fish that nine plants were rather few, and he wondered whether they could be supplemented by undertaking some experiments in sand culture.

DR. G. RIGBY (Manchester), referring to the variable factors encountered in the biological assay, said that in Manchester they had performed similar tests and found similar effects. The reason might be, as Dr. Rowson found, that alteration of ethanol concentration produced no alteration in its extinction. They, on the other hand, found that as the ethanol concentration was increased from 35 per cent. to 75 per cent. by volume, the extinction of a reaction mixture containing a constant weight of e.g., digitoxin, progressively fell. This, they felt, was related to the essentially ionic nature of the reaction itself, which might be retarded in the mixture containing the lower proportion of water. He suggested that the amount of water in the mixtures assayed varied considerably.

MR. K. L. SMITH (Nottingham) said that he was surprised the biological assays agreed so well. He had intended to recommend Dr. Rowson to forget about chemical assays but he would postpone making that recommendation until the chemists could agree among themselves.

PROFESSOR H. BRINDLE (Manchester) said that Dr. Fish had dismissed the use of tincture of digitalis too readily. Had he evidence to show that, in the human subject, powder of digitalis was equivalent in action to the tincture? When the powder was administered, the patient's alimentary tract was used as the extraction apparatus, and this varied considerably. A more constant result would be obtained from the tincture, of which the potency was known.

DR. F. J. ELLIOTT (Edinburgh) referred to the importance of root development.

MR. A. D. POWELL (Nottingham) suggested that the lime in Dr. Rowson's experiments might have acted as a soil corrective and improved the tilth of the soil, without itself being a growth substance.

DR. F. FISH (Glasgow) said that Professor Brindle, in observing that there was a considerable variation between patients, had put his finger on the main point. It was for the clinician to decide what kind of preparation was to be given to a patient. Most patients were left to dose themselves with digitalis. Nurses could be trusted to measure doses fairly

accurately, and the use of fresh tincture was therefore practicable in hospitals. But, if the tincture were diluted with water, as was often required, its potency would be lost in about a day.

DR. ROWSON, replying, said that he should apologise for having held up publication of the first paper for about two years, but he had published a summary in *The Pharmaceutical Journal* last year. He was sorry that the tincture was under a cloud, especially as tincture was by no means as liable to final breakdown of primary glycosides to secondary as they had thought two years ago. After an initial breakdown it was probably that the tincture remained constant for a long period. He thought that Dr. Fish went too far in his comments on the variation in results from one year to another. In 1952 they had had bad weather, and at Birdsgrove House the plants had not grown so well. In 1951 there was a good yield of leaf, and 1952 was poor. In 1953 the weights were lower because they had done their planting later. Concerning the number of plants, they tried to run three replicate blocks. Last year there were five, so they were getting a reasonable number of plants. The cultivation would be extended, and at a later stage they would have to go to the drug growers for help in cultivation. They were attempting a little sand culture this year, and were also trying to grow the plants in limestone country. He had tried planting straight on to lime soil, but without success. He did not think that changes in the tilth of the soil was the explanation for the effect of lime. He said that he would not commit himself as to the ionic nature of the dinitrobenzoic acid colour reaction. He did not believe that colour intensity was greatly influenced by change in the ethanol concentration of the reaction mixture, but all reaction mixtures quoted in the first paper had about the same ethanol content. He thought that under controlled conditions of plant cultivation and processing the chemical estimation gave results that were parallel with those of the biological estimation. He was pleased to note that Mr. Smith considered the results satisfactory and Dr. Rowson agreed that the chemical method might well replace the biological assay of digitalis leaf. The limits of error of the chemical method were lower than of the biological method.

THE ASSAY OF TINCTURE OF DIGITALIS AND THE DETERMINATION OF THE CONSTITUENTS OF DIGITALIS SPECIES

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Introduction

In our previously published work[1,2] we dealt with the 3 : 5-dinitrobenzoic acid (Kedde[3]) assay, the chromatographic behaviour and the frog assay of the pure constituents of *Digitalis purpurea* and of tinctures of digitalis. Guinea-pig assays have now been made and the results are here reported.

In the earlier chromatographic work[1] we dealt with the pure constituents of *D. purpurea* and used paper partition methods in which the stationary phase was water and the mobile phase was an organic solvent saturated with water. It was found impossible to separate the secondary glycosides from their respective aglycones, and for this reason we attempted to evolve a chemical assay which would allow us to measure with reasonable accuracy the concentrations of a secondary glycoside and its aglycone in a mixture of both like that obtained in chromatographic eluates.

Several assay processes were investigated and it was found that 33 per cent. hydrochloric acid[1] could be used to estimate a secondary glycoside in the presence of its aglycone. Assays of such mixtures have now been made. Subsequently, chromatographic separations employing formamide, in particular those described by Jensen[4–7], have been introduced in which a good separation of the aglycone from its secondary glycoside can be obtained—the 3 : 5-dinitrobenzoic acid reagent cannot be used to estimate the concentration of each constituent in the eluates from such separations. For this reason there is much to be said for the use of the earlier chromatographic systems when the separated chromatographic eluates are to be assayed.

It was felt that the formamide—chloroform system would be more suitable for investigating qualitatively the constituents of digitalis preparations and that the use of Whatman 3 MM paper would allow relatively large volumes of an extract, for example a tincture, to be chromatographed so that any constituents present in low concentration might be identified. For this purpose, the Standard Preparation of Digitalis was used and a practicable chromatographic method has now been attempted.

If a reliable chemical assay process for digitalis preparations is at all feasible it is almost certain that the separation of the constituents before assay and an interpretation of the concentration of each constituent in terms of biological activity will be necessary. Furthermore, the biological behaviour of a mixture of pure constituents must be investigated since the effect of the combination may be additive, so that in a mixture, one drug can be substituted at a constant ratio for any proportion of another without altering the toxicity of the combination. On the other hand, there may

be potentiation characterised by a toxicity greater than that predicted from experiments with the individual constituents, or there may be antagonism, characterised by a toxicity less than that expected if the ingredients acted independently[8,9].

Experimental

Small quantities of about 50 mg. of the constituents investigated were very kindly supplied by Professor Arthur Stoll. Standard solutions containing 10 mg. to 100 mg. of the constituents in 100 ml. were prepared in absolute ethanol. Similar solutions of commercial samples of digitoxin and gitoxin were also prepared. The solutions were stored at room temperature and were protected from light.

Hydrochloric Acid Assays of Pure Constituents

The purpurea glycoside A, purpurea glycoside B, digitoxin and gitoxin were assayed by the hydrochloric acid method[1] and calibration curves constructed (Figure 1). The reaction obeys the Beer-Lambert law within the range 0·1 mg. to 0·6 mg./10ml. of the reaction mixture.

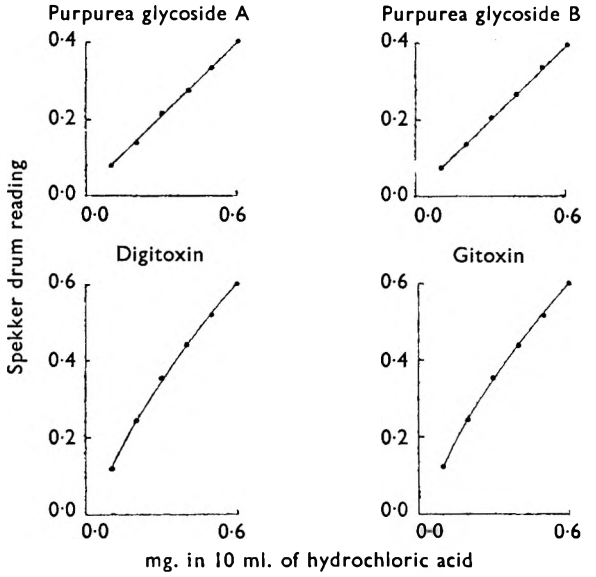

FIG. 1. Colour density/concentration calibration curves for the hydrochloric acid assay of digitalis constituents supplied by Professor Stoll.

Since each of the primary and secondary glycoside molecules contains 3 molecules of digitoxose, one would expect the molecular extinction coefficient for each glycoside to be constant and to be equal to 3 times that for digitoxose. Table I shows that it is approximately so in the case of the secondary glycosides but not in the case of the primary glycosides. This anomalous behaviour of the primary glycosides may be due to a non-quantitative reaction; the third digitoxose molecule in the primary

glycosides is combined with glucose and this linkage is not broken even by strong acid treatment[10].

TABLE I

MOLECULAR EXTINCTION COEFFICIENTS OF DIGITALIS CONSTITUENTS (STOLL). 0·5 MG. ASSAYED BY THE HYDROCHLORIC ACID METHOD

Glycoside or sugar	Molecular weight	Molecular extinction coefficient
Purpurea glycoside A	926	6190
Purpurea glycoside B	942	6250
Digitoxin	764	7950
Gitoxin	780	8060
Digitoxose (3 molecules)	444	8000

Assays of Mixtures containing Secondary Glycosides and the Respective Aglycones

Samples of commercial digitoxin and gitoxin were assayed by means of hydrochloric acid and the calibration curves (Fig. 2) constructed. These commercial samples of secondary glycosides were used in the assays of mixtures of secondary glycosides and their aglycones described below in order to conserve the purer samples obtained from Professor Stoll,

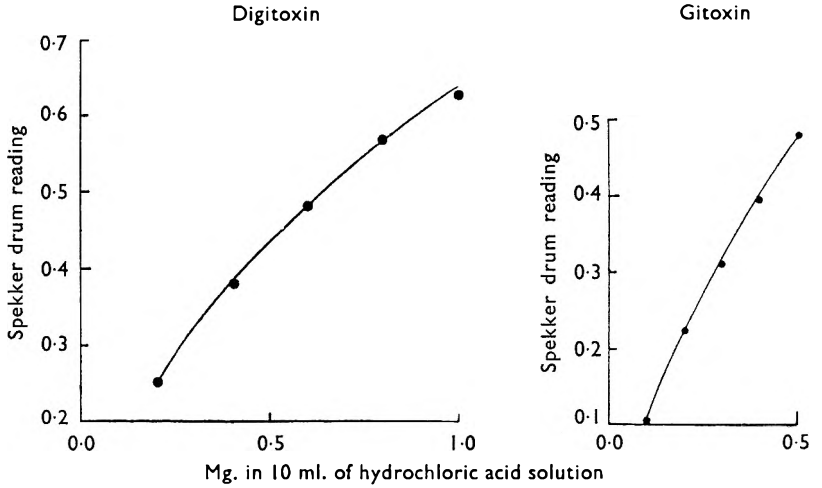

FIG. 2. Colour density/concentration calibration curves for the hydrochloric acid assay of commercial digitoxin and gitoxin.

since in assays of this type the results are not influenced by the purity of the sample. Furthermore, for the purpose of these assays, it is necessary to construct calibration curves for the secondary glycosides and for the aglycones when assayed by the 3:5-dinitrobenzoic acid reagent. Calibration curves of this type for the digitoxin and digitoxigenin used are reproduced in Figure 3 to illustrate the worked example shown below.

Solutions of a secondary glycoside and its respective aglycone were assayed by the alkaline 3:5 dinitrobenzoic acid and by the hydrochloric

acid methods. The former reagent estimates the glycoside and its aglycone and the latter reagent estimates the glycoside only. The reading obtained in the 3 : 5 dinitrobenzoic acid assay for the total aglycone content is expressed as mg. of the secondary glycoside and from this figure is

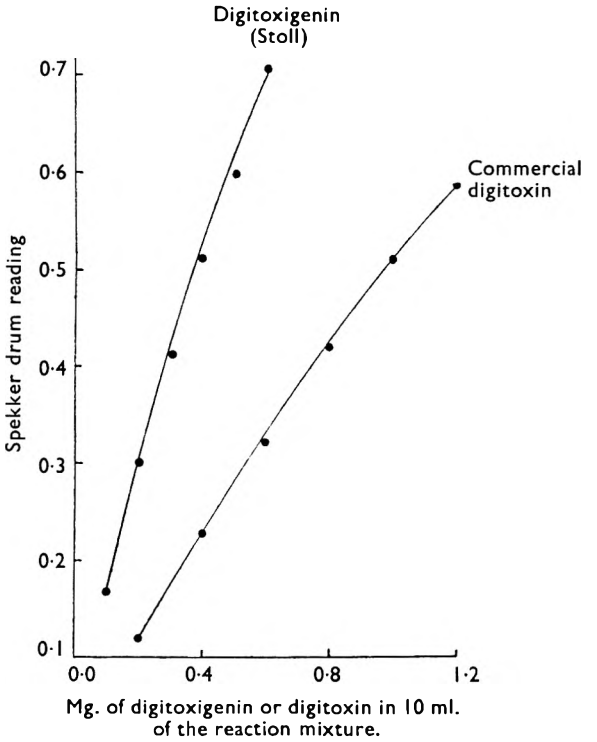

FIG. 3. Colour density/concentration calibration curves for the 3 : 5-dinitrobenzoic acid assay for digitoxigenin (Stoll) and commercial digitoxin.

subtracted the weight in mg. of the secondary glycoside estimated by the hydrochloric acid assay for secondary glycoside only. The difference is interpreted as weight of aglycone by reference to the standard curves for the glycoside and the aglycone. The example below illustrates the method.

A mixture containing 0·2 mg. of commercial digitoxin and 0·2 mg. of digitoxigenin was assayed. The Spekker drum reading 0·397 obtained by the aglycone assay (Fig. 3) is equivalent to 0·74 mg. of the digitoxin used, but by the hydrochloric acid assay (Spekker drum reading 0·242) only 0·2 mg. of secondary glycoside was found to be present (Fig. 2). Hence the residual absorption would be equivalent to 0·54 mg. of the digitoxin. Reference to the graph shows that 0·2 mg. of digitoxigenin gives the same colour density reading with the Kedde reagent as 0·54 mg. of digitoxin. Thus the calculated quantity of the aglycone agrees with the quantity actually taken.

Similarly a mixture containing 0·4 mg. of commercial digitoxin and 0·1 mg. of digitoxigenin was assayed as follows: Aglycone assay 0·370 ≡ 0·69 mg. of digitoxin, Glycoside assay 0·378 ≡ 0·40 mg. of digitoxin. The aglycone assay has estimated 0·40 mg. of digitoxin plus an amount of digitoxigenin equivalent to 0·29 mg. of digitoxin. Reference to the appropriate graph shows that 0·11 mg. of digitoxigenin produces the same colour density with the Kedde reagent as does 0·29 mg. of digitoxin.

The average results of triplicate assays are shown in Table II.

TABLE II

CHEMICAL ASSAYS OF MIXTURES OF SECONDARY GLYCOSIDES AND AGLYCONES

No.	Mixture assayed	Weight of constituents taken, mg.	Weight of constituents estimated, mg.
1	Digitoxin and digitoxigenin	0·10 0·30	0·11 0·31
2	" " "	0·20 0·20	0·20 0·20
3	" " "	0·40 0·10	0·40 0·11
4	Gitoxin and gitoxigenin	0·20 0·20	0·21 0·20
5	" " "	0·40 0·20	0·42 0·19
6	Digoxin and digoxigenin	0·20 0·30	0·22 0·29
7	" " "	0·40 0·20	0·40 0·19

BIOLOGICAL ASSAYS

Assays of some of the Constituents of D. Purpurea using Guinea-pigs

The lethal doses of purpurea glycoside A, purpurea glycoside B, digitoxin, gitoxin, digitoxigenin and gitoxigenin were experimentally determined by the procedure recommended by the British Pharmacopœia 1953 and Miles and Perry[11] for standardising tinctures of digitalis. Eight to 10 male guinea-pigs were used for each constituent and the solutions of the constituents were suitably diluted with sodium chloride solution so that the diluted solution caused the death of the animal 20 to 40 minutes after administration was commenced. 0·65 ml. per kg. weight, of the diluted solution was injected into the jugular vein every minute by means of a micro-burette. Digitoxigenin was found to be affecting the respiration of the animals and spasms were observed 8 to 10 minutes after the administration was started. The effect of digitoxigenin on respiration and its pharmacology are under investigation. Gitoxigenin was found to be of negligible potency which agrees with the results obtained with frogs[1]. The lethal doses of the constituents and their respective standard errors are given in Table III and for comparison the frog LD50 values obtained previously[1] are also tabulated.

Assays of Mixtures of Digitalis Constituents using Guinea-pigs

In order to study the effect of mixtures of these constituents, thereby simulating more nearly digitalis and its galenical preparations, 6 different mixtures were prepared, representing a mixture of the primary A and B

TABLE III

RESULTS OF THE BIOLOGICAL ASSAYS OF DIGITALIS CONSTITUENTS

Constituent or tincture	Frog assays		Guinea-pig assays		
	LD50 mg./kg.	Fiducial limits (P = 0·95)	Lethal dose mg./kg.	Fiducial limits (P = 0·95)	I.U. per mg.
Purpurea glycoside A	2·2	1·8–2·7	0·53	0·44–0·62	2·70
Purpurea glycoside B	5·7	4·8–6·7	1·44	1·25–1·63	0·99
Digitoxin	3·7	3·4–4·1	1·54	1·25–1·83	0·93
Gitoxin	16·3	12·6–21·2	13·34	10·97–15·71	0·11
Digitoxigenin	17·4	14·4–21·0	3·36	2·49–4·23	0·43
Gitoxigenin	>40	—	>80	—	—
Standard preparation of digitalis	—	—	109·0 (1·43) I.U.	79·0–139·0	0·01315

glycosides, a mixture of a primary glycoside and a secondary glycoside (purpurea glycoside A and digitoxin), a mixture of a primary glycoside and an aglycone (purpurea glycoside A and digitoxigenin), a mixture of a secondary glycoside and an aglycone (digitoxin and digitoxigenin), a mixture of an aglycone and a sugar (digitoxigenin and digitoxose) and a mixture of purpurea glycoside A, purpurea glycoside B, digitoxin, gitoxin and digitoxigenin. These mixtures contained equal volumes of those solutions of the respective constituents which caused death 20 to 40 minutes after administration.

The relative log potencies of the constituents (R_2, R_3 . . .) used in a particular mixture were calculated with reference to one of the constituents. The log potency of the mixture was calculated from the experimental data log $(\lambda_1 + \lambda_2 R_2 + \lambda_3 R_3 \ldots)$; its deviation (D) from the theoretical potency and its standard deviation (SD) were calculated. The values D/SD and the corresponding per cent. at which they are significant are shown in Table IV. The figure 5·24 (D/SD) in the second column is highly significant statistically. Other figures are not significant individually but all positive values taken together provide evidence of potentiation.

TABLE IV

RESULTS OF GUINEA-PIG ASSAYS WHEN DIGITALIS CONSTITUENTS WERE ADMINISTERED IN MIXTURES

Mixture	D/SD	Level at which significant, per cent.
1. Purpurea glycoside A and purpurea glycoside B	+0·60	55
2. Purpurea glycoside A and digitoxin	+0·90	37
3. Purpurea glycoside A and digitoxigenin	+5·24	0·0001
4. Digitoxin and digitoxigenin	+1·31	19
5. Purpurea glycoside A, purpurea glycoside B, digitoxin, gitoxin and digitoxigenin	+1·20	23
6. Digitoxigenin and digitoxose	No potentiation was observed	

Assays of Mixtures of Constituents using Frogs

In order to study the effect of the constituents in mixtures, 4 constituents (purpurea glycoside A, digitoxin, gitoxin and digitoxigenin i.e., a primary glycoside, 2 secondary glycosides and an aglycone) were mixed in suitable proportions.

Two dose values per kg. of frog which were expected to give 20 per cent. and 80 per cent. mortality were chosen for each of the constituents and also for the tincture prepared from the Standard Preparation of Digitalis, this information being obtained from their LD50 values. The lower and the higher doses were in each case in the ratio 2:3. The mixture contained a quarter of these quantities of each of the constituents. Thus 12 solutions were prepared for injection, 6 containing the lower dose (20 per cent. expected mortality) and 6 containing the higher dose (80 per cent. expected

TABLE V

COMPARISON OF THE ESTIMATED POTENCY TO FROGS OF A MIXTURE OF PURPUREA GLYCOSIDE A, DIGITOXIN, GITOXIN AND DIGITOXIGENIN WITH THE POTENCY OF THE MIXTURE PREDICTED ON THE HYPOTHESIS OF SIMILAR ACTION

Experiment	Potency ratio estimated: theoretical	Fiducial limits (P = 0·95)
First	1·30	1·09–1·57
Second	1·04	0·89–1·22
Pooled estimate	1·15	1·02–1·29

mortality) of the following—purpurea glycoside A, digitoxin, gitoxin, digitoxigenin, a mixture of the above 4 constituents in appropriate concentration and the tincture prepared from the Standard Preparation of Digitalis. The procedure adopted was the same as described for the 2 and 2, 18 hour frog assay method of the British Pharmacopœia 1953. Table V incorporates the results of two investigations made with 15 frogs for each dose of each constituent in the first experiment, and 20 frogs for each dose in the second. The individual slopes (b) and their respective variances (B) for the lines were calculated by the B.P. method. Since the figures for $X^2_{(4)}$, (3·12 and 7·0) were not significantly a line with a common slope $(\bar{b})$[12] was fitted and used in the calculations.

Log dose/probit curves were drawn for each of the constituents and for the mixture, plotting the logarithms of 2 and 3 (ratio of doses) against the probits of mortality corresponding to these doses. A line with a common slope $\bar{b}$ was drawn on the same graph sheet. Another line parallel to this was drawn to intersect the original line in the centre, and the log dose ($\lambda 5$) corresponding to probit 5 was read from this new line. Regression equations for all these constituents were then calculated and the potencies (R_2, R_3, R_4) of the other 3 constituents of the mixture were calculated from the fourth (digitoxin) which was regarded as unity. From the values of R, the equation for the mixture was calculated on the hypothesis of similar action[12,13]. The potency ratio (estimated potency: theoretical potency i.e., the ratio of the experimentally determined potency to the predicted potency) and its fiducial limits were then calculated by the equations described by Finney[12]. These results suggest a 15 per cent. potentiation which is statistically significant when these 4 constituents are mixed in these proportions.

Biological assays of tinctures. Six commercial tinctures[2] (B, C, D, E, G and H) were assayed by the official guinea-pig method mentioned above[11].

The results along with the frog assay figures (experiments performed at about the same time) are given in Table VI. Thus an interpretation of the potency of each constituent and each tincture can be made in terms of International Units per mg. of constituent, or per ml. of the tincture (See Tables III and VI). Throughout the whole series of guinea-pig assays, the Standard Preparation of Digitalis was assayed at intervals as a control on the uniformity of the animals etc.

TABLE VI

POTENCY OF COMMERCIAL TINCTURES OF DIGITALIS EXPRESSED IN I.U./ML.

Tincture	Potency expressed as I.U./ml.			
	Guinea-pig assay January-March 1955	Frog assay March 1955	Frog assay March 1954	Per cent. deterioration
A	—	—	0·54	—
B	0·51	0·46	0·48	4·2
C	0·42	—	0·39	—
D	0·57	0·55	0·62	11·2
E	0·55	—	0·51	—
F	—	—	0·47	—
G	0·51	0·55	0·58	5·1
H	0·49	0·45	0·51	11·7
*S	1·315	1·315	1·315	—

* Tincture prepared from the Standard preparation of digitalis.

CHROMATOGRAPHIC EXAMINATION

The R_F values of a number of *D. lanata* constituents obtained from Professor Stoll were studied using the formamide system. The results are set out in Table VII. The Standard Preparation of Digitalis was qualitatively analysed. A 1 in 10 tincture was prepared by the B.P. method and stored in a refrigerator below 5° C. In order to detect constituents which might be present in smaller quantity, relatively larger volumes of the tincture had to be chromatographed. For this purpose, Whatman 3MM paper was found to be more satisfactory than Whatman No. 1 paper. The tincture was applied along the starting line which was about 20 cm. in length by means of a pipette drawn to a capillary. The solvent was allowed to evaporate and the sheets were then impregnated with the stationary phase (formamide) using a mixture of 3 volumes of formamide and 7 volumes of acetone. Chromatography was then commenced in glass chambers previously saturated with the chloroform vapour of the mobile phase. The solvent was allowed to run down the paper and development stopped when the solvent front had travelled about 35 cm. On such chromatograms the primary glycosides are not completely separated from each other. Whenever it was desired to separate the

TABLE VII

R_F VALUES OF SOME OF THE CONSTITUENTS OF *Digitalis lanata* USING FORMAMIDE AS STATIONARY PHASE AND CHLOROFORM AS THE MOBILE PHASE

Constituent	Approximate R_F
Lanatoside A	0·31
Lanatoside B	0·05
Lanatoside C	0·02
Digoxin	0·33
Digoxigenin	0·11
Purpurea glycoside A / Purpurea glycoside B	0·02
Digitoxin	0·95
Digitoxigenin	0·85
Gitoxin	0·60
Gitoxigenin	0·34

primary glycosides development of the chromatograms was allowed to continue for about 60 hours—the mobile phase dripping from the bottom edge of the paper. After about 60 hours development, purpurea glycoside. A was found to have travelled about two thirds and purpurea glycoside B about one third the length of the paper. The chromatograms after appropriate development were removed and the mobile phase allowed to evaporate. The heavy stationary phase was removed by heating the sheets in an oven at 60° C. to 70° C. for 3 to 4 hours. Vertical $\frac{1}{2}$ in. strips were cut from the developed chromatograms, parallel to the direction of flow, and sprayed with 2 or 3 of the commoner reagents to localise and identify the constituents. Horizontal strips were then cut from the remainder of the sheet and each strip was eluted with 70 per cent. ethanol. The eluate from each was evaporated to small volume under reduced pressure and rechromatographed with the same phase system on 3 in. wide paper strips. On each strip a solution of the pure constituent having the corresponding R_F value was also applied as a reference spot.

The reagents used for spraying the chromatograms were antimony trichloride in chloroform, trichloroacetic acid in chloroform, chloramine-trichloroacetic acid reagent and xanthydrol reagent[14]. None of these reagents is satisfactory alone but the use of 2 or 3 reagents on strips cut from the same chromatogram is helpful in recognising the substance as a member of the A or B series and as a glycoside or an aglycone.

The following constituents were found to be present in a tincture prepared from the Standard Preparation of Digitalis when 6 ml. of the tincture was applied along the starting line of a sheet 22 cm. × 50 cm.—purpurea glycoside A, purpurea glycoside B, digitoxin and gitoxin. In addition a substance was detected at about R_F 0·2 which fluoresced under ultraviolet light after treatment with the chloramine-trichloroacetic acid reagent. When this substance was extracted from the paper and tested with alkaline 3:5 dinitrobenzoic acid reagent, it gave a positive reaction indicating the presence of a ketonic group in the molecule.

Discussion

Of the methods which have been used for the chemical estimation of the constituents of digitalis, the hydrochloric acid method of assay for digitoxose containing glycosides seems to be accurate. It can estimate a secondary glycoside in the presence of its aglycone. This reagent which reacts with the sugar moiety, digitoxose, appears to be applicable to the assay of digitalis preparations, but unfortunately this reagent cannot be used for assaying tinctures, the pigments of which are always difficult to remove by any of the standard decolourisation processes. Nevertheless the method is of value in estimating the concentration of a secondary glycoside in a chromatographic eluate containing both the secondary glycoside and its aglycone in a reasonable state of purity.

After chromatographic separation, the constituents may be estimated by a chemical process and the biological potency of the mixture calculated provided that the constituents have an additive biological action.

Investigations have been made with a few mixtures of constituents and the results suggest potentiation.

It has been observed that the aglycone digitoxigenin affects the respiration of the guinea-pig and that this effect causes the death of the animal. The pharmacological action of digitoxigenin needs further investigation. A similar unusual effect has been noticed in the frog assays of digitoxigenin —namely spasms occuring soon after injection, rapidly followed by cessation of respiration and reflexes and apparent death of the animal although the frog's heart continues to beat and the animal often recovers.

The constituents present in the Standard Preparation of Digitalis seem to be limited to the primary and secondary glycosides and no aglycones were found. This does not exclude the possibility of the presence of very small amounts of aglycones. The extra band R_F 0·2, which was repeatedly found indicates the presence of an unknown substance which may possibly be identical with one of the new constituents reported by Jensen[15].

Summary

1. A chemical assay process for digitoxin, gitoxin and digoxin in the presence of their respective aglycones using hydrochloric acid has been described.

2. Six constituents of *D. purpurea* have been biologically assayed by the frog and by the guinea-pig methods.

3. The biological behaviour of mixtures of these constituents has also been studied using frogs and guinea-pigs.

4. Six samples of commercial tinctures were assayed by the frog and guinea-pig method.

5. The constituents present in the Standard Preparation of Digitalis have been identified by paper partition chromatography.

The authors appreciate the interest taken and help given by Professor A. D. Macdonald during the biological investigation. Our thanks are due to Mr. A. M. Walker of the Statistics Department of Manchester University for his advice in statistical methods.

During these investigations one of us, S. N. Sharma, was the holder of an Imperial Chemical Industries' Fellowship in Pharmacy and Pharmacology, at Manchester University.

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DISCUSSION

The paper was presented by DR. G. RIGBY.

DR. F. FISH (Glasgow) said he agreed that in principle a chemical assay was good, but did it give any indication of the therapeutic activity in man? The chemistry of digitalis was very complex, but even if they got a clear picture of the chemical constituents, were they any further forward? He thought not, because they did not know which pharmacological assay gave a true indication of clinical activity. The authors had shown that the relative activities of the primary glycosides, secondary glycosides and aglycones in either the A or B series differed in the frog and the guinea-pig; and figures were available in the literature to show that cat assays gave yet a third set of ratios. He wondered if the use of commercial digitoxin (Fig. 3) led to discrepancy between the practical and theoretical results obtained in the assays of mixtures of glycoside and genin. The authors stated that, from the graphs in Figure 3, 0·2 mg. of digitoxigenin gave the same colour density as 0·54 mg. of digitoxin, whereas theoretically 0·2 mg. digitoxigenin should be equivalent to 0·48 mg. digitoxin. Referring to Table VI, he asked how old the tinctures were to begin with, and he suggested that they had already reached the stable condition that Dr. Rowson had mentioned earlier. In Table V, he did not know whether it was possible to make sound deductions from only two estimations: one showing an excess of 30 per cent. and the other of 4 per cent.

DR. ROWSON (London) made a plea for consistency in the terminology of reagents, for example, the use of "3:5-dinitrobenzoic acid" instead of "Kedde" reagent. He wished the authors had been more precise in stating the reason why the molar extinctions of primary glycosides with hydrochloric acid were lower than for the secondary glycosides. This was surely due to the interaction of two of the three digitoxose molecules present only. The same phenomenon occurred when primary glycosides are estimated with other reagents, e.g., Keller-Kiliani and the phosphoric-sulphuric acid reagent of Tattje. He asked the authors to state the molar extinction values for different glycosides and their genins with dinitrobenzoic acid. The deviations in Figure 3 between the two graphs would be more clearly apparent from such molar extinction values. The high value quoted for digitoxigenin was probably due to the influence of acid used in the preparation of that genin producing "exaltation of colour." The wide deviation of results between the frog and the guinea-pig methods of assay was worrying, and it would appear that the more constant and reproduceable results from chemical methods were to be preferred. He asked for more details of the methods of chromatographic separation used to decolorise the tinctures before the hydrochloric acid assay was applied. Was the unknown glycoside, found chromatograhically in powdered digitalis leaf, digitalinum verum?

PROFESSOR BRINDLE said that he agreed with Dr. Fish that they ought to be able to decide which particular action of digitalis they wished to use in evaluating the drug. He did not, however, agree with his conclusion. In bioassays they were testing on animals, and up to the death of those

animals, which was quite different from the therapeutic use of the drug. They should try to analyse the constituents of digitalis and judge the potency by the amount of each individual active substance present.

DR. RIGBY, replying to points raised by Dr. Fish, said that Professor Brindle had answered him in some degree, and he had no wish to enter into a discussion on which bioassay should be preferred. His own opinion was that the cat and guinea-pig assays were the best. The literature of the past 20 years showed that there was not much hope of agreement as between chemical and bioassay. On the difference between primary and secondary glycosides, their method of approach was by chromatographic separation, and it was very easy to separate them in pure form. He had used commercial products for economy's sake. With regard to the tinctures, the labels from the bottles were available but Dr. Sharma, who had assayed the tinctures by the guinea-pig method, was unfortunately not available to report their age. It was true that there were only two estimations shown in Table V but they involved the use of 400 frogs, and the results had been checked statistically. Dr. Rowson had made a point about nomenclature of reagents which had always bothered them a little. The hydrochloric acid method was developed originally because at the time they could not separate clearly the secondary glycoside from the aglycone, so they had tried to get round it by estimating the two in a mixture. Personally, he had found results with the Bial reagent unreproducible. The molar extinction of the genins to dinitrobenzoic acid, they felt, might be due to the formation of anhydro compounds. On digitalinum verum, they had been thinking along the lines of Jensen's work, who had found a number of unidentified compounds. The hydrochloric acid assay for quantitative estimations after chromatography had not yet been used. In his view if one could rely on a good quality dried leaf—carefully collected and processed—one could probably find a high proportion of primary glycosides present. That could be checked chromatographically, and if one used a large volume one could test for smaller amounts of degradation products. Then the problem became simpler: the estimation of the potency of a dried leaf containing, roughly speaking, two active constituents.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Aureomycin Hydrochloride, Spectrophotometric Assay for. L. J. Ravin and A. E. James. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 215.) The method depends on the development of a bluish-green colour when phosphomolybdic acid and sodium hydroxide are added to solutions of aureomycin hydrochloride. The hydrogen ion concentration and quantity of phosphomolybdic acid required for the development of the maximum intensity of colour were determined experimentally, and the following assay method was evolved. Dissolve a sample containing about 25 mg. of aureomycin hydrochloride in 150 ml. of water, add 20 ml. of a 2 per cent. solution of phosphomolybdic acid, adjust the reaction to *p*H 5·5 to 6·5 by the addition of sodium hydroxide and add sufficient water to produce 1000 ml. Determine the light absorption at 405 mμ, the wavelength of maximum absorption. Calculate the quantity of aureomycin by reference to a standard curve based on the results of experiments with a sample of pure aureomycin hydrochloride. The method may be applied to tablets, capsules and surgical powder. Good agreement was obtained between this method and the assay based on the light absorption of aureomycin solutions at 440 mμ. G. B.

Creatinine in Alcoholic Solution, Spectrophotometric Determination of. L. Jacobbson and L. Paulsen. (*Scand. J. clin. Lab. Invest.*, 1955, **7**, 32.) Creatinine was dissolved in various solvents and the absorption in the ultra-violet region was recorded, using a Beckman spectrophotometer. The solvents used were distilled water, methanol, ethanol, propanol, butanol, heptane, pyridine and 0·1N hydrochloric acid. Creatinine dissolved in either 4 parts methanol or ethanol and 1 part water or in pure butanol showed absorption in the ultra-violet region with a distinct maximum at 235–236 mμ. Either no maximum or no absorption was obtained with the other solvents. The standard curve for creatinine in the ethanol-water solvent was a straight line passing through the origin and is thus in accordance with Lambert-Beer's law. Under identical conditions no maximum was obtained with creatine. M. M.

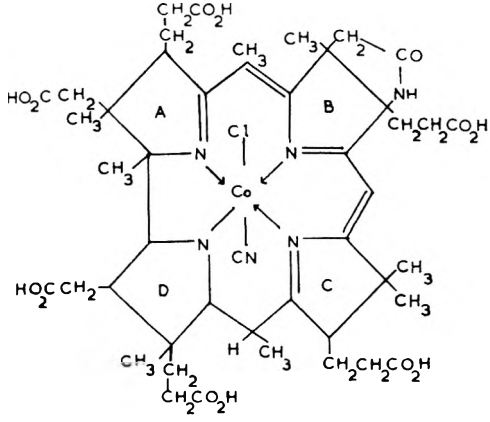
Erythromycin, Spectrophotometric Determination of. J. B. Tepe and C. V. St. John. (*Analyt. Chem.*, 1955, **27**, 744.) The characteristic ultra-violet absorption spectrum of hydrolysed erythromycin was found to offer the most promising method of analysis. Erythromycin itself showed a broad absorption band of weak intensity at 285 mμ; after hydrolysis by strong acid at elevated temperatures, maxima at 226, 267, and 485 mμ were produced, the absorption at 226 mμ having $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ approximately 150 and obeying Beer's law. Other degradation products of erythromycin absorb in the 226 mμ range and limit the usefulness of acid hydrolysis as an assay method, although a dilute acid-inactivated blank can be use to correct for the ultra-violet absorption of degradation products and impurities. After dilute alkaline hydrolysis, erythromycin exhibits strong absorption at 236 mμ, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 85. The procedure described was used for the determination of the potency of fermentation samples. Erythromycin was extracted from broth at *p*H 10 with amyl acetate or trichloroethylene, after which a procedure for nonaqueous samples was used. It was necessary to

restandardise the test on the basis of recoveries of erythromycin in the extraction procedure, which varied from 90 to 94 per cent. Good agreement was obtained between ultra-violet and microbiological assay methods. R. E. S.

Riboflavine, Determination of, by Light Absorption and Polarographic Methods. A. J. Zimmer and C. L. Huyck. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 344.) The extinctions of solutions of riboflavine in 0·02N acetic acid were determined at 267, 270, 440 and 445 mμ. Beer's law was obeyed at concentrations from 2 to 7·5 μg./ml. Samples were assayed by comparison with a standard curve based on measurements of the extinctions at 270 and 440 mμ of solutions of riboflavine reference standard (2, 3 and 5 μg./ml.) in 0·02N acetic acid. It was established that the solutions are not affected by light under ordinary laboratory conditions, although prolonged exposure to the light of a "daylight" fluorescent lamp, caused decomposition of riboflavine, demonstrated by changes in the ratio of extinctions at 267 and 270 mμ, the latter being the more sensitive to the decomposition of riboflavine by light. Solutions of riboflavine in 0·02N acetic acid were polarographed from 0 to 1·5 volt, using a saturated calomel cell as reference. 0·1N potassium chloride was used as supporting electrolyte, and oxygen was removed by bubbling nitrogen through the solution. The diffusion current at 0·4 volt was measured and the residual current subtracted from this figure. The quantity of riboflavine in the sample was then read from a standard curve based on measurements in solutions of riboflavine reference standard containing 10 to 100 μg./ml. This method was suitable for tablets and some liquid preparations. Both methods were of good precision, but gave results slightly lower than the U.S. Pharmacopeia (fluorimetric) method. G. B.

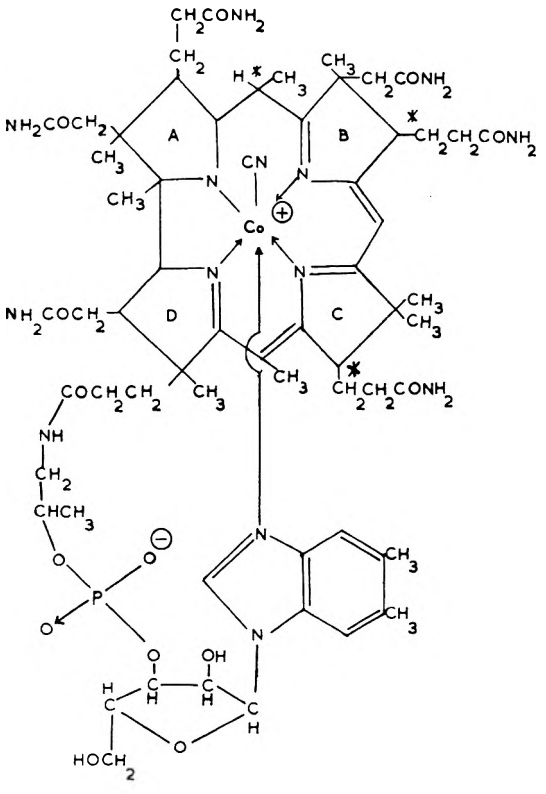
ORGANIC CHEMISTRY

Vitamin B_{12} and its Hexacarboxylic Acid Degradation Product, The Structure of. R. Bonnett, J. R. Cannon, A. W. Johnson, I. Sutherland, A. R. Todd and E. Lester Smith. (*Nature, Lond.*, 1955, **176**, 328.) Degradation of the crystalline nucleotide-free hexacarboxylic acid, from the alkaline hydrolysis of vitamin B_{12} previously described, has been carried out to fill in details of the molecular structure not revealed by X-ray crystallography. (*Nature, Lond.*, 1954, **174**, 1169.) A further communication (*Nature, Lond.*, 1955, **176**, 325) from Crowfoot Hodgkin, Pickworth, Robertson, Trueblood, Prosen and White published simultaneously with the present one describes the determination by X-ray crystallographic methods of the relative positions of all the atoms (excluding hydrogen) in the hexacarboxylic acid. These results taken with the chemical evidence have led to the structures proposed for the hexacarboxylic acid (I) and vitamin B_{12} (II). The formula of the hexacarboxylic acid has been revised to $C_{46}H_{60}O_{13}N_6CoCl,2H_2O$. The analytical figures are best accounted



(I)

for by the assumption that all eight single substituent atoms are present as carbon in methyl groups. Independent evidence for the existence of ring C has already been obtained by the isolation of 1 : 1-dimethyl-2-(2′-carboxyethyl) succinimide from chromic acid oxidation of a crude vitamin B_{12} hydrolysate, and the same substance has now been obtained by a similar oxidation of the pure hexacarboxylic acid. The five-membered ring attached to ring B of the hexacarboxylic acid, which X-ray examination reveals is not present in the vitamin itself, has been assigned a lactum structure on the basis of elementary analysis, infra-red data and the fact that the hexacarboxylic acid does not acquire an additional negative charge even at *p*H 11. An explanation is provided of the known conversion of B_{12} to a biologically-inactive crystalline substance when submitted to the limited action of alkali



(II)

in the presence of air, in terms of ring hydroxylation of the activated β-positions of the pyrrole rings, similar to that observed with chlorophyll derivatives. In support of this thesis it has been shown that acid hydrolysis of the oxidation product gives a mixture of penta- and hexa-carboxylic acid but no heptacarboxylic acid. Differences between Vitamin B_{12} and the porphorin series, as in the visible spectra and the failure of the former to yield maleimides on oxidation are explained by the fact that the rings A, B, C and D each contain at least one tetra-substituted β-carbon atom, so that the pyrrole rings are partly reduced. Vigorous hydrogen peroxide oxidation of the hexacarboxylic acid, however, does yield oxamide, the formation of which is in agreement with the placing of the conjugated double bond system in the molecule of the vitamin. The positioning of this system is confirmed by the action of *N*-chloroamides on vitamin B_{12}, when three moles of reagent are consumed in the formation of a stable product, a dichloro compound with a visible spectra displaced into the red. Chlorination is assumed to occur at the three positions marked*, followed by elimination of hydrochloric acid from the meso position between rings A and B to extend the conjugated system by formation of a sixth double bond. Biogenetic considerations support the proposed structure for vitamin B_{12}, for which a simple scheme of biogenesis can be formulated based on that already accepted for many natural porphorins. J. B. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

α-Peltatin Glucoside, The Isolation of, from the Rhizomes of *Podophyllum peltatum* L. A. Stoll, A. von Wartburg and J. Renz. (*J. Amer. chem. Soc.*, 1955, **77**, 1710.) A new glucoside was isolated from the more water-soluble fractions obtained by partition chromatography between different solvents during the isolation of the glucosides of podophyllotoxin and β-peltatin from the rhizomes of *P. peltatum*. It crystallises from acetone in colourless, long prisms m.p. 168 to 171° C. (decomp.), $[\alpha]_D^{20}$ −128·9° (*c*, 0·5 in methanol), formula $C_{27}H_{30}O_{13}$. It is readily soluble in ethanol and fairly readily soluble in water, and gives a positive reaction with ferric chloride. This glucoside is easily hydrolysed by β-glucosidase at *p*H 5 into aglucone and glucose. The aglucone was found to be identical with α-peltatin. α-Peltatin glucoside exhibits antimitotic activity. A. H. B.

BIOCHEMISTRY

BIOCHEMICAL ANALYSIS

Bile Pigments in Serum, Chromatographic Determination of. B. H. Billing. (*J. clin. Path.*, 1955, **8**, 126.) Bile pigments ("bilirubin") in the serum of patients with obstructive jaundice consists of three related substances, bilirubin and pigments I and II. A quantitative method is described for their determination based on their separation by reverse phase partition chromatography. To 1 ml. of serum add 0·18 ml. of saturated ammonium sulphate solution and 2·5 ml. of ethanol. Stand in the dark for 30 minutes and remove the precipitate by centrifuging. A sample of the supernatant, containing 50 to 100 μg. of total bile pigments (previously determined by the method of Malloy and Evelyn), is evaporated to dryness *in vacuo* at room temperature. The dried supernatant is transferred quantitatively to the top of a kieselguhr chromatographic column, prepared in a special tube allowing easy separation of the bands, using not more than 0·5 ml. aqueous phase of a butanol-water system. The pigmented solution is run into the kieselguhr and when the top is almost dry 4·5 ml. of aqueous solution is added. The solution is allowed to flow through at a rate of not more than 1 ml. every 3 minutes until separation of the three pigments is achieved. The portions of the column containing the pigments are separated and each transferred to glass stoppered tubes. The pigments are converted to the corresponding azo-compound, extracted by shaking with 5 to 10 ml. of ethanolic diazotized sulphanilic acid and after centrifuging the kieselguhr residue is re-extracted with 5 ml. of ethanol. The solutions are combined for each pigment, the volumes measured and after 30 minutes read in a spectrophotometer at 525 mμ, from which the proportions of bilirubin, pigment I and pigment II are calculated. Methyl red is used as the standard. G. F. S.

Blood Serum, Determination of Total Lipides in. W. M. Sperry and F. G. Brand. (*J. biol. Chem.*, 1955, **213**, 69.) A method is described for the direct gravimetric determination of the unmodified total lipides of blood serum. For the extraction add 1 ml. of blood or plasma to 8·3 ml. of pure methanol in a 25 ml. volumetric flask, add an approximately equal volume of chloroform, bring just to the boil, cool, add chloroform to the mark, mix and filter. For purification two methods are described. In the first, pipette 20 ml. of extract into a vial 2 cm. in diameter and 8 cm. high, add water slowly until the vial is full and then lower into a 1 litre flask of water. Allow to diffuse for 18 hours,

removing the "fluff" which collects at the interface. In the second method, pipette 20 ml. of extract into a 25 ml. glass stoppered cylinder, add 4 ml. of water and shake vigorously for 1 minute. Allow to stand overnight to separate the two phases and wash the residue with 3 ml. of a chloroform-methanol-calcium chloride solution. The lipide extracts are then evaporated to dryness *in vacuo* or in an atmosphere of nitrogen, dissolved in chloroform-methanol, filtered into a 5 ml. volumetric flask and dried and weighed. The weight of lipides multiplied by 125 gives the concentration in mg. per 100 ml.

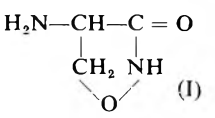
G. F. S.

Human Hæmoglobin, Estimation of. H. K. Prins and T. H. J. Huisman. (*Nature, Lond.*, 1955, **175**, 903.) Two methods have been developed for the separation and quantitative estimation of four different kinds of human hæmoglobin, namely, carboxyhæmoglobin-A (adult), B (sickle cell), C and F (fœtal). The best separation, using Amberlite IRC-50, was obtained at *p*H 6·50 (citrate buffer with a constant amount of citric acid and different sodium ion concentrations. Fœtal hæmoglobin was practically unabsorbed and separation of carboxyhæmoglobin-F was helped by a relatively low rate of flow of the effluent. A chromatogram is given of the four different hæmoglobins using a column of Amberlite IRC-50 (XE-64) of 13 cm. × 0·9 cm. at 10° C.; the yields were about 85 per cent. at 10° C., but could be improved by working at lower temperatures. The second method (cuvette method) employed a flat cuvette ("Lucite") of 3·0 cm. × 0·5 cm. × 20 cm. filled up to 15 cm. with resin; about 10–15 mg. carboxyhæmoglobin (in 1·0 ml.) was chromatographed using a sodium citrate-citric acid buffer (sodium ion concentration 0·15) of *p*H about 6 at 0° C. After the elution of 200 ml. effluent, siphoned into the cuvette at a rate of about 20 ml. per hr. a good separation of the four components was obtained. The mean rates of displacement were different for each component in the proportions of 1·33 (F): 1·00 (A): 0·72 (B): 0·34 (C). R. E. S.

CHEMOTHERAPY

Antirabies Vaccine for Human Use. F. B. Peck, Jr., H. M. Powell and C. G. Culbertson. (*J. Lab. clin. Med.*, 1955, **45**, 679.) A rabies vaccine prepared from embryonated duck eggs compared favourably with commercial vaccine from brain tissue, when tested in mice and monkeys. In dogs, a dose of 3 ml. of duck embryo vaccine produced the same percentage of antibody responses as a 5-ml. dose of commercial vaccine. During a clinical trial of duck embryo vaccine, antibody responses were demonstrated in 12 out of 13 subjects tested. The vaccine contained little if any of the brain antigen which is present in the rabbit brain vaccine, which may cause allergic encephalomyelitis. Duck embryo vaccine should not be given to persons sensitive to chicken egg albumin, on account of its antigenic similarity to duck egg albumin. G. B.

Cycloserine, Structure and Reactions of. P. H. Hidy, E. B. Hodge, V. V. Young, R. L. Harned, G. A. Brewer, W. F. Phillips, W. F. Runge, H. E. Stavely, A. Pohland, H. Boaz and H. R. Sullivan. (*J. Amer. chem. Soc.*, 1955, **77**, 2345.) Cycloserine is a new broad spectrum antibiotic elaborated by the soil organism *Streptomyces orchidaceus*. Isolation from culture filtrates was accomplished by absorption on anion exchange resins, elution with dilute mineral acid, and formation of a crystalline silver salt ($C_3H_5N_2O_2Ag$), from which the crystalline antibiotic was obtained as fine white needles from aqueous ethanol ($C_3H_6N_2O_2$), m.pt. 156° C. (decomp.) $[\alpha]_{5461}^{25}$ 137 ± 2° (*c*, 5 in



(I)

2N NaOH), $[\alpha]_D^{25}$ 112°, (*c*, 5 in 2N NaOH). Potentiometric titration (pKa′ 4·4 and 7·3) indicates that cycloserine exists in aqueous solution as a dipolar ion. These data, together with the infra-red spectrum, are consistent with structure I, D-4-amino-3-isoxazolidinone, for cycloserine. The reactions of cycloserine are recorded. A. H. B.

PHARMACY

NOTES AND FORMULÆ

Ascorbic Acid in Liquid Media, Stability of. F. J. Bandelin and J. V. Tuschhoff. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 241.) Solutions containing ascorbic acid were assayed by the dichlorophenolindophenol method before and after storage at 25° C. and 40° C. for various periods up to 3 years. The effect of varying the *p*H and degree of aeration and of the presence of other substances was studied. The rate of decomposition was greatest in weak solutions, and increased with increasing *p*H value, being markedly accelerated above *p*H 4·0. Sucrose, sorbitol and propylene glycol had a stabilising effect, and ethanol, corn sugar and dextrose were less effective. Tragacanth, pectin, carboxymethylcellulose and methylcellulose, added to the solutions to increase their viscosity and thereby hinder oxygen exchange, appeared to accelerate the decomposition. The stability of ascorbic acid in syrup or sorbitol was increased by the presence of synthetic compounds of the vitamin B complex. G. B.

Carrageenin, Emulsifying Properties of. B. W. Fitzgerald and D. M. Skauen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 358.) Carrageenin (purified dry extract of Irish moss) was mixed with water in a mortar and allowed to stand for one hour to form a suitable mucilage. Oil was added in small quantities, triturating after each addition. The coarse emulsion formed by this process was improved by passing through a homogeniser or colloid mill. The best results were obtained with mucilages containing 0·2 to 0·4 per cent. of carrageenin. The emulsions were inferior to those prepared with acacia or, in the case of benzyl benzoate, with soap. Good results were obtained by using 0·4 per cent. of carrageenin to emulsify oil of turpentine. Since very low concentrations of carrageenin are required it is suggested that economies may be effected by using it in combination with other substances to reduce the total quantity of emulsifying agent required. G. B.

Dihydroxy Aluminium Sodium Carbonate, Preparation and Properties of. I. W. Grote, J. M. Holbert and M. Fox. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 219.) Dihydroxyaluminium sodium carbonate was prepared by dissolving 42 g. of sodium bicarbonate in 350 ml. of warm water, and adding 102 g. of aluminium *iso*propylate, stirring vigorously. Stirring was continued until the precipitate became granular, when it was separated by filtration, washed to remove traces of sodium bicarbonate, and dried to constant weight. The substance was non-toxic when administered orally. When tested for antacid effect it rapidly neutralised the added acid and maintained the reaction above *p*H 3 for 2 hours under the conditions of test. The form of the neutralisation curve differed considerably from that given by a mixture of equimolecular quantities of sodium bicarbonate and dried aluminium hydroxide gel. G. B.

PHARMACOLOGY AND THERAPEUTICS

Aspirin and Ulcer. A. Muir and I. A. Cossar. (*Brit. med. J.*, 1955, **2**, 7.) The object of this investigation was to study the possible harmful effects of aspirin on the stomach, particularly in patients with peptic ulcer. As a result of fractional test meals and fæcal occult blood tests carried out on 20 patients suffering from peptic ulceration it was shown that aspirin tended to increase gastric acidity, and that evidence of gastric irritation, as shown by the presence of bloodstained mucus in the test-meal specimens, was commonly present. In order to study directly the effect of aspirin on the stomach 2 uncrushed 5-grain aspirin tablets were given with a little water to a series of patients 2 hours prior to gastrectomy for peptic ulceration. Three groups of 20 patients were given respectively tablets of an ordinary commercial brand of aspirin, tablets of a specially hard type, and tablets of soluble calcium aspirin. In the first group 12 patients showed evidence of mucosal irritation (3 severe); in the second group 8 showed typical acute erosive gastritis (moderately severe in 5); in the third group, receiving soluble aspirin only, 2 showed a very mild reaction, not regarded as abnormal. The specimens in this series showing the most severe acute erosive gastritis were all from patients with duodenal ulceration. Moreover, the erosions were obviously the result of the local irritant action of aspirin since they often took the shape of the aspirin granule, which remained *in situ*. Prior to the carrying out of clinical investigations 300 people were questioned about aspirin intolerance, without reference to their previous medical history. Approximately 1 in 20 confessed that aspirin gave them dyspepsia-heartburn, sometimes nausea, and occasionally epigastric discomfort. Of a series of 318 patients suffering from peptic ulcer who were questioned as to aspirin intolerance 110 were well aware that aspirin could only be taken at the risk of dyspepsia. Of 83 patients suffering with recurrence of ulcer pain 34 admitted to taking aspirin less than 24 hours before the recurrence of symptoms. Over a period of 7 years 15 patients with major dyspepsia were seen who were habitual aspirin takers and who were cured of their dyspepsia by its withdrawal. Of 166 cases of hæmatemesis, in 21 aspirin was clearly indicated as the major factor in precipitating the hæmorrhage. The authors conclude that aspirin should never be given to patients with peptic ulceration or to any who have gastric intolerance to it, however mild, and such an instruction should be given a prominent place in peptic ulcer advice charts. Calcium aspirin, especially the soluble form, does not have the same irritant action and can be used with impunity. S. L. W.

Bacterial Pyrogens on Splanchnic Metabolism and Cardiac Output, Effect of Subfebrile Doses of. L. W. Hamrick, Jr., and J. D. Myers. (*J. Lab. clin. Med.*, 1955, **45**, 568.) Erratic results are occasionally obtained in the study of splanchnic metabolism by the sulphobromophthalein extraction method, especially after intravenous injections of various materials, and since fever-producing doses of pyrogens increase hepatic blood flow and decrease removal of the dye by the liver an investigation was made to ascertain whether small doses of pyrogens, insufficient to produce fever, affected splanchnic metabolism. The pyrogenic material used was Pyromen, a purified polysaccharide from *Pseudomonas pyocyanea*, which was given intravenously in doses of 5 μg., and the study was carried out on 21 healthy hospital patients in the fasting state. Hepatic blood flow was determined by the sulphobromophthalein method, and the sulphobromophthalein clearance in ml./minute/sq. metre of body surface area was derived from the ratio of the clearance rate to the arterial

concentration. Splanchnic oxygen consumption was derived from the product of the hepatic blood flow and the average of two determinations of the arterial-hepatic venous oxygen difference. Cardiac output was determined by the dye-dilution technique using azovan blue. In 3 patients studied before, and 1½ hours after, pyrogen administration the sulphobromophthalein clearance decreased, but there was no other consistent alteration. In 11 patients observed 2 or more hours after pyrogen administration there were striking changes from the values obtained in 63 controls. The mean decrease in sulphobromophthalein clearance was 33 per cent., the mean elevation of hepatic blood flow was 47 per cent., and the mean increase in splanchnic oxygen consumption was 18 per cent. Splanchnic glucose production was unaffected. The elevation of hepatic blood flow was not accompanied by a proportionate increase in cardiac output. Contamination of parenterally injected materials with small amounts of bacterial pyrogens is therefore a possible cause of erratic alterations of splanchnic metabolism. H. T. B.

Barbiturate Intoxication, New Treatment of. A. Shulman, F. H. Shaw, N. M. Cass and H. M. Whyte. (*Brit. med. J.*, 1955, **1**, 1238.) The treatment suggested involves the use of two new barbiturate antagonists, β-methyl-β-ethylglutarimide (bemegride NP13) and 2:4-diamino-5-phenylthiazole hydrobromide or hydrochloride (amiphenazole D.A.P.T.). Bemegride is the more active antagonist but amiphenazole, although a weak antagonist, is a good synergist to bemegride and an excellent respiratory stimulant. High dosage of the substances, especially of bemegride, can cause convulsions in normal or barbiturized animals but virtually no signs of toxicity were observed in any of the authors' series of 41 cases. One death occurred but was not thought to be due to the treatment. The recommended treatment of barbiturate poisoning is as follows. If laryngeal and pharyngeal reflexes are absent, as ascertained by passing a laryngoscope, a cuffed tube is inserted and the stomach contents aspirated, but gastric lavage is not attempted. A clear airway and adequate oxygenation are ensured. Bemegride is given as a 0·5 per cent. solution in normal saline, and amiphenazole as a 1·5 per cent. solution in saline. A 5 per cent. dextrose intravenous drip is set up and every 3 to 5 minutes, by means of two 20 ml. syringes, there is injected into the rubber tubing of the apparatus 1 ml. amiphenazole solution and 10 ml. bemegride solution. If the response causes concern, dosage is halved. The injections are continued until the patient is brought to a "safe state," a state of light anæsthesia denoted by a return of tone and reflexes; in a deeply comatose patient this may take 2 hours. Total dosage of 200 ml. bemegride solution and 20 ml. amiphenazole solution is usually adequate. Once the safe state is attained, the endotracheal tube is removed and the patient treated as if recovering from light anæsthesia. Toxic effects are unlikely with the specified dosage. Vomiting and retching, perhaps with slight flickers of the fingers, are the earliest signs; if they occur treatment is suspended, and if they are severe a small intravenous dose of thiopentone sodium is given. If the patient's condition regresses after reaching the safe state, further treatment is necessary. The advantages of this procedure are that it minimizes the duration of endotracheal intubation, it minimizes the immediate and remoter risks to the patient's life and it avoids the need for prolonged strict nursing. H. T. B.

Bis-Quaternary Hypotensive Agents, Sites of Action of. C. J. Cavallito, A. P. Gray and T. B. O'Dell. (*Arch. int. Pharmacodyn.*, 1955, **101**, 38.) A study of a series of 1:3-bis-quaternary ammonium compounds of type $A^{-}(CH_2)_3B^{+}$ are described in which A is a β-carboline nucleus joined through

the pyrido-*N* and B is a small cationic group. Compounds in which B is smaller than triethylammonium are potent hypotensive agents with predominantly a central action accompanied by varying degrees of peripheral activity. The trimethylammonium compound has an intense hypotensive activity mediated through a probable central action and ganglionic blockade. The *N*-methyl pyrrolidinium derivative shows a greater central activity and less ganglionic blocking activity. The triethylammonium derivative shows little central and ganglionic activity but parasympathetic blocking activity is still present. The diethylmethyl derivative shows intermediate activity. The relationship of chemical structure to pharmacological activity is described. G. F. S.

Chlorpromazine in Acute Alcoholism. E. H. Mitchell. (*J. Amer. med. Sci.*, 1955, **229**, 363.) In the treatment of 400 cases of acute alcoholism equally divided into chlorpromazine-treated and barbiturate-treated groups the following procedures were carried out. On admission the patients in the chlorpromazine group were given 50 mg. intramuscularly or intravenously; after this they were given 50 to 100 mg. orally every 4 hours. The patients in the barbiturate group were given 250 mg. of quinalbarbitone intravenously and 60 mg. of butobarbitone and 1·5 g. of mephenesin every 3 hours until bedtime. In the chlorpromazine group effective sedation was achieved in 85 per cent. of the patients. Moreover, in this group, as compared with the barbiturate group, the nursing was simplified in that the patients were more amenable to suggestions, there were fewer falls from bed, the patients could retain food sooner, and they were ready to be discharged from the hospital on an average 24 hours earlier. There was no appreciable difference in the readmission rate for the two groups. In the patients receiving chlorpromazine mild postural hypotention was noted in 43 cases, and severe hypotention with syncope in 7 additional cases. There was one death in this series in a poor-risk cardiac patient with a cirrhotic liver who died suddenly following an attack of syncope 2 days after admission. Tachycaria was noted in 22; the heart rate returned to normal on discontinuance of chlorpromazine. S. L. W.

Chlorpromazine in Psychiatry. G. F. Vaughan, D. M. Leiberman and L. C. Cook. (*Lancet*, 1955, **268**, 1083.) The effects of chlorpromazine on 224 recent and chronic neurotic and psychotic patients were studied. The drug was given either by intramuscular injection, followed by oral administration of tablets, or by the oral route alone. With the former method, up to 150 mg. daily, usually in three doses, was injected for 4 days. Owing to the low *p*H the solution is irritant to the tissues, but if the drug is given by deep intramuscular injection there are few complaints of soreness, though indurated areas are produced in some and may persist. In 1 case out of 33 so treated a cold abscess formed. There would appear to be some evidence that the parenteral route may be more potent than the oral. Dosage by mouth was started at 75 or 150 mg. daily in 3 doses, and varied according to response up to 450 mg. daily. Chlorpromazine is not a curative drug but it effectively controls the symptoms of psychomotor over-activity, aggressiveness, agitation, and psychitic tension, and is particularly valuable in the treatment of chronic over-active deteriorated schizophrenics, mania, schizophrenic excitement, and patients who are unsuitable for electroconvulsion therapy or relapse after an initial improvement after it; it is not a substitute for this latter therapy or other forms of physical treatment where these are indicated. It is of considerable value in the large mental hospital ward; patients who are a continual source of disturbance may become quiet, co-operative, occupied and able to leave the hospital for short periods of

leave. Of the 224 patients in this series 22 per cent. developed sufficiently severe complications to stop treatment. Major symptoms occurring either alone or in combination were severe collapse (9), persistent pyrexia (8), persistent pains in legs or abdomen (7), persistent rashes (4), gross confusion (4), severe headaches (4), jaundice (3), vomiting (3), severe nausea (3), persistent tachycardia (3), constipation (3), convulsions (2), facial œdema (2), parotitis (2), severe cyanosis of the legs and trophic ulcers (2), marked depression (2), cardiac failure and œdema of the ankles (2), auricular fibrillation (1), diarrhœa (1), and hæmatemesis (1). The authors conclude that chlorpromazine is a valuable though limited addition to the therapeutic armamentarium of the psychiatrist. S. L. W.

Cortisone in Pyrogen-induced Fever, Antipyretic Action of. E. Atkins, F. Allison, Jr., M. R. Smith and W. B. Wood, Jr. (*J. exp. Med.*, 1955, **101**, 353.) The authors have investigated the 3 possible ways in which, on the basis of existing knowledge, cortisone may act in suppressing a febrile response to pyrogens. It may interfere with the leucopænia which precedes the onset of fever. It may alter the composition of the recipient's serum and thus modify the effect of the serum factor on which the action of pyrogen appears to depend. Thirdly, it may influence some later stage of the fever-producing sequence. The pyrogens used were Pyromen, a purified polysaccharide from a *Pseudomonas* species, and native dextran having an average molecular weight between 200,000 and 300,000, the dose (5 μg./kg. body weight of Pyromen and 200 mg. of dextran) being sufficient to give a 1° to 2° F. temperature rise in normal rabbits. Using 10 control rabbits, and 10 rabbits given 25 mg. of cortisone intramuscularly twice a day for 3 days preceding the experiment and a further 25 mg. on the morning of the experiment, it was found that the temperature response to Pyromen was effectively blocked whereas the fall in leucocyte count was not significant. With dextran the temperature response of 7 untreated rabbits was greater than the response to Pyromen but the response in the cortisone-treated rabbits was even more effectively blocked while the fall in the leucocyte count was the same in each group. It follows that the antipyretic effect of cortisone is not due to any effect on the reaction of leucocytes to the injected pyrogen. The serum factor shortens the time lag between injection of a pyrogen and the resulting rise in temperature; it is present in normal serum but not in the serum of animals rendered tolerant to pyrogens by a course of injections. In order to determine whether cortisone has any effect on the serum factor, experiments were carried out to determine whether there was a depression of the activity of serum factor in cortisone-treated rabbits and whether cortisone failed to suppress a response to pyrogen previously exposed to the active factor. In the first experiment, a pyrogen consisting of typhoid vaccine incubated with serum from cortisone-treated rabbits was injected into 7 rabbits rendered tolerant to the vaccine by daily injections. There was a marked shortening in the duration of the latent period but the shortening was the same as was obtained with a similar vaccine incubated with serum from normal rabbits, showing that cortisone does not significantly alter the serum factor activity. In the second experiment, cortisone-treated rabbits were challenged with typhoid vaccine incubated in normal serum, the results being compared with those obtained previously on the same animals using a suspension of typhoid bacilli in pyrogen-free saline. Cortisone was just as effective in suppressing the fever due to the incubated vaccine as that due to the saline suspension, showing that the antipyretic effect is not due to any action on the serum factor. It follows by exclusion that the antipyretic action of cortisone is exerted at some later stage of the fever-producing mechanism. H. T. B.

Deoxycorticosterone, Effect of, on the Reticulo-endothelial System. T. Nicol and R. S. Snell. (*Nature, Lond.*, 1955, **175**, 995.) Deoxycorticosterone acetate had little or no effect on the activity of the reticulo-endothelial macrophages in the spleen, liver and lymph-nodes of the guinea-pig. In this respect it differed from cortisone, which depressed phagocytic activity, especially in the spleen. The state of activity of the macrophages was measured by histological examination of their uptake of trypan blue, administered daily over a period of six days before the animals were killed. Treated animals received 2 or 5 mg. deoxycorticosterone daily by intramuscular injection for one to two weeks before being killed. G. P.

Dexamphetamine, Anticonvulsant Properties of. W. M. Alexander and L. C. Weaver. (*Arch. int. Pharmacodyn.*, 1955, **100**, 472.) Experiments in mice show that dexamphetamine itself has no anticonvulsant properties against electrical and chemical induced convulsions, and in conjunction with phenobarbitone did not alter the anticonvulsant potency of this barbiturate. It did reduce the toxicity of phenobarbitone, thus improving its safety index. G. F. S.

Dimercaprol, Hypoglycæmic Effect of. W. J. H. Butterfield. (*Lancet*, 1955, **268**, 489.) 15 severely burned patients were observed to develop hyperglycæmia and glycosuria about 5 days after their injury when force-fed to offset the expected loss of weight. Abnormal carbohydrate metabolism lasted for days, weeks, or months, the duration being shorter in children than adults. The abnormality seemed to be related at first to increased adrenocortical activity. The hyperglycæmia was shown to be insulin-resistant, the resistance being most pronounced within 3 weeks of injury, and less from 3 to 13 weeks after injury, the diabetes becoming insulin-sensitive if it lasted over 13 weeks. Since it has been suggested that the effects of increased adrenocortical activity in animals may be related to altered thiol (SH) metabolism, and since several SH enzyme systems take part in glycolysis, it was decided to study the effect of dimercaprol, an SH compound, on the glucose tolerance of burned patients. Dimercaprol, 250 mg. in oil intramuscularly, was given to 9 burned patients with hyperglycæmia at the start of a glucose tolerance or a glucose-insulin-tolerance test. The results showed that the dimercaprol injection slightly improved glucose tolerance in these patients when given alone in the early phase of greatest insulin resistance and when given with insulin from 3 to 13 weeks after injury. In these patients the blood keto-acid levels showed a smaller rise an hour after the start of tests involving dimercaprol than in corresponding tests without dimercaprol. Longer trials were then carried out with 7 diabetics whose daily insulin requirements varied from 24 to more than 320 units; in 2 cases the insulin requirements were sufficiently high to indicate insulin resistance. After control periods lasting from 2 to 5 days the patients were given intramuscular dimercaprol 5 per cent. in oil 4 ml. four times daily for 3 days and insulin. In all but 2 cases the glycosuria diminished on the 3rd day of treatment with dimercaprol and insulin; the same trends were observed in the blood sugar levels. Preliminary studies of blood-pyruvate levels showed high values an hour after insulin was given in the glucose-insulin-tolerance tests; these values were lower after dimercaprol treatment. In one of the 2 insulin-resistant cases on a diet excluding the supplementary glucose there was a daily urinary excretion of 11 g. of glucose on insulin 280 units a day; on the same amount of insulin, with 4 ml. of dimercaprol 5 per cent. in oil daily, the glucose excretion was 8 g.; and this was reduced to 2 g. when the insulin was given with 8 ml. of dimercaprol daily. It would seem therefore that dimercaprol given in conjunction with insulin may improve glucose tolerance in the circumstances of these investigations, especially in patients with possible insulin resistance. S. L. W.

Noradrenaline and Adrenaline in Urine, Excretion in Normal and Pathological Conditions. A. Pekkarinen and M-E. Pitkänen. (*Scand. J. clin. Lab. Invest.*, 1955, **7**, 8.) Using the fluorescence method described in *Scand. J. clin. Lab. Invest.*, 1955, **7**, 1 the mean daily excretion of adrenaline and noradrenaline (as noradrenaline equivalent) in the urine of normal, healthy adults was 81 μg. The individual results varied between 31 and 185 μg. The concentration in the urine varied between 3 and 17 μg. per cent. In 26 patients with hypertension and some other pathological conditions, one had a paraganglioma with a urinary excretion of 1354 μg. of noradrenaline per day. In two other patients there was an increased output but the other 23 showed a normal excretion. In patients receiving an infusion of noradrenaline in surgical shock, a small percentage of the amount of noradrenaline infused was found in the urine during the following 3 days. M. M.

Pentolinium Tartrate in the Long-term Treatment of Hypertension. A. Agrest and S. W. Hoobler. (*J. Amer. med. Ass.*, 1955, **157**, 999.) 31 patients with severe or complicated hypertensive disease were treated with orally administered pentolinium tartrate, with or without reserpine. Treatment was started by the administration of 20 mg. of pentolinium tartrate at 8 a.m., 3 p.m. and 10 p.m., the dose being increased by 20 mg. increments daily until a lowest systolic blood pressure of 110 to 130 mm. Hg with the patient standing was achieved consistently. It was later found possible to lower this minimal systolic blood pressure to 90 to 100 mm. Hg if the medicament was well tolerated. Constipation enhanced the effect of the drug and strict attention to bowel function early in the treatment was most important. The combined effects of a dose of pentolinium tartrate taken in the morning plus the noon meal produced a blood pressure "trough" at about 2 to 3 p.m. if the drug was taken immediately before breakfast and luncheon is eaten at 1 p.m. A 20 mg. increment in the dose was recommended if "trough" blood pressures had fallen almost to the desired level. Doses were increased by 20 mg. at 3-day intervals if results were unsatisfactory. Reserpine, 0·25 mg. 3 times daily, was also given in most cases. Hydrallazine was occasionally given in addition to pentolinium, but it rarely produced additive effects and its use was abandoned. A median reduction of 38 mg. Hg in the mean daytime standing blood pressure and of 23 mm. Hg in the mean recumbent blood pressure was estimated to have occurred in this series, and a notable improvement in the manifestations of congestive heart failure and of hypertensive retinopathy was observed; the effect on cerebrovascular manifestations was less certain but seemed beneficial. No marked relief of moderate uræmia or of angina pectoris was seen. There were no serious complications, and tolerance was slow to develop. S. L. W.

Rauwolfia in Hypertension. S. Locket. (*Brit. med. J.*, 1955, **1**, 809.) This is a report on a group of 39 severely hypertensive patients (38 of whom had essential hypertension) treated for a minimum period of 6 months and a maximum period of 20 months with oral preparations of rauwolfia. All the patients were ambulatory and all had a minimum diastolic pressure of never less than 130 mm. Hg. Each patient was used as his own control, the treatment being alternated between active drug and control tablets without his, or the clinician's, knowledge. In the first 10 months of the trial the active preparation used was total root extract, 1000 mg. daily; subsequently total active alkaloids were used, 8 mg. daily. 16 of the patients received, in addition, 8 to 10 mg. daily of veratrum viride extract during the entire observation. Little difference in hypotensive effect could be observed between the total root extract and the total active alkaloids.

Of the 39 patients, 16 showed no consistent fall in diastolic blood pressure, 7 a slight fall, 12 an appreciable fall, and 4 a fall to below 100 mm. Hg. Of the 16 patients with no consistent fall 4 had during the entire trial received either the control tablets alone or with veratrum viride. In the other 23 cases the fall in blood pressure occurred only while the patient was receiving the active rauwolfia preparation. Of the 34 patients receiving active rauwolfia tablets at some period during the trial 22 (67 per cent.) showed a fall in blood pressure of varying degree whilst taking the active preparation but not when on control. The combination of veratrum viride extract and rauwolfia gave 6 patients out of 13 who showed a hypotensive effect, as against 16 who showed a fall in blood pressure out of 21 receiving rauwolfia alone. The average age of patients who failed to respond to rauwolfia was 50 (range 34 to 64) whereas the average age of all patients who responded was 56 (44 to 64). There was evidence of a better response in males than in females. In every case in which the patient responded to the drug there was a delay in onset of the hypotensive effect, usually of 7 to 14 days, but sometimes as long as 4 weeks. In about half the patients several further weeks' treatment were necessary before the diastolic blood pressure reached its lowest level. Side-effects were seldom severe enough to necessitate cessation of treatment. Those complained of included diarrhœa, depression, fatigue, lack of energy, drowsiness, visual disturbances, fullness in the head, nasal congestion, nausea and vomiting. All patients with angina of effort found it necessary to continue to use nitroglycerin. The author concludes that rauwolfia is by far the most effective and useful orally administered hypotensive agent he has yet used, and is worthy of trial in every case of essential hypertension requiring treatment. S. L. W.

Tetracycline, Clinical Report on. R. L. McCorry and J. A. Weaver. (*Lancet*, 1955, **268**, 1102.) *In vitro* studies of antibacterial activity showed that tetracycline is effective against both Gram-positive and Gram-negative organisms, though the latter tend to have higher minimal inhibitory concentrations than do the former. Among the most sensitive organisms are certain strains of staphylococci, the pneumococci, the β-hæmolytic streptococci, some of the α-hæmolytic streptococci, and some enterococci. Among the resistant organisms are all strains of *Ps. pyocyanea* and some strains of *Staph. pyogenes*, *Strep. fæcalis* and *E. coli*. Most of the 87 patients in the series under review were treated for acute infections of the respiratory system. The best form of dosage was found to consist of an initial loading dose of 1 g. followed by 0·25 g. 6-hourly; this mode of treatment produced good blood levels. In pneumoccal lobar pneumonia the results obtained were good and in every respect equal to those following the use of chlortetracycline and oxytetracycline. On acute bronchopneumonia the results were variable and difficult to assess, but generally the results were again comparable with those of the two analogues. Of the 39 cases of pneumonia of all types it was considered that in 10 cases the response had been less than expected. In 17 cases of chronic bronchitis a good response was obtained in 13, the results being usually better than those following inhalational therapy with either streptomycin or penicillin. In 8 cases of bronchiectasis there was temporary improvement, with lessening of sputum in 6. The side-effects observed were mainly intestinal, flatulence, nausea and vomiting, diarrhœa, and pruritus ani. The authors conclude that tetracycline justifies further trial in that the incidence of side-effects appears to be less than that with the other substances of the tetracycline series and it seems to be quite as effective clinically. They consider, however, that penicillin should still remain the drug

(ABSTRACTS *continued on p. 968.*)

LETTER TO THE EDITOR

A New Graphical Way to Indicate the Preparation of Solutions Isoosmotic with Blood, Tears and Tissue

SIR,—In the Danish Pharmacopœia and addenda graphs are given, which for a large number of compounds permit the preparation of solutions which are isoosmotic with blood, tears and tissue by indicating the addition of suitable amounts of sodium chloride or in a few cases of potassium nitrate. The curves are based largely on experimental evidence given by Lund, Peülicke Nielsen and Pedersen-Bjergaard[1], who have also proposed the graphical way in which the data are presented. For each compound the concentration of aqueous solutions is plotted as ordinate against the freezing point depression, together with a curve for sodium chloride (potassium nitrate) showing the concentration plotted against the difference between 0·52° C. and the freezing point depression. Several curves are drawn on the same graph in order to save space. A vertical line which meets the curve for a certain compound at the ordinate corresponding to a prescribed concentration will meet the sodium chloride (potassium nitrate) curve at an ordinate that indicates the concentration of a sodium chloride (potassium nitrate) solution in which the prescribed compound should be dissolved in order to obtain a solution isoosmotic with blood, tears or tissue.

Another way of presenting the same data in a pharmacopœia is to furnish the monograph of each substance from which eye-drops or injections are prepared with a graph, which directly shows the composition of solutions of any concentration of the compound in question, that are isoosmotic with blood, tears and tissue (Fig. 1). These graphs may be used in the following way.

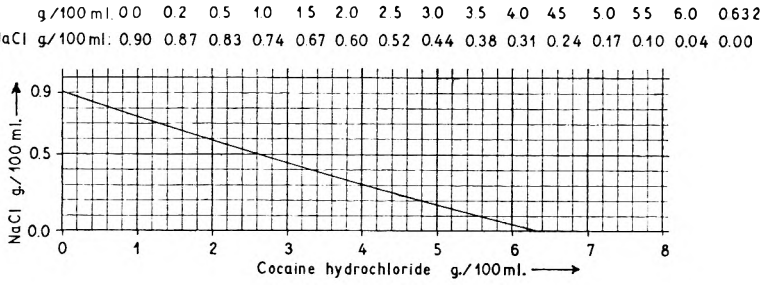


FIG. 1.

(*a*) *Solutions of a single prescribed compound.* The concentration of the described compound (in g./100 ml.) is sought along the abscissa. The ordinate of the curve corresponding to this abscissa gives the amount of sodium chloride (potassium nitrate) in g./100 ml. which has to be added to obtain a solution which is isoosmotic with blood.

(*b*) *Solutions containing more than one prescribed compound.* If the solution contains *n* prescribed compounds, the amounts of sodium chloride to be added in order to make a solution of each single compound in the prescribed concentration isoosmotic with blood, are found as described under (*a*). The sum of the found *n* amounts of sodium chloride in g./100 ml. minus (*n*-1) 0·9 g./100 ml. gives the amount of sodium chloride which renders the prescribed solution isoosmotic with blood.

The amount of sodium chloride (potassium nitrate) to be added to any

solution for which a curve is shown may also be given in an accompanying figure, such as that illustrated, though this is not imperative.

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BODIL JERSLEV.

Royal Danish School of Pharmacy,
Copenhagen Ø.
August 12, 1955.

REFERENCE

1. Lund, Peülicke Nielsen and Pedersen-Bjergaard, *The Preparation of Solutions Isoosmotic with Blood, Tears and Tissue.* Contribution from The Danish Pharmacopœia Commission, Vol. II, 1947.

(ABSTRACTS *continued from p. 966.*)

of choice in the treatment of pneumonia and that tetracycline should be reserved for cases that do not respond to penicillin or where the causal agent is penicillin-resistant. S. L. W.

Toxoids, Enhancement of Antigenic Activity by Sympathomimetic Drugs. J. Ungar. (*Brit. med. J.*, 1955, **2**, 20.) The investigation was conducted with purified diphtheria toxoid, the sympathomimetic drugs employed being (1) adrenaline hydrochloride, 1 : 1000 or 1 : 2000; (2) amphetamine hydrochloride, 1 : 250; and (3) ephedrine hydrochloride, 1 : 250. In addition, two amines with no vasoconstrictor action, β-phenylethylamine, 1 : 250, and phenylethylamine, 1 : 250, were tested but were found to be without stimulating effect on antigenicity. The method of testing the antigens was that used in earlier experiments (Ungar, *Proc. roy. Soc. Med.*, 1954, **47**, 355). The results were as follows:

MEAN ANTITOXIN TITRES IN GROUPS OF 10 GUINEA-PIGS AFTER GIVING PURIFIED DIPTHERIA TOXOID WITH VARIOUS SUBSTANCES

Group No.	Substance added to toxoid	Antitoxin Response (u./ml.) (Geometric mean of 10 animals)
1	Control, plain toxoid	0·25
2	β-Phenylethylamine, 1 : 250	0·17
3	Phenylethylamine acetate, 1 : 250	0·23
4	Amphetamine hydrochloride, 1 : 250	1·96
5	Ephedrine hydrochloride, 1 : 250	2·21
6	Adrenaline, 1 : 1000	2·87
7	Adrenaline, 1 : 2000	3·36
8	Histamine, 0·5 μg./ml.	0·70

Doses: 1 ml. of diphtheria toxoid (2·5 Lf./ml.) injected subcutaneously. Two doses with four-weeks interval. Animals bled 2 weeks after second dose.

The amount of vasoconstrictor added to the toxoid must be carefully chosen since higher concentrations of vasoconstrictors than those quoted may cause local tissue damage. The results show that vasoconstrictors have a considerable enhancing effect on the antigenic action of diphtheria toxoid. As adrenaline hydrochloride is stable only in an acid medium it cannot be kept in contact with the toxoid for any length of time. On the other hand, ephedrine and amphetamine, being stable at a neutral *p*H, can be mixed with the toxoid and stored at a suitable temperature. The addition of a vasoconstrictor to a toxoid may not only be useful for immunising children against diphtheria, but has the additional advantage that its anti-allergic action would be of value for children subject to allergic manifestations after injections of antigens. S. L. W.

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