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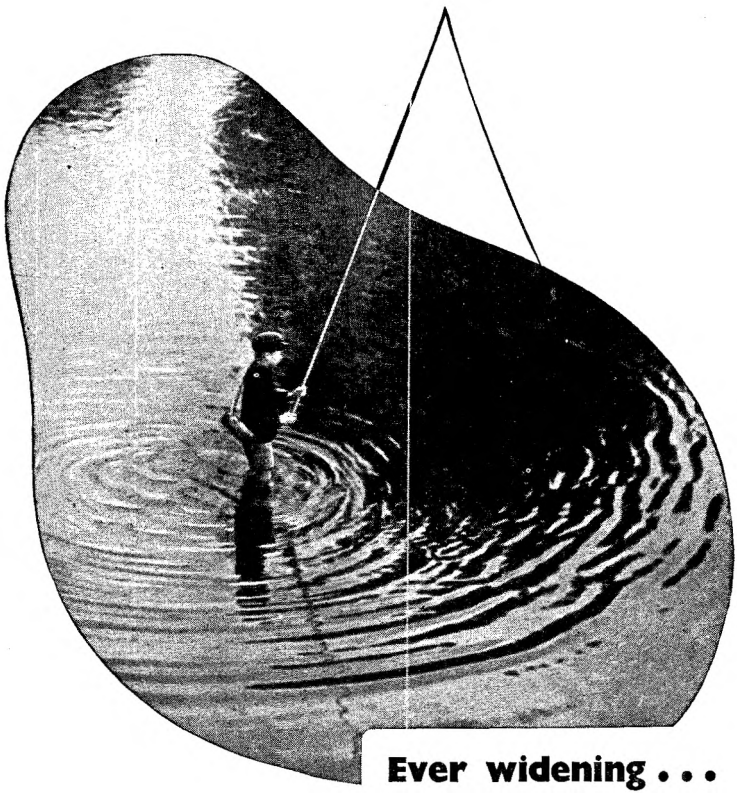

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CONTENTS

REVIEW ARTICLE

GLASS FOR PHARMACEUTICAL PURPOSES

By Violet Dimbleby, M.Sc., F.S.G.T.
From the Department of Glass Technology, University of Sheffield

Introduction

To most of us the term "glass" implies a brilliant, brittle, amorphous substance, usually transparent but sometimes translucent, and capable of production in many colours. Specimens have come down to us through centuries without being seriously impaired in appearance as may be seen in the windows of cathedrals and churches, or in museums. The widespread use of glass at the present day, as for instance in windows, building blocks, mirrors, cooking vessels, optical and artistic ware and containers, testifies to its possession of some great advantages as compared with other materials. The survival of window glass through the centuries at once indicates resistance to the chemical action of atmospheric agents as well as mechanical strength. Some of these old glasses have, however, become dimmed or covered by a thin, whitish or irridescent film, due to surface decomposition. In nature many rocks slowly disintegrate, as instanced by the slow decomposition of felspars into clays of much lower alkali content, so it is perhaps not surprising that the "silicate" glasses suffer slow change when exposed to all weathers over a long period.

But to-day, glasses are called upon to give good service under a great variety of conditions, some of which may involve exposure to chemical reagents, or to water and steam at temperatures well above the normal boiling point of water, or to the action of solutions over long periods, often in tropical climates. Again, some glasses are required to exercise selective absorption of radiations as for protection against ultra-violet or infra-red rays, or in the production of definite colours.

Under all these many and varied conditions it is essential that glasses retain their characteristic brilliance, but the pharmaceutical chemist makes even greater demands. In addition, he requires glass containers to preserve, without contamination, preparations differing considerably in chemical composition. This involves careful cleaning and sterilisation of the containers before use, and glass is satisfactory in all these respects. But commercial glasses, like many other materials, differ in chemical composition and as a result they differ in their properties, including absorption of radiation and chemical behaviour, two properties of paramount importance to the pharmaceutical chemist. Other factors such as mechanical strength, resistance to thermal shock, ease of moulding into pleasing yet functional shapes capable of secure and ready closure, and steady supply at a reasonable price are also of importance but not peculiarly to the pharmaceutical trade. In this review, therefore, attention will primarily be paid to the consideration of chemical resistance

and absorption of radiation, but it may be well to note here that, broadly speaking, glasses which exhibit the greatest all-round chemical resistance also have great thermal resistance and mechanical strength. They are not, however, as easy to melt and mould, nor are they as cheap to produce, as glasses of higher alkali content but resultant lower chemical stability.

Typical Chemical Compositions and Coefficients of Linear Expansion of Glasses

Although several substances, when fused, can form glasses, the glasses of commerce are produced by fusing at 1300° C, or above, silica in the form of sand, with two or more other materials such as, for instance, limestone and soda ash in the production of containers, and red lead with potash for high quality artistic "crystal" ware. Other constituents may be barium oxide, alumina, zinc oxide, magnesia, boric oxide, lithia, arsenic or antimony oxides, with oxides of copper, cobalt, iron, manganese, chromium, and nickel, as colouring agents. Selenium and cadmium, gold, or copper are used in making ruby glasses. Optical glasses and glasses used in the electrical industry differ widely in composition but need not receive further notice here.

A few typical percentage compositions with some coefficients of linear expansion of glasses are given in Tables I and II, from which it will be seen that container glasses are generally of the simple soda-lime-magnesia-alumina-silica type, with relatively high expansion coefficients, whilst "neutral" tubing for ampoules may contain considerably more alumina, with boric oxide, and lower alkali content and somewhat lower expansion. "Lead crystal" is of an entirely different composition, whilst chemical or cooking ware generally contain several per cent. of boric oxide with low alkali but high silica content and much lower expansion coefficient. The resistant glass "Vycor" approaches the composition of fused silica, and the special conditions necessary to the production of these two glasses render them too expensive for general use as containers. Moreover, fused silica cannot be "worked" or moulded as easily as the more usual glasses. Of all the glasses which are commercially available it has the lowest coefficient of expansion and is famed for its thermal shock resistance.

The Chemical Behaviour of Glass

(1) *Historical Note.*

During the last 50 years much research work has been directed towards an understanding of the chemical behaviour of glass, in fact the literature of this subject is now vast. The superiority of glass over competing materials as containers for foods and pharmaceutical preparations, enabling the purchaser to see what he buys, and at the same time preserving the contents uncontaminated, testifies to the resistance offered by glass surfaces to decomposition when in contact with atmospheric agents (mainly moisture), as well as with solids and liquids differing widely in chemical composition, some solutions being neutral, others alkaline, or acidic. But, as has already been stated, some old glasses show a marked

TABLE I

PERCENTAGE COMPOSITIONS OF TYPICAL CONTAINER GLASSES, TUBINGS FOR VARIOUS PURPOSES, AND SOME SPECIAL PURPOSE GLASSES

Constituent oxides	Colourless containers†		Soft soda tubing* and electric bulbs	Thermometer stem‡† tubing	Colourless "neutral" ampoule*	"Neutral" amber tubing*	Electric lamp tubing† and bulbs	High pressure mercury vapour lamp* (alkali-free)
	British	U.S.A.						
SiO_2	73·4	73·3	70·12	55·7	67·0	64·1	56·6	54·25
B_2O_3	—	—	0·78	—	7·5	7·1	0·2	7·5
TiO_2	0·04	0·48	2·58	0·03	n.s.	n.s.	0·8	22·0
Al_2O_3	0·75			0·22	8·5	7·1		
Fe_2O_3	0·045			0·016	n.s.	4·1	0·05	n.s.
MnO	—	—	—	—	n.s.	1·4	—	—
CaO	8·9	5·3	5·4	0·15	4·0	6·8	—	13·25
MgO	0·1	3·9	3·6	—	0·3	0·1	—	—
BaO	—	—	—	—	—	—	—	3·0
PbO	—	—	—	31·4	—	—	30·2	—
Na_2O	15·9	16·31	16·82	0·1	8·7	6·3	5·1	nil
K_2O	0·4		0·35	12·04	4·0	3·0	7·2	nil
As_2O_5	0·01	—	—	—	—	—	n.s.	—
SO_3	n.d.	n.d.	0·20	—	n.s.	—	n.s.	—
Coefficient of linear expansion $\times 10^6$ . .	—	—	9·6	—	7·3	7·5	—	—

* "Glass, Osram, G.E.C. Glass Works Products brochure, July 1950.
† Turner, *Outline of the Properties of Glass*, 1942. Glass Manufacturers' Federation.
‡ This glass (or slight modifications of it) is the "English lead crystal" glass.
n.s. = not stated. n.d. = not determined.

TABLE II

PERCENTAGE COMPOSITIONS OF SOME CHEMICAL AND HEAT RESISTANT GLASSES AND SOME COLOURED GLASSES

Constituent oxides	Fused silica	"Vycor" (U.S.A.)*	"Pyrex"‡	"Flamerex"*	Kimble Laboratory No. N51a†	Dark green (British)*	Amber		Cobalt blue*
							Fe-Mn*	C-S*	
SiO_2	100	96·3	80·7	57·52	74·7	67·22	67·78	70·48	71·82
B_2O_3	—	2·9	12·0–12·6	5·74	9·6	—	—	—	—
TiO_2	—	—	0·05	—	5·6	0·09	0·1	—	0·03
Al_2O_3	—	0·4	2·2–3·0	19·42		1·81	1·06	2·08	1·08
Fe_2O_3	—	—	0·08	0·10		2·38	2·31		0·13
MnO	—	—	—	—	—	2·47	3·80	n.d.	0·03
CoO	—	—	—	0·01	—	—	—	—	0·29
CaO	—	—	0·20	6·53	0·9	8·14	8·58	8·85	12·54
MgO	—	—	—	9·20	—	1·47	1·32	1·38	1·13
BaO	—	—	—	—	2·2	—	—	—	—
ZnO	—	—	—	—	0·1	—	—	—	—
Na_2O	—	<0·02	3·9–4·1	1·10	6·4	14·81	13·85	15·98	11·80
K_2O	—	<0·02	—	—	0·5	0·96	0·41	0·56	0·31
As_2O_5	—	0·005	<0·01	—	0·03	—	—	n.d.	—
SO_3	—	—	—	—	Sb_2O_3 0·009	0·37	0·42	0·25	0·43
Coefficient of linear expansion × 10^6	0·5	0·8	3·3†	—	4·	<····· probably 8 to 10 ·····>			

* Turner, *Outline of the Properties of Glass*, 1942.
† Wichers, Finn and Clabaugh, *J. Res. Nat. Bur. Standards*, 1941, 26, 537.
‡ Typical results.

dimming or iridescence on the surface, and as far back as 1770, Lavoisier[1] reported that some material was extracted from glass vessels in which water was boiled for long periods. Sir Humphry Davy found that alkaline matter was extracted from glass by water, whilst later, the contamination of precipitates by matter derived from glass vessels was noted by Dumas and Berzelius. Towards the end of the nineteenth century several empirical formulæ were proposed for controlling the composition of glasses to achieve resistance to the action of aqueous solutions but now, thanks to recent researches, the atomic structure of alkali-silica glasses has been revealed and it is possible to assess, at least approximately, the nature and strength of the chemical bonds existing between the various constituents, and hence to choose that composition most likely to yield any result desired in the properties of a glass. It was not until the closing years of the last century that the boron-containing glasses were developed commercially, particularly at Jena, whilst Pyrex glass was produced at Corning, U.S.A. in 1915. A number of other new types of laboratory apparatus glass, including some of the alumina-zinc oxide-borosilicate type, appeared in Europe and in America at this period and considerable activity centred around investigations into the respective merits of these glasses and into the whole subject of the chemical properties of glasses in general.

The replacement during the last 50 years of the centuries-old "hand" methods of common glass container fashioning by automatic mechanical methods has meant modification of chemical compositions and stringent control, not only of chemical homogeneity but also of viscosity. Thus, careful technical supervision of raw materials and of every operation of manufacture is essential to satisfactory production, and not least among such controls is that of chemical properties.

(2) *Factors Influencing the Chemical Properties of Glass.*

The resistance which a glass offers to the action of any attacking agent in contact with it is generally termed the chemical durability of the glass, or precisely, its durability towards the particular agent. Methods of measuring this property will be dealt with later (page 981). Several factors influence this chemical resistance and these may be listed as:—

(A) Chemical composition.
(B) Temperature of attacking agent.
(C) Length of period of contact.
(D) Previous history of the glass, e.g., mode of production, annealing, special treatments and storage.

(*A*) *The Influence of Chemical Composition.*

(i) *Theoretical Considerations.* The most important factor controlling the chemical durability of glass towards any agent is its chemical composition, and in order to obtain some idea of the influence of the various constituents it is useful to consider briefly the atomic structure of glasses. Starting with the simple vitreous silica, this is now regarded as being a

random, three dimensional network, in which each small silicon cation is bonded tetrahedrally to 4 larger oxygen ions, each oxygen being linked to two silicon ions. Such a bonding is predominantly covalent and is strong. If, now, a flux such as sodium oxide be introduced, some of the oxygen-silicon links are broken, resulting in a weaker bonding, the alkali-oxygen bond being much more electrovalent or ionic. It is to be expected, therefore, that the greater the proportion of alkali in such a glass the less will be its resistance to chemical attack. Further, if some of the alkali be replaced by another oxide, for example, BaO, CaO, MgO, PbO, Al_2O_3, ZnO, TiO_2, the bond strengths will be altered, and, from known data which cannot be given here, in every case, the replacement of the monovalent alkali by a divalent, trivalent, or quadrivalent element results in a stronger bonding with resultant increase in chemical resistance. The acidic oxides SiO_2, P_2O_5 and B_2O_3 are network-formers, whilst the amphoteric oxides such as Al_2O_3 contain cations which can either enter the silica network or modify it. The alkali cations, and those of the basic oxides do not generally enter the network, and are termed "modifiers." From theoretical considerations it is expected, therefore, that fused silica will resist the attack of water and acids, except hydrofluoric acid, and that, of all the other constituents of silicate glasses the alkalis will be most potent in reducing chemical durability, whilst alumina, titania, zirconia and zinc oxide should be less potent in this respect than the alkaline earths, provided that comparison be made on a molecular basis. Boric oxide presents an anomoly, for if it be present beyond some 12 to 14 per cent. it reduces the chemical resistance of glasses so far studied. The reason for this is still not fully agreed. Alkalis disrupt the silica network, forming alkali silicates so all silica-containing glasses, including fused silica itself, are attacked by alkaline solutions. For a full consideration of the atomic structure of glasses and assessment of "chemical stability" reference may be made to the works of Zachariasen[2], Warren[3], Warren *et al.*[4,5], Fajans[6], Barber and Fajans[7], Weyl[8], Cole[9], Stanworth[10] and Stevels[11].

(ii) *The Action of Water, Acids and Alkalis (Including "Weathering").* The ease with which the alkali ions can be extracted from a glass can result in chemical decomposition of the surface at relatively low temperatures, in fact, free alkali can be detected on the surface of some freshly-made glasses immediately on exposure to atmospheric moisture. When glasses are stored, especially if in damp atmosphere with alternate rise and fall in temperature, as in the tropics, the wetting of the surface by condensed moisture results in extraction of alkali and when the surface dries again, a whitish deposit can be seen. On subsequent condensation an alkaline solution is at once formed which then dissolves out some of the surface silica. This decomposition may seriously impair the brilliance of the surface and water, or even acid, washing will not restore the original appearance, but acid washing of the soluble products of decomposition can expose a whitish film of silica.

This slow surface decomposition of a glass exposed to the action of atmospheric agents only, is known as "weathering" and is manifest in the

early stages by the appearance on the glass surface of fine crystals, mainly of sodium carbonate (carbonation due to carbon dioxide from the air) which can at this stage be removed by water or acid washing. The removal of this alkali leaves the glass surface poorer in alkali and the decomposition process will, in commercial glasses, be slowed up. In some cases, due to prolonged, undisturbed storage in damp atmosphere, crystals of calcium carbonate have been detected along with the sodium carbonate on the surface of soda-lime-silica glass. Sulphates may be produced if sulphur-containing gases be present in the atmosphere. "Weathering" proceeds most rapidly, of course, with glasses of high alkali content. No "weathering" occurs in an atmosphere free from moisture, as demonstrated by Dimbleby and Turner[12].

When water is in contact with a glass the alkali in the surface reacts, but to preserve the electrical balance something must enter the glass in return. Thus, hydrogen ions replace the alkali, some water accompanying the hydrogen. If the glass has an appreciable alkali content its surface will swell and may become cracked and broken on subsequent drying due to expulsion of this water. This exchange of H^+ for Na^+ occurs in the preparation of glass electrodes.

The action of water, of acids and of aqueous solutions upon glass is primarily a preferential extraction of alkali and to a much less extent, of the other most basic constituents and if by this action the reagent becomes alkaline then the silica "framework" of the glass is decomposed thus exposing fresh glass to attack and decomposition will proceed. If, however, the reagent be continuously removed and replenished, or if it remain acidic, the attack will slow down due to the formation of a surface film, richer in silica than the original surface, and this film may be indicated by faint iridescence.

The rate of decomposition of glasses by alkaline solutions is much greater than by water or acids, and the glass may, in prolonged contact with hot caustic alkali solutions, appear to be unaffected but may be dissolving as a whole. The alkali silicates formed by the reaction of the glass silica and alkali of the reagent are soluble in water and diffuse away, thus in no way producing a protective coating. But, if the solution becomes loaded with salts extracted, or if certain salts be added the reaction can be slowed down, as found by Geffkin and Berger[13]. Silicates, aluminates and salts of zinc exert this influence and are used to decrease the attack of alkaline cleaning solutions upon glass as in the patent of Cooper[14]. On the other hand, phosphates were reported by Brown and Watts[15] to be corrosive towards glass.

(iii) *Experimental Data* (a) *Behaviour of Fused Silica, Simple Soda Silica Glasses, and Three Component Glasses based on a Parent 6* SiO_2, *2* Na_2O. Fused silica is practically unattacked by water or by acids except hydrofluoric, but is attacked by alkalis, by caustic solutions more than by the carbonates, as proved by Mylius and Maüsser[16]. Dimbleby and Turner[17] prepared series of glasses to include molecular compositions ranging from 2 SiO_2, Na_2O to 4 SiO_2, Na_2O, and 3-component series based on 6 SiO_2, 2 Na_2O in which Na_2O was systematically replaced in

steps of 0·1 molecule by the other oxides BaO, CaO, MgO, PbO, ZnO, Al_2O_3 or Fe_2O_3, TiO_2 or ZrO_2.

In the soda-silica series a decrease in the soda content resulted in increased resistance to the action of boiling water, hydrochloric acid (constant boiling strength) and sodium carbonate (2N) as measured by percentage losses in the weight of the glasses. The results for the 3-component glasses showed that replacement of Na_2O by any of the other oxides, even in very small proportions, resulted in great improvement in resistance towards boiling water, hydrochloric acid and sodium carbonate (2N). With greater substitutions the improvement continued but the rate fell off until, in many cases an almost constant value was reached at compositions approximately 6 SiO_2, (R_2O_3) (RO) (RO_2), Na_2O where (R_2O_3) (RO) (RO_2) represents the substituted oxide. Figures 1 and 2 show the results of the water tests, and Figure 3 shows those of the sodium carbonate tests. The zirconia glasses exhibited marked resistance to all the reagents and to caustic soda.

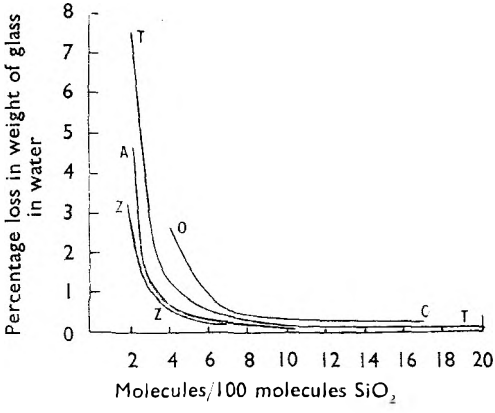

FIG. 1. Glass series based on $6SiO_2 2Na_2O$. Other oxides substituted for Na_2O in 0·1 mol. steps.

O. Substituting CaO
A. ,, Al_2O_3
T. ,, TiO_2
Z. ,, ZrO_2

(Dimbleby and Turner.)

Towards acid and water the order of decreasing effectiveness of oxides, was Fe_2O_3, ZrO_2; Al_2O_3, TiO_2; ZnO; MgO, PbO, CaO, and BaO thus agreeing with theoretical expectations as described above.

(*b*) *Boric Oxide-containing Glasses.* In 2 series of glasses developed by substituting B_2O_3 for SiO_2 on a percentage basis in parent glasses of respective percentage compositions 80 SiO_2, 20 Na_2O and 90 SiO_2, 10 Na_2O, Dimbleby and Turner found that when the B_2O_3 exceeded some 14 per cent. the glasses were seriously attacked by water, hydrochloric acid and alkaline solutions. A faint maximum durability was indicated at about 9 to 11 per cent. of B_2O_3 in the 20 Na_2O series and Enss[18] obtained a maximum towards water at about 7 per cent. of B_2O_3 in a similar series. Winks and Turner[19] reported no maximum when they systematically substituted B_2O_3 for SiO_2 in a mixed alkali-lime-silica glass (the old Kavalier resistance glass) but they reported a fall in durability when B_2O_3 exceeded some 11 to 12 per cent. In these glasses, as in those of Dimbleby and Turner containing more than about 15 per cent. of B_2O_3, digestion with hydrochloric acid extracted all constituents but SiO_2. This fact is utilised in the production of "Vycor" glass. (See Table II).

From Table III in which are quoted some results of Dimbleby and Turner using a "grain" method, and from Table IV, giving some results of Wichers, Finn and Clabaugh[20], the comparative actions of water, acids and alkaline solutions are evident. Hydrochloric acid has proved to be the most corrosive of the common acids excluding hydrofluoric, concentrated sulphuric and nitric acids having little action on most glasses. Nitric acid readily decomposes some special high lead or phosphate glasses.

TABLE III

VARIOUS GLASSES TESTED IN GRAIN FORM (LIMITS 25 TO 26 B.S.I. SIEVES). BOILED 1 HOUR IN WATER, OR IN THE REAGENT STATED

(Dimbleby and Turner, *J. Soc. Glass Tech.*, 1926, **10**, T. 304)

Glass type	Na_2O extracted by water per cent.	Percentage loss in weight in			
		Water	20 to 24 per cent. hydrochloric acid	2N sodium carbonate	2N sodium hydroxide
Bottle	0·04	0·09	0·08	0·53	1·42
Machine drawn sheet	0·008	0·01	—	—	—
Resistant boiler gauge	0·014	0·019	—	—	—
		5 hours' boiling			
Chemical Resistant No. 1	0·002	0·01	—	—	—
" " No. 2	0·001	0·009	—	—	—

TABLE IV

CHEMICAL GLASSWARE (AMERICAN) IN FORM OF 250-ML. FLASKS TREATED WITH 200 ML. OF GENTLY BOILING REAGENTS FOR 6 HOURS

(Wichers, Finn and Clabaugh[20])

Reagent	Losses in weight. Average of 3 successive treatments			
	Pyrex mg.	Vycor mg.	Kimble mg.	Glasbake mg.
Water	0·2	0·1	0·1	0·3
N sulphuric acid	0·4	0·1	0·5	0·8
N phosphoric acid	0·5	—	0·5	0·7
Hydrochloric acid, 20·24 per cent.	2·2	0·4	2·6	4·6
Sulphuric acid 95 per cent.	1·6	1·3	1·5	2·1
Perchloric acid	0·1	0·02	—	0·3
Sodium chloride, 5 per cent. unbuffered	2·2	0·5	0·7	12·8
Sodium chloride, 5 per cent. in 0·001N HCl	0·1	—	0·03	0·4
Sodium chloride, 5 per cent. buffered at *p*H 6·2	4·0	—	1·9	6·3
" " " " " " " 8·4	10·2	7·4	4·7	22·1
Buffer solution at *p*H 6·8, 10·85 g. $Na_2HPO_4 12H_2O$ + 4·55 g. KH_2PO_4 per l.	2·6	—	0·8	4·9
0·05N sodium hydroxide	90·9	43·3	55·3	94·1
0·5N " "	287	—	173	251
0·5N potassium hydroxide	166	—	93·6	156
0·5N sodium hydroxide	84·1	—	53·2	94·2

(B) *The Influence of Temperature of Reagent.*

As might be expected, the temperature at which glass is exposed to any reagent has a great influence upon the extent of attack especially above some 60° C. for all types of reagent. This was investigated (20° to 100°C.) for caustic soda (2N) by Cauwood, Way and Turner[21]; for water (80° to 102° C.) by Rexer[22], and for water (25° and 90° C.) sulphuric acid (0·02N) and caustic soda (0·02N) by Taylor[23]. This effect is more pronounced

above 100° C. and whenever such treatments must be given to glass, as in sterilisation in an autoclave, precise control should be maintained upon temperature and time.

(C) *The Influence of Period of Contact.*

Generally speaking, in service, glass is very slowly decomposed on the surface in contact with any reagent, the extraction being preferential and at first involves only the alkali ions. Later, the decomposition will depend upon whether the reagent is replenished, and whether the new compounds formed by the decomposition can readily diffuse away from the surface. The reactions may in some cases become complex, but, Turner *et al.*[24], Rexer[22], and Douglas and Isard[25] have shown that, for good, commercial, soda-lime- (or lime-magnesia)-silica glasses, the relationship between extent of attack by water, and time, was parabolic, resembling that for the passage of an electrolyte through a gel. At first the extraction proceeds most rapidly, the rate falling off gradually, until after some 10 to 30 hours a constant and much lower rate is attained. Rexer found that at 80° C. the extraction in $3\frac{1}{2}$ hours was equal to 80 per cent. of a 7 hour extraction. For a less durable glass the decrease in rate may not be so great.

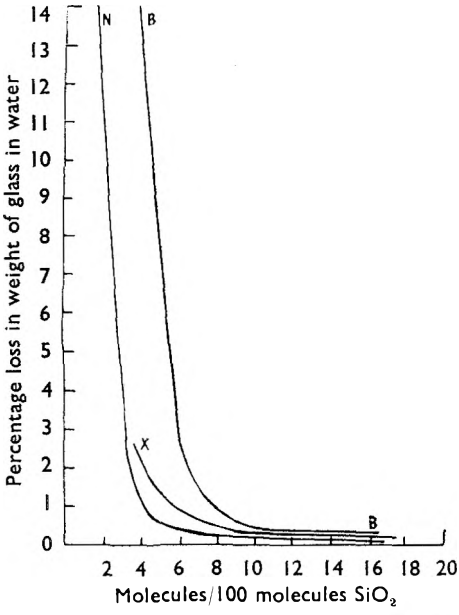

FIG. 2. Glass series based on $6SiO_2 2Na_2O$. Other oxides substituted for Na_2O in 0·1 mol. steps.

B. Substituting BaO
N. ,, ZnO
X. ,, MgO

(Dimbleby and Turner.)

In this connection it is interesting to note the work of Hinson, Smith and Greene[26] who investigated the storage of distilled water in 5 types of glass ampoules autoclaved and un-autoclaved before storage which extended to 24 months. They found (1) differences in total extracted matter and in *p*H of the water in the different glasses, (2) that *p*H values were unreliable for following the course of the attack during storage after autoclaving, due to the buffering effect of dissolved matter, (3) that, except in one case, the total matter extracted into the water after 24 months was the same whether samples were autoclaved at 121° C. for half an hour or not before storage, (4) that the storage times at room temperature at which the *p*H increase and total dissolved matter were the same as after autoclaving differed for the different glasses and there was

no general rule for predicting storage results from those of autoclaving.

Turner *et al.*[24] and Rexer[22] have also shown that water in contact with bottle glass changes in composition as time progresses, At first the solution contains alkali only but later calcium and even silica appear; the total extracted matter differs considerably in percentage composition from the glass itself, usually being much richer in alkali and much poorer in silica and lime.

(D) *The Influence of the Previous History of the Glass.*

Any treatment which can alter the condition of a glass surface will affect its surface reactions, and it is known that furnace atmosphere, fire-finishing, mechanical or acid-polishing, acid or water-washing, atmosphere during annealing, conditions and length of storage, as well as special surface treatments such as coating with water-repellent substances or "sulphuring" can all affect the chemical activity of a finished glass surface. Unannealed glasses have been found to be more "reactive" than well annealed, but it is difficult to eliminate other influences in such investigations. Gehlhoff and Schmidt[27] found that well annealed bulbs resisted weathering two or three times as well as unannealed samples.

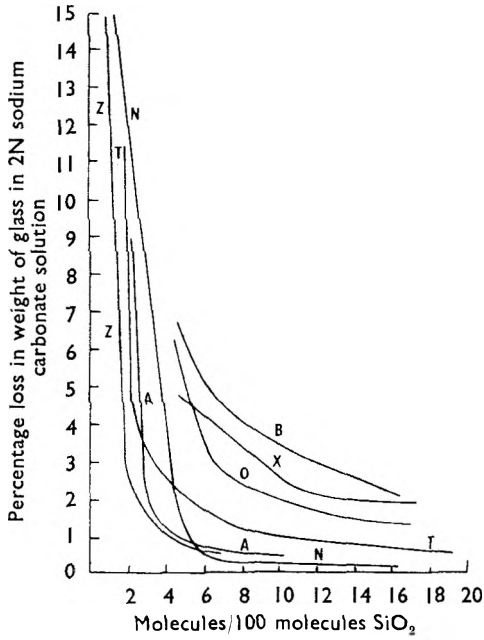

FIG. 3. Glass series based on $6SiO_2 2Na_2O$ as in Figures 1 and 2.

B.	Substituting	BaO
X.	,,	MgO
O.	,,	CaO
A.	,,	Al_2O_3
T.	,,	TiO_2
N.	,,	ZnO
Z.	,,	ZrO_2

(Dimbleby and Turner.)

The effects of damp storage conditions upon glass and general "weathering" have already been considered (page 973). Dimbleby and Turner[12] have shown that moisture is the cause of this slow decomposition and, further, that bottle glasses which have been stored and then washed so as to remove the alkaline debris, yielded less alkali to boiling water than the glasses when new, but in one case after 9 months' storage the glass tended to produce insoluble flakes more readily. Water or acid-washing of glasses followed by drying, renders the glass more resistant to weathering and is sometimes used when glasses must be packed and stored for long periods. Gehlhoff and

Schmidt recommend open, dry storing, with good ventilation rather than wrapping in any paper.

The fire-polished "skin" of a glass is more resistant, generally, than the inner layers of a glass, hence mechanical abrasion or chipping of bottle surfaces should be avoided, particularly when the bottles must be sterilised.

The Effect of Sulphuring. It is now well established that exposure of a glass to an atmosphere containing water vapour and acidic gases, particularly sulphur dioxide as in the old type of direct-fired lehr, or in "sulphuring," results in reaction between the gases and some of the surface alkali and, therefore, the surface becomes more resistant for a period, to attack by water. The following values for 5-hour extractions by boiling water from washed wide-mouth jars quite alike except for annealing atmosphere, show this.

	Extraction as mg. Na_2O
Annealed in absence of SO_2 = ..	2·9
Annealed in presence of SO_2 = ..	0·9

Unless the ware be wide-mouthed so that the gases can readily penetrate, steps must be taken to ensure entry of the SO_2, to produce this effect, as found by Cousen[28]. Douglas and Isard proved that this "sulphuring" treatment in gas saturated with water vapour at 20° to 100° C. produced a white deposit of sodium sulphate on a soda-lime glass, the amount of sodium removed from the glass varying as the square root of time as for a water-leaching (page 978), the two processes as also the electrical conductivity depending upon the diffusion of sodium ions in the glass. They suggested that the production of a layer of "H^- glass" as in this action of sulphur dioxide in presence of water vapour, resulted in the formation of a "compacted" layer on drying and expulsion of the water. The diffusion of sodium through this layer was greatly retarded, hence extraction by water would be considerably reduced. Heating at temperatures above 500° C. could aid diffusion and restore the rate of extraction. It must be remembered that this reaction generally proceeds to but a slight depth in the glass surface, and that, in long term usage the resulting silica-rich layer may be penetrated or removed. (See also effect of cream of magnesia, page 983.)

Holland, some years ago in the Department of Glass Technology at Sheffield, found that long term heating of flat glasses at 500° to 550° C. caused sodium to diffuse to the surface whence it could be removed by washing, leaving a more water-resistant layer. The present writer has observed that the surfaces of bottles annealed in an atmosphere of sulphur dioxide were difficult to scratch with a diamond and had a different texture from that of untreated bottles, whilst Boow and Turner[29] reported that sulphur dioxide treatment above 500° C. increased the mechanical strength of soda-lime-silica glass surfaces.

The Effect of Water-Repellent Coatings.

The coating of a glass surface with a water-repellent substance such as a silicone appears attractive on first thoughts but the writer does not feel

competent to reach definite conclusions without further experience. She has seen some coatings which were streaky and patchy, certainly not enhancing the beauty of the glass, and likely to arouse suspicion. Questions that immediately arise concern the preparation of the glass, the uniformity of the coating, its freedom from pin-holes; its behaviour towards alkaline solutions, to cleaning agents (acid and alkaline), and towards dry sterilisation; its impermeability, and its resistance to aqueous solutions in presence of any type of closure at temperatures above 100° C. If under any service conditions the coating be liable to peel off then its remnants may appear as "flakes" which may be more harmful than alkali extracted from the glass and the glass will become exposed. It is to be hoped that all these points will be investigated before putting such treated glasses into use.

An interesting paper upon silicones was published in 1950 by Tod[30], who dealt with their early application to glass for electrical purposes.

(3) *Methods Used for Measuring the Chemical "Durability" of Glass.*

From what has already been written it will be obvious that there is no actual value of the "durability" of any glass as there is for some of its properties, for at temperatures ordinarily employed glasses have no true solubility but are very slowly decomposing on the surface. The rate of this decomposition is influenced by all conditions, in fact, an increase of temperature alone, can alter the order of merit of glasses under test. The best way to test a glass is, of course, to subject it to the conditions it will meet in service but this often involves too long a time and is impracticable. Recourse must be had, therefore, to accelerated methods. These should as nearly as possible simulate service conditions but give a reasonable "safety margin."

Acceleration can be achieved by increasing temperature, or by increasing the surface area of contact of reagent and glass by using small cubes or plates or even grains of definitely controlled size rather than a glass vessel. But such increase in area involves the use of a different type of surface and one which does not represent truly the ware as it would be used. Further, if the glass be strained it fractures irregularly, giving grains of rougher surface than an annealed glass thereby enhancing attack. So, if the behaviour of finished ware is in question the test pieces should preferably be that ware, not grains, but if, for research purposes, glass is being studied as a substance only, then the grain test is most useful as the effects of surface peculiarities are eliminated.

Test methods are exceedingly numerous and standardisation has not progressed far in any one country, and far less internationally, though there are moves in this direction now. Generally, the attacking media are water or water and steam at temperatures above 100° C., or acid or alkaline solutions of definite strength at various specified temperatures. In all tests all conditions, including temperature, time, and ratio of volume of attacking medium to surface area exposed, should be precisely specified and maintained.

Measurement of the extent of attack demands sensitive methods and is made in various ways such, for example, by:—

(i) Change in appearance of the glass coupled with production of flakes. This is not reliable as the only measurement.

(ii) Change in *p*H or electrical conductivity of water. *p*H determinations may be affected by a "buffering" effect as found by Hinson *et al.*[26].

(iii) Rate of formation of a precipitate in an alkaloid solution, as in the narcotine hydrochloride test used in Germany but not to any extent elsewhere. (See Kroeber[31], and Blackmore, Dimbleby, and Turner[32].)

(iv) The determination of the total alkalinity of water by titration. Bases may be mixed alkalis, lime, magnesia, but are generally reported as mg. of Na_2O, as in the Standard 5-hour boiling test for medicine bottles, adopted by the Society of Glass Technology[33] and in the Tentative Standards of the American Society for Testing Materials[34]. Limits were proposed in these Standards.

(v) The determination of total solids extracted. Usually the solution is made acid with sulphuric acid then evaporated in platinum, the residue finally ignited at 500° C. or so. This is similar to the British Pharmacopœia test upon water for injection.

(vi) The loss in weight of the glass, but high-alkali-containing glasses can gain in weight due to absorption of water.

(vii) Combination of (i) with (iv)–(vi) but this is not often practicable.

(viii) Rate of neutralisation of an acid solution, as in the B.P. ampoule test, or in the Tentative Standards of the American Society for Testing Materials[34].

It is beyond the scope of this paper to discuss in detail the methods of test which have been adopted as standards in the various countries but emphasis should be given to the following points:—

(*a*) The rates of reaction of glass are highly susceptible to slight differences in experimental conditions.

(*b*) No one test can safely be chosen for application to all types of glass.

(*c*) The choice of test should be governed by service requirements, and before its adoption comprehensive experimental work should be carried out to establish that the method does distinguish between glasses which are known to be satisfactory, and those which are unsatisfactory in service.

(*d*) Test procedures should be as simple as possible to facilitate wide application and they should be specified precisely to ensure faithful reproduction.* Any procedure should include the careful preparation of the glass for test. Since glass can become weathered on storage the surface should be cleansed of all alkaline weathering

* In the British Pharmacopœia whole ampoule test no instructions are given as to the orientation of the ampoules in the autoclave yet, if the acid test solution gets into the necks it can become yellow, whereas it remains pink if the samples be kept upright.

debris before test, and before use—a fact rarely recognised, yet of great importance as in the storage of distilled water for injection.

The Extraction of Arsenic and Lead from Container Glass

Some years ago there was considerable concern lest arsenic, which was introduced into glass as a decolourising aid, or lead, was extracted in such quantity as to render medicinal preparations poisonous. The bonding of arsenic and of lead in commercial glasses would not be expected to be weak like that of the alkalis, but if the silica framework were attacked as by an alkaline reagent then all constituents would be involved. Very little work on this matter has been published but at one glass-making firm at least, the question of arsenic was investigated and I am indebted to Mr. Hodkin of Messrs. Bagley and Co. Limited for permission to quote the results of this work which included the use of bottles "sulphured" and "unsulphured," some containing arsenic, others not. The results are summarised briefly in Table V. It is obvious that cream

TABLE V

EXTRACTION OF ARSENIC FROM GLASS

(From a note read by S. Chaplin, B.Sc., A.R.I.C., at a meeting of the Yorkshire Section of the Society of Glass Technology, on November 4th, 1944.)

Bottle No.	Contents	As_2O_3 extracted p.p.m. as by B.P. method
Test No. 1, 4 *oz. medicine bottles kept at* 60 *to* 70° *C. for* 8 *hours daily for over* 2 *months*		
1	Cream of magnesia (1) As_2O_3 0·1 p.p.m.	1·0
2	do.	0·9
3	Cream of magnesia (2)	0·4
4	Cream of magnesia (1) + 0·1 per cent. of sodium hydroxide	2·0
5	Cream of magnesia (1) + 1·0 per cent. of sodium hydroxide	2·0
6	Cream of magnesia (1) + 2·0 per cent. of sodium hydroxide	2·0
7	Sodium hydroxide solution (0·1 per cent.)	0·1
8	Sodium hydroxide solution (1·0 per cent.)	0·1
9	Sodium hydroxide solution (2·0 per cent.)	0·1
Test No. 2. 4 *oz. medicine bottles sulphured* (*S*) *and unsulphured** (*U*) *kept at* 35 *to* 90° *C. daily for about* 2 *months.*		
1 (U)	Cream of magnesia (1)	3·0
2 (U)	" " "	3·5
3 (U)	" " "	3·0
4 (S)	" " "	3·5
5 (S)	" " "	3·0
6 (S)	" " "	3·0
Test No. 3. *Using blue and white bottles of two different makes* (*B or C*) *and some arsenic-free "medicals" stored for* 7½ *months, with some heating, probably* 35 *to* 40° *C. for a quarter of the time.*		
1, 2, 3 (4 oz. B. white)	Cream of magnesia (1)	0·3, 0·4, 0·5 (glass inner surfaces iridescent).
4, 5, 6 (B. pale blue)	" " "	1·1, 0·5, 0·5 (surfaces iridescent)
7, 8, 9 (4 oz. Win. dark blue less resistant)	" " "	2·5, 2·0/2·5; 2·0/2·5
10, 11, 12 (4 oz. As-free)	" " "	0·1, 0·1, 0·1 (surfaces iridescent)
13, 14, 15 (4 oz. C. As-free)	" " "	0·1, 0·2, 0·1 (surfaces iridescent)

* Sulphured and unsulphured bottles, subjected to the Standard 5-hour boiling water test of the Society of Glass Technology, yielded average values for Na_2O extracted, U = 2·45 mg. S = 0·74 mg. Limit = 5·0 mg.

of magnesia did attack the glasses tested during long storage periods or if heated to 85° to 90° C. for about 400 hours, with 900 hours at room temperature, and if the glass contained arsenic this could be removed in appreciable quantity. Sulphuring the inner surfaces of the bottles does not prevent this extraction. Under normal storage during 7½ months it is unlikely that many glasses would yield arsenic in excess of the British Pharmacopœia limit (1 p.p.m. of As_2O_3), but the use of arsenic-free glass is advisable for storing all alkaline reagents. The extraction of arsenic by the caustic soda solutions used was not as great as that of the cream of magnesia, which fact raises an interesting query.

Most colourless container glasses on the British market to-day contain very low proportions of arsenic if any, and extraction by neutral or acidic contents would be considerably less than by alkaline solutions or hygroscopic, alkaline solids. Dry solids generally do not attack glass, although some vapours, e.g., iodine can be absorbed into a glass surface, particularly into a ground one.

With regard to the extraction of lead, this is not likely to occur now that container glasses in this country, rarely, if ever, contain lead except as a trace impurity.

The Production of Insoluble Matter (Flakes) in Solutions Stored in Glass

Insoluble flakes sometimes "appear" when solutions are stored in glass and these may arise from several causes such as (i) decomposition of the contents due to oxidation or under the influence of radiation; (ii) the formation of a precipitate due to reaction between the contents and closure, or between contents and glass; (iii) the presence of minute glass splinters which were not noticed in the contents when filled into the glass, or which were produced in handling or closing.

Particles due to cause (iii) are proof of careless or wrong operations in filling and should never get beyond inspection in the filling department, whilst those due to (i) could be avoided by study of the character of the preparation and correct choice of container and closure. Cause (ii) involves reaction with closure or container, and careful investigation may be necessary to decide which. The writer has encountered closures which were adequate so long as the preparation did not wet them for some time but the bottles had been stored horizontally, with disastrous results. Again, thin protecting layers on closures may become loosened or pinholed, with resultant trouble if a liquid reaches the closure. As stated previously, water and aqueous solutions can react very slowly with glass, alkali being preferentially extracted from the glass leaving a minute decomposed layer richer in silica than the body of the glass. This layer may, under suitable conditions, develop until it falls away and is seen as "flakes" in the solution. If a glass has suffered to this extent its surface often shows iridescent or whitish patches after rinsing and drying. Analysis of the separated flakes reveals their origin. Some samples investigated by the writer, resulting from the storage of neutral aqueous solutions or water, have contained quite 80 per cent. of SiO_2 with CaO, MgO, and

Al_2O_3, the flakes differing in composition from the glass, as expected after removal of alkali. When these flakes were washed with hot hydrochloric acid nothing but SiO_2 remained, again showing that they were not of the glass itself. Flakes examined from acidic solutions in glass have been found to consist almost entirely of SiO_2, and they have appeared as minute, thin, iridescent, glistening particles which settled slowly. Quite different in appearance and settling tendency are precipitates produced by reaction of a liquid with the matter extracted from glass as in the precipitation of alkaloid solutions by alkali.

Sodium phosphate and citrate, and caustic alkali solutions relatively quickly produce flakes from some glasses. In some cases decomposition of the glass may be proceeding without visible sign, as the writer has known of some boric oxide-containing glasses being dissolved slowly but completely in boiling strong caustic soda solution, remaining bright until final collapse on reaching wafer thickness. This susceptibility of glass to alkaline solutions can be reduced by choice of composition as will be shown in a paper soon to be published by the writer and others, and as indicated by the results represented in Figure 3, but when it is known that interaction is likely it might be helpful if a warning be issued to keep the packages cool. It is not generally realised that temperatures inside containers exhibited in windows may rise to 30° C. in this country.

The Protection of Light-Sensitive Preparations

The solar radiation transmissions of glasses can be controlled by adjustment of chemical composition and conditions of production, hence, by the choice of the right glass as container adequate protection can be given to sensitive materials. The famous "Crookes" glass and those of the "Noviol" type[35] absorb ultra-violet radiation; common "colourless" glass transmits throughout the visible range but absorbs in the ultra-violet, whilst glasses have been developed to absorb infra-red rays. Recently, attempts have been made to produce glasses transmitting well in the visible whilst absorbing in the ultra-violet, as reported, for instance, by Čtyroky[36]. Amber, blue and green glasses are often used in the pharmaceutical industry and in Figures 4 and 5 are given the transmission curves (determined by Mr. D. K. Hill in this Department) for such glasses, for a colourless glass, and a medium-green bottle glass, taken from recent trade supplies.

These curves show clearly that, quite unlike the colourless glass (Fig. 5, curve 9) each of the others exerts considerable selective absorption, the "actinic" or yellow green (curves 5 and 6) "cutting off" completely at 400 mμ, the amber (Fe-Mn) (curves 1 and 2) transmitting slightly at this wavelength, with increasing transmission until the infra-red region is reached, the medium-green bottle glass (curves 3 and 4) transmitting to some extent throughout the visible range but absorbing somewhat in the infra-red, whilst the blue (curves 7 and 8) transmits over 80 per cent. at 400 mμ with a rapid drop in transmission to about 540 mμ and again at 600 to 650, but with considerable transmission in the infra-red.

Thus, for protection against ultra-violet radiation the yellow-green glass

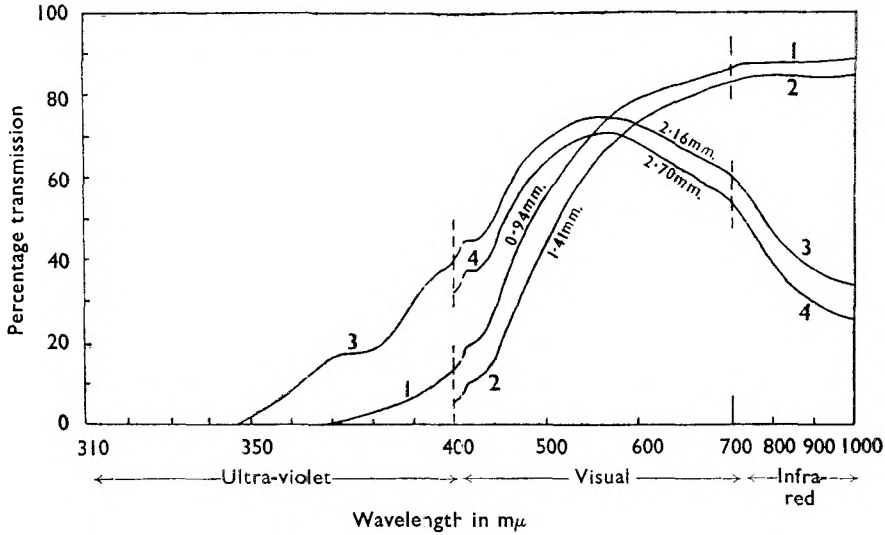

FIG. 4. Transmission curves.

1. Amber vial (Fe–Mn) .. .. thickness 0·94 mm.
2. ,, ,, ,, ,, 1·41 mm.
3. Medium green bottle (Fe^{++}–Fe^{+++}) thickness 2·16 mm.
4. ,, ,, ,, ,, ,, 2·70 mm.

(Hill.)

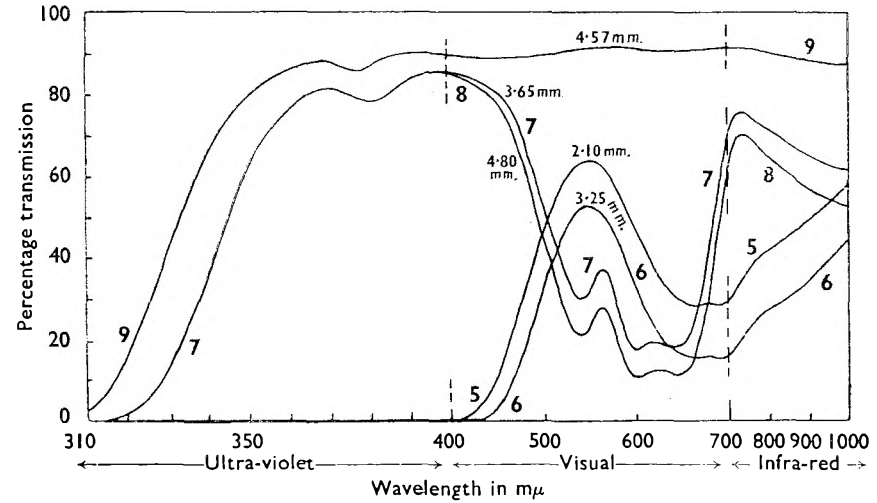

FIG. 5. Transmission curves

5. "Actinic" green bottle (Cr) thickness .. 2·10 mm.
6. ,, ,, ,, ,, ,, .. 3·25 mm.
7. Blue bottle (Co) thickness .. .. .. 3·65 mm.
8. ,, ,, ,, ,, ... 4·80 mm.
9. Colourless medicine bottle, thickness .. 4·57 mm.

(Hill.)

is the best tested here, followed by the iron-manganese amber. The green glasses would give the greatest protection from infra-red rays. Of course, an increase in the thickness of a glass specimen increases its absorption.

In order to facilitate the comparison of the absorbing powers per unit (1 mm.) thickness of the glasses studied, the extinction coefficients (k) have been calculated from the curves given in Figures 4 and 5, and the coefficients have been plotted against wavelength in Figure 6. The equation relating k with the percentage transmission (T) is, $T = (1-R)^2 \times 10^{-kt}$ where t is the glass thickness in mm. and R is the reflection loss from one glass surface.

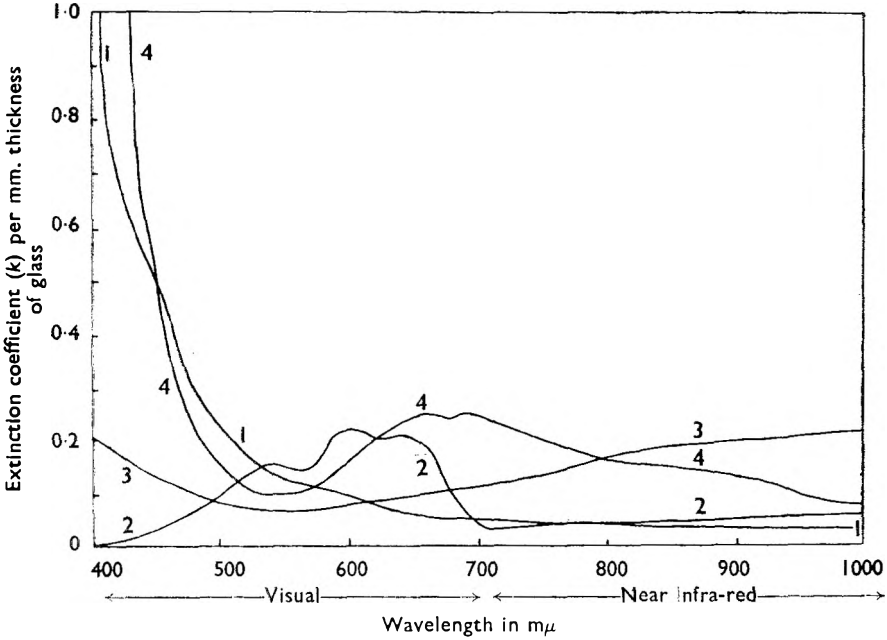

FIG. 6. Extinction coefficients

1. Amber vial (Fe–Mn)
2. Blue bottle (Co)
3. Medium green bottle (Fe^{++}–Fe^{+++})
4. Actinic green bottle (Cr)

(Hill.)

A high value of k at any wavelength indicates high absorption, or low transmission. Thus, the actinic green and the amber glasses have high values for k at 400 mμ. (Fig. 6, curves 1 and 4.)

Comprehensive, recent publications dealing with coloured glasses, giving transmission curves for a wide range of glasses, are due to Weyl[37], and to Moore *et al.*[38,39,40].

THE CLEANING OF GLASS

For cleaning of unused glassware for testing it should be sufficient to use tap water followed by 3 washes with acetic acid (0·1N) being sure to

treat all the surface, then finally rinsing thoroughly, once with tap water then 4 times with distilled water, draining for 1 minute between washes. The vessels should be cleaned immediately before use. If used glass is to be cleaned for re-use some special reagent may be necessary, such as one of the proprietary detergents or even "chromic acid." The writer has limited experience of the latest types of synthetic detergents but remarks already made upon the action of alkaline solutions upon glass should be kept in mind. Rounsfell[41] discussed the relative efficiency of caustic soda, sodium carbonate and solutions containing these in addition to phosphates including sodium hexametaphosphate and favoured the inclusion of the last named for exerting a satisfactory softening effect upon hard waters without producing scaling. The germicidal character of caustic soda is well known but it cannot be readily rinsed from glass, neither can potassium chromate in sulphuric acid, and the use of "chromic acid" is to be discouraged except in those rare cases where a strong oxidant is necessary to remove residues from previous use. Abrasion or chipping of the glass should be avoided.

Conclusion

In concluding I would say that glass possesses just those properties required of containers for the pharmaceutical industry. It is robust, transparent, or coloured to give any radiation-absorption desired; it can be moulded to please the eye and yet to provide a neck which can readily be closed to prevent ingress of gas and dust or leakage from within, or it can be sealed in a flame; it has a smooth, brilliant surface which can readily be cleaned, and when the type is correctly chosen it does not contaminate preparations stored in it, under normal conditions.

If close co-education and co-operation between the pharmaceutical and glass industries be attained there should be very few, if any, problems which defy solution.

I wish to express my thanks to Mr. D. K. Hill for the curves in Figures 4 to 6, and to Professor H. Moore for allowing the work to be done in the Department, to Mr. F. W. Hodkin for the data upon the extraction of arsenic from glass, and for permission to publish, and to Dr. J. Boow, Editor of the *Journal of the Society of Glass Technology*, for permission to reproduce the data in Figures 1 to 3.

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BRITISH PHARMACEUTICAL CONFERENCE LONDON, 1953

(*continued from page* 955)

REPORT OF A SYMPOSIUM ON CONTAINERS AND CLOSURES

AT the Symposium Session the Chairman, Dr. G. R. Boyes, presided, and introductory papers were read, in abstract, by Miss Violet Dimbleby, Mr. James Haworth, Mr. D. Stephenson, and Professor H. Berry.

For Miss Dimbleby's address see *Review Article, pages* 969 to 989. The other papers are printed below in abridged form.

THE TECHNOLOGY OF RUBBER

BY JAMES HAWORTH, B.SC., A.R.I.C., A.Inst.P.

J. G. Ingram and Sons, Ltd.

INTRODUCTION

IN spite of continuous contact between rubber users and the manufacturer since 1822, when Thomas Hancock was covering corks with rubber to improve their sealing properties, many misunderstandings have existed between the two parties. The causes of these appear to be twofold. In the first place, while rubber has found a wide range of application owing to its remarkable properties, little is really known of the basic physics and chemistry of its structure and properties. Empiric methods rather than scientific techniques play the major part in dealing with problems arising in the use of rubber. Secondly, owing to the fact that rubber technology is more an art than a systematised science, much of its terminology lacks the precision of more scientific techniques. The use of the word "rubber" for a wide assortment of materials including raw rubber of many botanical species, the infinite number of vulcanised rubbers of varying composition and the newer synthetic rubbers, illustrates how even the key word of the industry can confuse.

The basic raw materials of rubber technology are materials possessing properties intermediate between solid and liquid whose behaviour cannot be explained by laws appropriate for either ideal solids or liquids. Since the properties of the materials can be moved over the range between the ideal states, showing under some conditions properties akin to a liquid and under others more characteristic of a solid, it is perhaps not suprising that many perplexing results are obtained in service.

WHAT RUBBER IS

Natural Rubber.

Raw rubber is obtained as an aqueous dispersion (rubber latex) which exudes from the trunk of the tree when shallow cuts are made in the bark. Rubber latex contains 30 to 45 per cent. dry rubber and is subject to some considerable variation as is usual with materials of botanical origin.

The dried raw rubber, prepared by coagulation of the latex, consists of about 90 to 93 per cent. of a hydrocarbon of empirical formula C_5H_8 having an unsaturated double bond to each unit of C_5H_8 which are linked together in the *cis* position to form long chains[1]. The remainder of the raw rubber consists of resins to an extent of about 3 per cent. and between 2 and 3 per cent. of sugars and proteins.

In spite of all the work which has been carried out on the non-rubber components, there is not as yet, a full understanding of the part they play in modifying the properties of raw rubber.

Raw natural rubber as used in industry is of two main types. The essential difference between them arising from the methods of converting the wet coagulum to the dried sheet. The type used in greatest quantity is smoked sheet rubber. This is prepared by drying the washed coagulum over the smoke of wood fires when phenolic bodies in the smoke are absorbed which, besides colouring the rubber a brown amber shade, act as inhibitors of mould growth during storage. Smoked sheet rubber has always a characteristic odour which persists into the final vulcanisate. The second type, pale crepe rubber is washed more thoroughly than other grades and so tends to contain less non-rubber material. Sodium bisulphite is added to this grade during preparation to preserve the pale colour and inhibit mould growth, drying being carried out at or near atmospheric temperature in air.

Synthetic Rubber.

While the synthetic rubbers are similar in molecular structure to natural rubber, their often very great differences in chemical composition give rise to important differences in properties. Most synthetic rubbers resist heat and oxidation better than natural rubber, while some show remarkable inertness to the swelling effects of oils which so readily attack natural rubber. They are usually more difficult to process than natural rubber and tend to crystallise at much higher temperatures. Types used include polymers of chloroprene valuable for oil and light resistance, silicone polymers which have great heat resistance as well as copolymers of butadiene with styrene, *iso*butylene with isoprene and butadiene with acrylonitrile.

The synthetic rubbers nearly all contain added chemicals which act as stabilisers during storage. These include phenyl β-naphthylamine in some butadiene/styrene rubbers, while, in the chloroprenes, sulphur compounds such as thiuram disulphides are used.

The Properties of Raw Rubber

Raw rubbers exhibit a marked variation in physical properties with temperature. At low temperatures they become stiff and hard in the so-called frozen state. As the temperature increases they soften and become flexible, while at higher temperatures, they become plastic rather than elastic so that any deformation under stress becomes permanent.

From the standpoint of solubility, rubber is a liquid. This may seem

surprising, but a study of the mechanical properties indicates that the molecules of the rubber structure are free to slip past one another[2], the characteristic of the liquid state. The absorption of liquids and solids by rubber can thus be regarded as the mixing of two liquids or the solution of a solid in a liquid. Gee[3] has shown that in many cases, liquids and rubbers with the same cohesive energy density will mix to the greatest extent and the degree to which a liquid will mix with a rubber is related to the difference between the cohesive energy densities of the two. Many exceptions to these relationships occur, so that for practical purposes the extent to which a given liquid will be absorbed by a rubber can only be determined by experiment. Large and unexpected effects are known to occur when rubbers are subjected to contact with mixtures of liquids.

The absorption of water by natural raw rubber is influenced mainly by the amount of non-rubber constituents present. Specially purified rubbers with low non-rubber content absorb very little water[4]. There is little systematic information on the properties of synthetic rubbers in relation to this property. Silicone rubbers appear to be fairly resistant[5], while *iso*butylene rubber has been found to show less water absorption than butadiene/styrene[6].

Raw rubbers are permeable to gases to varying degrees dependent on the rubber type and the gas. The permeability of natural rubber to oxygen being about 12×10^{-8} c.c./sec. at 63° F., for a test specimen 1 cm. thick and 1 sq. cm. in area under a pressure of one atmosphere. The process involved appears to be a function of both solubility and diffusion[7].

The chemical properties of the raw rubbers are determined essentially by the constitution of the unit in the polymer, the double bonds in the hydrocarbon chains of many of the rubbers being the main point of reaction. The most important reaction is with sulphur which is the basis of the process known as vulcanisation or cure. The process is usually considered as the formation of cross links between the long chains in the structure with a consequent depression of plastic and liquid properties[8] and enhancement of elastic properties

The second important reaction is one that occurs spontaneously with oxygen of the atmosphere and also involves the centres of unsaturation in the polymer. This causes a breaking up of the hydrocarbon chains and ultimately all rubber-like properties are destroyed. In spite of the loss of some saturation accompanying vulcanisation, the vulcanisate is even more subject to oxygen attack than raw rubber. It is considered that this is due to the possibility of attack at the sulphur bridges. The process is catalysed by light, heat and traces of certain metals such as copper.

The ready reaction of many rubbers with halogens is also a consequence of centres of unsaturation in their structure.

The Rubber Compound

The essential composition of the majority of rubber articles is the "rubber compound" made up of raw rubber with selections from a wide range of materials chosen to serve either or both of two purposes, viz.:—

(1) To facilitate manufacture.

(2) To control the ultimate physical and chemical properties and service behaviour of the rubber article.

In spite of the fact that many of the ingredients have effects other than the one intended, which results in every rubber compound being a compromise between competing claims, it is possible to classify them according to their main function in the rubber compound[9]. Table I illustrates the main types of compounding ingredients with typical practical examples.

TABLE I

Classes of ingredient	Soft vulcanised rubber	Ebonite	Synthetic rubber
Rubber	100 Raw natural rubber	100 Raw natural rubber	100 Chloroprene
Vulcanising agent	2 Sulphur	45 Sulphur	10 Zinc oxide 4 Magnesia
Activator	1 Stearic acid 4 Zinc oxide		
Accelerator	0·75 Diphenylguanidine	2 Aldehyde amine condensate	
Extending filler	60 Whiting		
Reinforcing filler	75 China clay		30 Carbon black
Softener	1 Mineral oil		7 Tricresyl phosphate
Antioxidant	1 Phenyl β-naphthyl-amine		1 Phenyl β-naphthyl-amine
Pigment	5 Iron oxide		
Special components	3 Paraffin wax	3 Cotton seed oil	

Compounding Natural Rubber

Vulcanising Agent.

The essential ingredient in all rubber mixes after the rubber has been selected, is the vulcanising agent which is usually sulphur. In order to show a marked improvement over uncured rubber by treatment with sulphur it is necessary to mix into the raw rubber about 8 per cent. of sulphur and heat the mixture for as long as 6 hours at 300° F. Consequences of the process include an increase in tension strength, stiffness, improved resistance to changes in temperature and decreased solvent solubility. The simple vulcanisation process has, however, a number of limitations. Resistance to oxidation is bad since the process of heating for a long time causes chain scission. Further, since the non-rubber components markedly influence rate of reaction with sulphur, variability due to differences in rubber quality is reflected in the quality of the vulcanisate.

Activators.

Early experience in use of the vulcanisation process soon showed that inclusion of some metallic oxide in the compound had a beneficial effect. Since it was shown that fatty acids occurring naturally in the raw rubber played such an important part in the process, a small amount of stearic or other fatty acid is added to the mix to swamp variations due to changes in amount of naturally occurring acid present. Metallic oxides, used as activators, include litharge, zinc oxide, lime and magnesia of which zinc oxide is most common.

Accelerators.

Accelerators have the effect not only of markedly increasing the rate at which vulcanisation takes place, but also of allowing a surprising degree

of control over the physical properties. It has become possible by the use of such compounds so to reduce the time of heating to bring about proper vulcanisation that factors such as thermal conductivity have become serious obstacles to obtaining uniform vulcanisation in the fastest curing mixes. Other consequences of the use of accelerators include reduction of the amount of sulphur in the mix and with particular types the vulcanising temperature can be lowered considerably.

Accelerators in general use include guanidines, e.g., diphenyl guanidine, di-*o*-tolyl guanidine; aldehyde-amine condensates. Many accelerators are derived from carbon disulphide of which the most common types are thiazoles, e.g., 2-mercaptobenzthiazole, thiurams, e.g., tetraethylthiuram disulphide and dithiocarbamates, e.g., zinc diethyl dithiocarbamate. The amount of accelerator (usually from 0·5 to 1·5 parts to 100 parts of rubber) added to the rubber not only effects the rate at which vulcanisation proceeds, but has a marked influence on the physical properties of the vulcanisate.

All the properties of a vulcanised rubber compound vary with the time of vulcanisation, some changing continuously without inflection, while others pass through a maximum or minimum value. The process of vulcanisation can be followed by such changes in properties and it is a regular practice to use tension strength as a means of fixing the best time of cure. Many of the properties which change with time of vulcanisation do not reach their optimum values at the same time so that when times of heating have to be chosen to give the best service properties choice is often a matter of compromise. If the amount of free sulphur left in the compound is excessive, it will tend to migrate to the surface on storage forming a characteristic white or yellow film often noticed with compounds of the older high sulphur containing type. When it is necessary to have a very low amount of unreacted sulphur in the compound, use is made of high dosages of tetramethylthiuram disulphide as an accelerator without the addition of elemental sulphur. Such compounds are often referred to as sulphurless compounds. As the time of vulcanisation increases the rubber shows less liquid properties and thus is decreasingly affected by liquids[10] which act as solvents. Water absorption is also reduced[11,12].

Accelerators have important side effects which influence their choice in particular applications. Many can cause changes in colour of the vulcanised rubber[13], and other effects of importance include taste, odour and toxicity. Some are not greatly affected in respect of their chemical structure by the vulcanisation process and thus, if used in large amounts, can migrate to form objectionable surface blooms on the rubber surface. Extensive use is made by the rubber industry of mixtures of accelerators to obtain special effects.

Fillers.

Fillers are added to the rubber compound to modify such properties as hardness, tension strength, stiffness of the raw compound and resistance to abrasion. They also influence such properties as liquid absorption and permeability to gases. The effect of the filler, added to the rubber as a

finely divided powder, depends on the size, shape and surface properties of the particles. It is considered by some authorities that the more active fillers do actually combine in some way with the hydrocarbon chains of the rubber structure producing an effect on the raw rubber similar to that obtained by vulcanisation. The fillers of this type are known as reinforcing fillers, they include carbon black, clays, magnesium carbonate, and zinc oxide. Extending fillers can be added to the rubber in varying amounts without detracting seriously from the tensile properties. These are used to facilitate manufacture owing to their influence on the plastic properties of the raw compound, and to control hardness and cut resistance and to reduce cost. Typical examples include barytes, whiting and talc.

Softeners.

Softeners are used to facilitate the processing of the raw compound and control the final hardness of the vulcanisate. In compounds based on synthetic rubbers which are prone to crystallisation at higher temperatures than natural rubber they also serve to depress the freezing temperature. Raw natural rubber can be softened by mechanical working in the presence of air or by heating; the process adversely affects resistance to oxidation in service. Many oils such as pine oil, petroleum or coal tar fractions and pine tar when added to the rubber bring about softening and also facilitate the incorporation of fillers. In synthetic rubber compounds, esters are used as softeners, such materials as dibutyl phthalate and dibutyl sebacate not only soften the compound but improve such properties as resilience.

Antioxidants.

It has been mentioned that vulcanised rubber is readily attacked by oxygen. Both heat and light accelerate the effect and since the reaction is autocatalytic, a very small amount of oxygen can produce a very marked deterioration in properties. The detailed behaviour of a vulcanised rubber in this respect depends markedly on the ingredients in the compound, the method of processing and the manner and degree of vulcanisation. The chemicals which are effective in slowing down the rate of deterioration are known as antioxidants and are normally used in amounts of the order of 1 per cent. on the rubber content of the compound. Types in general use include secondary aromatic amines, aldehyde or ketone/amine condensates and phenolic derivatives. Important side effects which influence antioxidant choice for particular applications include the tendency of some types to migrate and form a surface bloom while others cause marked changes in colour when the rubber is exposed to light[13].

Pigments.

Pigments used to obtain coloured effects include oxides of iron, sulphides of antimony and cadmium selenides and sulphides. Organic pigments are being used in increasing variety.

Special Ingredients.

Special ingredients used in the rubber compound serve a number of purposes. Paraffin and other waxes are widely used because of their

tendency to migrate to the rubber surface and so form a physical barrier over the rubber against oxygen attack. They also help to reduce water absorption[14]. Other ingredients used include tack producers, such as wood rosin, to enhance surface stickiness, stiffeners such as *p*-amino phenol as well as blowing agents for sponge manufacture.

The synthetic rubbers are compounded in a similar manner to natural rubber. There are, however, a number of notable differences. The former are usually more inert than natural rubber, vulcanisation is more difficult necessitating higher accelerator dosages and longer times of vulcanisation. They are usually much tougher in the raw state than natural rubber so that softeners are nearly always necessary to facilitate processing.

Manufacture of Rubber Articles

The essential processes of manufacture of articles for closures consist of:

(*a*) The mixing of the rubber compound;
(*b*) Formation of blanks of a suitable shape for loading the mould;
(*c*) Moulding and vulcanising;
(*d*) Trimming the moulded article.

The process of mixing and moulding has been admirably described in a form well suited for those not well versed in the art[15,16]. There are, however, one or two points worthy of mention as these have some bearing on the application of rubber to the manufacture of closures. The essential processes of mixing, forming and moulding the rubber all necessitate some treatment of the surface of the compound at each stage to prevent surface adhesion of the various raw rubber parts and to facilitate opening of the mould and removal of the finished article when vulcanisation is complete. It is usual to dust the raw compound with zinc stearate or french chalk prior to moulding to reduce surface stickiness. Use of gross excess can leave some material loosely bound to the surface and this can be easily dispersed in solutions coming in contact with it.

Mould release agents are always likely to be left on the surface of the rubber as a film. This is a very common cause of turbidity in solutions coming into contact with rubber articles.

Variations in the quality of the vulcanisate which arise from the processes of manufacture include variations of hardness and of other physical properties. These arise from variations in, the time and efficiency of the mixing operation, the times and temperature of vulcanisation, and the time of storage of the rubber compound between each stage of the process. The length of time between that of manufacture and that of testing can be of importance in this connection.

Specifications

The question of devising specifications[17] for vulcanised rubber articles is difficult and is often a source of great difficulty between users and makers of rubber articles. The main contribution that can be made by the pharmacist is one of accurate specification of requirements.

The main difficulty in drawing up a specification for a rubber article is

that the preliminary operations through which the rubber is put in the process of manufacture are "remembered" in the cured products. Hence identical compositions cured in the same way can have very different properties arising from variations in the processes through which they have passed. Specification of a rubber article by detail of the composition and final properties would thus involve the specification of both the methods and machinery of manufacture.

Attempts at basing specifications on chemical tests are also fraught with danger. For example, an acetone extract clause designed to prevent the use of rubber diluents could prevent the necessary inclusion of an antioxidant or softener in the formula. Of physical tests, tension strength is perhaps too freely used by the rubber manufacturer as a criterion of quality.

Accelerated tests intended to give information to be expected from the article in service can be misleading. For example, the very beneficial effects conferred on the rubber vulcanisate by inclusion of an antioxidant in the compound as shown by ageing tests conducted in oxygen under pressure at an elevated temperature are rarely as marked under natural conditions of ageing.

The most useful guidance which can be given to the manufacturer of rubber articles for use in pharmacy is to indicate the type of ingredients likely to cause trouble in service, so that these, if possible, can be excluded from the compound. More attention will have to be paid by both users and makers to the properties of rubber in contact with liquids and the formation of extractable matter by water and other fluids. Some consideration will have to be given to the possibility of the rubber absorbing materials from solutions in contact with them as well as the susceptibility of some rubbers to react with oxidising and halogenating agents.

Physical properties meriting attention include hardness and modulus which can influence resistance to piercing by needles, while tear resistance, compression set and gas permeability are of importance. Resistance to sterilisation procedures is of importance and the possibility of absorption in the rubber of phenolic disinfectants which can cause softening and surface stickiness should be considered. Some note of the change in properties over long storage periods will have to be taken and provided against when necessary.

Future Trends

It seems likely that with the increasing understanding of the properties of rubber-like materials due to the continual development of the synthetic rubber manufacturing industry, marked improvements in the compounding of natural rubber will result in better vulcanisates for pharmaceutical purposes. The synthetic rubbers will be of increasing interest particularly as better methods of stabilisation, rendering the use of objectional chemical additives unnecessary, become available and as increasing exprience is gained in compounding the new rubbers.

The improvement of rubber compounds for use in pharmacy is not, however, a one-sided affair solely concerning the rubber manufacturer,

there is a constant need for the pharmacist to play his part in continually pointing out the problems and difficulties raised as developments in his field proceed.

As Raven has pointed out in his recent Gifford Lectures[18], the relative stagnation of medieval industry was due in some measure to the hierarchial orderings of the separate callings and professions which repelled all attempts to pass discoveries in one field to others and so contribute to the solution of problems in other fields. In a similar way, the problems raised by the application of rubbers in pharmacy are the joint concern of both rubber technicians and pharmacists and it is only by working together, each contributing his special knowledge, that real progress can be made.

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SOME EXPERIENCES WITH CONTAINERS AND CLOSURES IN THE PHARMACEUTICAL INDUSTRY

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There are few standards available for containers as such. The British Pharmacopœia and the British Pharmaceutical Codex describe containers for injections; the United States Pharmacopeia XIV goes a little further and gives general definitions for containers and classifies them under the categories of well-closed, tight, hermetic and light-resistant containers. Standards for paper and board wrappers and containers and for films and foils are published by the British Standards Institution[1,2]. When the pharmacist has consulted all the available information he will still need to satisfy himself by suitable tests that the container and its closure are likely to maintain the product for a reasonable time without change.

The following notes are based upon some years of experience of the use of containers for pharmaceutical products by the author and his colleagues, past and present, and of tests carried out in the laboratory.

It is proposed to discuss the testing of pharmaceutical containers, relating some experiences with containers of materials other than glass and rubber, and then to discuss in more detail the subject of closures.

Tests for Efficacy of Containers

1. *Test for Inertness to Product.* In the words of the U.S.P. XIV, "the container shall not interact physically or chemically with the drug which it holds so as to alter the strength, quality or purity of the drug beyond the official requirements."

Storage at normal, low, and elevated temperatures of container and product will be routine practice in pharmaceutical laboratories concerned with the development of medicinal products intended for widespread distribution in small containers. Periodic assays of the active ingredients will frequently be necessary. With dry solids an inspection of the drug and the inside of the container will generally be sufficient to indicate when interaction with the container is affecting the product. In the case of liquids a change in colour, clarity, or *p*H is frequently the first indication of reaction.

When liquids, jellies, creams, or ointments are enclosed in metallic containers evidence of corrosion of the metal lining should be sought—particularly at the liquid-air interface. The use of metallic collapsible tubes for liquids as well as ointments, creams, and jellies has been increasing; they are used for eye lotions, for unit dose containers for the convenient administration of veterinary intramammary injections[3] and for ampoule syringes for hypodermic and intramuscular injections.

An experience with ampoule syringes of tin illustrates very vividly how small and unsuspected changes can have far-reaching effects. For some years during the Second World War injection of morphine hydrochloride was successfully presented in such devices. The hydrochloride

was chosen because less tin was dissolved (in laboratory tests) than when other salts such as tartrate were used, and because the hydrochloride solution was known to be more stable. The entire ampoule syringes were sterilised by dry heat at 140° C. for one hour, filled, and the tubes folded and electrically welded. Later attempts to repeat the process were not successful; local corrosion occurred, frequently resulting in perforation of the tubes in 2 or 3 months. No difference could be found in the composition of the tin of the tubes. Corrosion of tin is said to be accelerated by the presence of chlorides and by increasing acidity[4,5], but the fact remains that for several years injection of morphine hydrochloride, *p*H 3·7, was stored in tin tubes without corrosion taking place. No satisfactory explanation has been found for this immunity from corrosion.

Because of the relatively high cost of tin tubes, lead tubes, tin-coated lead tubes and aluminium tubes have been in wider use for pharmaceutical preparations. Whilst lead tubes might be satisfactory for use with certain products for external use, such as veterinary obstetric lubricants, tin-coated lead would be preferable for many medicinal products, but great care must be taken to ensure that contamination and discoloration of the contents of the tubes does not occur—it not infrequently happens that electrochemical reaction between the lead and tin in the presence of an electrolyte results in more contamination than would have arisen from an untinned lead tube.

The danger of concluding that a container of this type is suitable because of absence of evidence of deterioration in a few test specimens was well illustrated in 1943, when our laboratories were consulted about the condition of collapsible tubes of silver picrate tragacanth jelly of foreign origin imported and stored for 2 years in this country. In some of the tubes there was a darkening in the mass of the jelly and even complete coagulation had taken place so that nothing could be squeezed from the nozzle except a little watery fluid. Examination of the tubes revealed that they were of lead with a tin coating. Where the surface was cracked there was a dark stain indicating reaction with the product and possible deposition of metallic silver. Some of the silver picrate jelly from the tubes was examined and found to contain lead which had evidently displaced silver from solution. It was concluded that the product had been packed in an unsuitable container.

Aluminium tubes should not be used for aqueous base preparations or oil-in-water emulsions in which the continuous phase has a *p*H of less than 6·5 or more than 8·0. Aluminium tubes are now being sprayed internally with lacquers or microcrystalline waxes but the danger of cracks, scratches, or pin-holes occurring in the coating is still great.

2. *Strength of Container.* The adequacy of the strength of the container can usually be judged by handling or a simple drop test. The degree of protection provided from shock by the immediate container will determine the extent of the protection required by the use of further packages such as carton, carton liner, outer, etc.

3. *Tests for Leakage.* These are preferably carried out at an elevated temperature rather higher than that which the container is likely to

experience; periodic temperature variations should be arranged. Containers should be inverted and laid sideways as well as upright, they should be weighed before and after the test period as well as examined for leakage. Vacuum tests should be used with discretion. They are most useful for eliminating unsatisfactory containers.

4. *Permeability Tests.* Containers should be sufficiently impervious to prevent loss of volatile constituents by evaporation or deterioration by absorption of moisture. If the product or one of its constituents is volatile, storage at an elevated temperature, with periodic temperature variations, of filled and partially filled containers previously weighed, with re-weighing at intervals, will be a useful guide to the suitability of the container.

When the product is to be protected from moisture the containers may be tested by storing filled and partially filled containers under conditions of elevated temperature and high humidity. The standard conditions which are generally used are: (1) 100° F., 90 per cent. relative humidity continuously. (2) 100° F. 90 per cent. relative humidity with a temperature fall to about 60° F. for approximately 8 hours out of the 24. A further test which may be found useful if the product is to be sent to the tropics is: (3) 100° F., 100 per cent. relative humidity with temperature changes as in (2). In this test, condensation occurs during the cooling period.

If the container in question is required for a number of different products, or if a quick answer is required to the question of its suitability, an accelerated test using a desiccant may be devised. (See closure test below.)

The value of such experiments can be greatly increased by the use of a tried and proved container as a control. If the container under test gives a performance equal to or better than that of the control, one is reasonably happy in its use. When the performance is less good than that of the control the difficult question to be decided is how significant the difference is. The most satisfactory assessment of a container or closure is to send experimental packages containing the product for storage in the countries where it will be used. After a suitable interval these are returned for examination.

Closures

During the Second World War tabletted products were marketed in two types of container. One type was the corked bottle and the other was a screw-capped bottle with aluminium screw-cap having a composition cork wad faced with "resistol" or "ceresine". Both types of container were widely considered to be satisfactory for all climates. In common with many other things at that time, these bottles were exposed at the docks to blast, fire, and the water of the fire-fighters. Many cases of goods were returned in which the two types of bottle containing products from the same batch had been packed side by side. In every instance the tablets in the corked bottles were in better condition than similar products in the screw-capped bottles. One variety of tablet contained sodium nitrite which is very slowly decomposed under normal conditions of storage.

The products from the corked bottles still assayed 100 per cent. of the labelled strength, whilst those in the screw-capped bottles had fallen to 55 per cent. of the labelled strength. This series of unpremeditated large-scale experiments impressed us to such an extent that we have since that time used the corked bottle as a control when carrying out closure tests on other types of bottles.

1. *Glass Bottles with Corks.* The taper of the cork and its resilience reduces the importance of variations in the internal diameter of the bottle neck. If the closure is being used for tablets or capsules which are wadded into the bottle with cotton or other wadding material the lower end of the cork usually pushes before it the strands of cotton and enables the upper conical surface to make close contact with the inner surface of the bottle neck. Dangers of an incomplete seal due to projecting wadding or crevices in the cork may be minimised by dipping the inserted cork and the bottle rim into a wax bath. Some of the new microcrystalline waxes are very resistant to the passage of moisture vapour, but have the disadvantage that being plastic they tend to collect dust particles, and become rather unsightly. Corks have for many years been protected from the attack of insects in tropical countries by dipping in sealing wax.

2. *Glass Bottles with Screw-caps and Lining Wads.* Here the closure is formed between the rim of the bottle and the face of the lining wad, which are pressed together by the tightening of the cap. It is most important when wadding material is used to ensure that wisps of the material are not left over-hanging the rim of the bottle, or a channel may be left through which moisture may be transmitted to the product.

Types of Bottle Rim. We have usually obtained the most effective closures using bottles in which the rims were flat and smooth. Other types are made, for example, corrugated, domed, and angular.

The presence of mould marks on the rim is to be avoided as these tend to tear the smooth surface of the wad as the caps are tightened and also prevent the face of the wad fitting closely to the entire circle of the rim.

Kinds of Screw-caps. (i) There is one type of screw-cap which eliminates some of the dangers of the mould mark and the irregular rim. The screw-cap is supplied without screw-thread—the thread being formed by the special capping machine pressing the metal of the cap into the thread of the bottle whilst the former is pressed firmly against the bottle rim. Bottles must be specially made to take the "roll-on" caps; the screw-threads join the neck ring of the bottle to enable the formers of the machine to be spun off the cap and the cap to be unscrewed from the bottle. In closure tests using a desiccant this type of screw-cap with certain kinds of wad has given performances closely comparable with that of corked bottles waxed over.

(ii) Screw-caps which are screwed onto the bottle are usually used with bottles the screw-head of which is not connected with the neck-ring. It is extremely important that the caps should be tightened sufficiently to obtain the maximum efficiency of the closure, but not so tightly as to overcome the elasticity of the backing material of the wad or to deform the caps. One company manufacturing bottles and caps has introduced

from America an instrument for measuring the torque applied to caps during tightening and the torque required to loosen the caps[6]. (The latter is about 50 per cent. of the former.) This apparatus promises to be useful in experimental work and in departments using large numbers of screw-capped bottles or pots.

The caps are usually of metal (aluminium or tinplate) or of plastic. Aluminium being softer and less resilient than tinplate it is more easily permanently deformed. Aluminium needs to be made from thicker sheet than tinplate and the presence of a strong beading is probably more important. Both kinds of sheet are frequently enamelled—the protection afforded is important in the case of tinplate to prevent the rusting which is frequently such a nuisance particularly in humid climates.

The plastics usually used in the manufacture of screw-caps are phenol formaldehyde resins or urea formaldehyde resins. Both are thermosetting resins. The former is brown and is usually made up only in brown or black moulding powders. Urea formaldehyde resin being pale can be used for the preparation of white or pale colours. Caps which can be autoclaved are made from phenol formaldehyde resin: the fillers and pigments used affect the properties of the caps but wood or paper fillers will both give satisfactory heat sterilisable caps.

Plastic screw-caps on glass bottles have a tendency to become loose on standing. This has been ascribed at different times to the swelling and contraction of the caps because of (i) temperature changes and, (ii) humidity changes. (The coefficient of thermal expansion of formaldehyde resins may be 6 times that of glass; that of aluminium twice and that for tinplate about $1\frac{1}{2}$ times.) Some recent experiments in our laboratory using wood-filled phenol formaldehyde resin caps and paper filled urea formaldehyde caps gave the following results. When caps which had been dried at 50° C. were put into air with a relative humidity of 90 per cent. they increased in weight by 3·5 to 4 per cent. and their dimensions increased by about 2 per cent. It seems from these results that humidity changes are the predominating factor.

It is usual to use with screw-caps a lining wad which makes the closure with the bottle rim when the cap is tightened. When metal screw-caps are made a recess is usually formed into which the wad can be pressed so that it is held and will not be separated from the cap by jostling in packaging machinery or fall out in use. The method of moulding plastic caps rules out the provision of a recess and wads are generally fixed into such caps with an adhesive.

The subject of screw-caps and liners was well discussed by Boardman at the Symposium on the Storage of Drugs and Medicines[7], but it should be reviewed in more detail here. Lining wads are usually stamped out of sheet material which is frequently in two layers, the backing layer or resilient layer and the facing layer, the latter usually provides the closure and must be resistant to attack by the product. The screw-caps should also be resistant to the product so that there is no danger of unsightly corrosion whilst the contents are being used or if leakage occurs during storage. The resilient layer is usually either pulpboard or cork—generally

cork granules pressed together with an adhesive. Cork backing is considered to be liable to give rise to mould growth when used with aqueous preparations but is more resilient than pulpboard. The resistant surface may be of metal (tin or aluminium) or may be formed from suitable plastic materials spread on sulphite paper. The paper helps in smoothing out the irregular surface of pressed cork composition. Liner wads in general use are [8]:—

(i) *Tinfaced Pulpboard or Cork Composition.* Such wads are very useful in preventing loss of water or other volatile materials, and also frequently in preventing absorption of water vapour.

(ii) "*Blackol*" is a cashew nut oil polymerised resin pigmented with carbon black on a super calendered high varnishable kraft paper. The acid resistance of "blackol" is better than that of "ceresine" but this material is used generally for alkaline materials up to a *p*H of 9 or a little over.

(iii) "*Ceresine*" is an oil varnish base prepared with tung oil and linseed oil and coated onto paper. The acid resistance of "ceresine" is medium. These wads have occasionally developed a strong linseed oil odour. They can be satisfactorily sterilised by dry heat.

(iv) "*Resistol*" is a paper impregnated with melamine formaldehyde resin combined with an alkyd type resin. It is pale in colour and is widely used for pharmaceutical products.

"*Crystal Cap*" is a white form of "resistol".

(v) "*Vinylite*" is a paper coated with a copolymer of vinyl chloride and vinyl acetate. The material is soluble in ketones but is very acid and alkali resistant.

(vi) "*Permaceal*" is a rubber hydrochloride similar to "pliofilm" spread on paper. It is slightly more brittle than "vinylite" and is not now widely used.

(vii) "*Polythene.*" Polyethylene coated paper on cork composition is being widely used, giving excellent protection against moisture absorption. Polyethylene coated directly on to polyethylene bonded cork should be very moisture proof and resilient. Wads cut from polyethylene sheet have proved disappointing as liners.

(viii) *Rubber.* When rubber is used with a good rim, an excellent moisture-proof closure is obtained. Rubber washers are used in aluminium screw-capped tubes to protect photographic film in tropical climates. The disadvantages in our experience with rubber are: (*a*) The odour which seems to be inseparable from rubber. (*b*) There is a tendency for caps to spring back when being tightened on to rubber wads. (*c*) There is no means of sticking rubber wads into plastic caps.

Rubber, being a compounded natural product, is subject to change by the manufacturer. If changes in composition are made without reference to the user unexpected and even serious results can follow.

3. *Glass Tubes with Corks or Rubber Stoppers* are giving place to tubes of glass or plastic with polyethylene stoppers or with screw-caps.

Polyethylene stoppers of varied design have been used for some years in the U.S.A. and are becoming more readily available here. Moulded

neck tubes and bottles are used with the stoppers, the wide variations in diameter of tubes made by cutting drawn glass tubing is too great to allow of a good closure. The stoppers are usually hollow; they have a short pronounced taper to permit of ready entry into the tube or bottle neck and then a slighter long taper to make the contact with the moulded neck which is the closure proper. In tests we have carried out polyethylene stoppered tubes have been shown to have a remarkably efficient closure (see Table I).

TABLE I

CLOSURE TEST ON GLASS TUBES WITH POLYETHYLENE STOPPERS

37° C.—SATURATED ATMOSPHERE

2 g. OF ANHYDRONE PER CONTAINER

No. of replicates	Type of closure		Months		
			2	4	12
20	Plastic stoppers	Mean increase in weight (mg.) ± standard deviation	27·1 ± 26·8	45·3 ± 36·0	136·4 ± 131·4
12	Waxed corks (controls)		49·1 ± 16·4	80·2 ± 27·3	255·8 ± 70·5
Probability of difference between means ("t" test)			0·008	0·007	0·004
"Efficiency" of polyethylene closure, per cent.			182	177	187

Screw-capped tubes may be internally or externally threaded but the efficiency of the seal decreases with the diameter of the tube. With internally threaded tubes the closure is obtained by a small washer which is fitted over the screw thread of the cap and rests against the projecting rim of the cap: in our experience this type of closure is not good.

4. *Plastic Tubes.* Tubes of cellulose acetate, polystyrene, polymethylmethacrylate, polyethylene, nylon, etc., are available with screw-caps or stoppers. They are most used for solid materials; their limitations are dependent upon their design and upon the properties of the plastics from which they have been formed.

5. *Aluminium Screw-capped Tubes* prepared by impact extrusion are used largely for tablets. The closure obtained is not so efficient as the corresponding closure of a screw-capped glass bottle. These containers provide the only example I can recall in which the results of closure tests with a desiccant did not predict the behaviour of the packing when used for a hygroscopic product.

6. In considering "*strip packs*," which are film or foil envelopes sealed at the edges, we may be concerned with consideration of the permeability of the material itself. Tables of relative permeability to water vapour of many of the films, laminates, and foils now available are given in British Standard Specification No. 1133[1], together with a standard method for the determination of permeability as g./sq. m./24 hours. In the Tables aluminium foil laminates are characterised as having "high resistance". We have found that aluminium foil made from 99·5 per cent. pure aluminium in 0·032 mm. fully annealed sheet coated with heat sealing lacquer gave for long periods—i.e., for more than 6 months—protection to hygroscopic material equivalent to that of a corked bottle.

If one regards the foil as the container the closure might be considered to be the sealed join between the 2 layers. We have also found that a well-sealed British product would withstand being submerged in water—a vacuum being applied until about 500 mm. Hg. was reached, this being held for half an hour, the vacuum then broken and re-applied for a further half-hour. The tablets inside the foil were all quite dry and unaffected. Tablets in some foil packs of foreign origin—indistinguishable at sight from the British material were not protected, 9 out of 10 products being softened by water.

TABLE II

	Polyethylene	Polymethyl methacrylate	Polystyrene	Nylon
Transparency	*Translucent-transparent in thin film*	*Transparent-bright*	*Transparent*	*Translucent in thin film*
Heat distortion temperature	80 to 100° C.	90° C.	Less than 100° C.	180 to 200° C. contracts
Water absorption	Nil	1·2 per cent.	Nil	7·6 per cent. at saturation
Permeability to: (1) oxygen (2) water vapour	Slightly permeable to both	Slightly permeable to both	Slightly permeable to both	Very permeable to both
Coefficient of thermal expansion	2×10^{-4} cm./cm./° C.	9×10^{-5}/ cm./cm./° C.	$8{\cdot}0 \times 10^{-5}$/ cm./cm./° C. (−10 to +45° C.)	10×10^{-6}/ cm./cm./° C.
Effect of dilute acids	Nil	Slightly affected	Nil	Nil
Effect of dilute alkalis	Nil	Nil	Nil	Nil
Effect of solutions of bactericides etc. In 0·5 per cent. phenol	No absorption in 3 months	No absorption in 3 months	—	Absorption of about 45 per cent. within 15 hours
Physical appearance of plastic sheet after 1½ months:—				
In 0·3 per cent. cresol	No change	Slight clouding of surface	No change	No change
In 0·1 per cent. chlorocresol	"	Surface appreciably clouded	"	"
In 0·5 per cent. chlorbutol	"	No change	"	"
In 0·001 per cent. phenylmercuric nitrate	"	"	"	"
In 0·001 per cent. phenyl*mercuric acetate*	"	"	"	"
In ethyl oleate	Slightly permeated	"	Complete deterioration	"
In arachis oil	"	"	Slight distortion commencing	"
In liquid paraffin	"	"	"	"
In 1 per cent. aqueous methylene blue	No change	"	No change	Colouration throughout

Closure Test. It might here be of interest to describe in detail the method we have used. The desiccants preferred are freshly dehydrated *magnesium perchlorate or anhydrous* fused calcium chloride previously heated to 150° C. for 3 hours; usually the latter in granular form, 10 mesh B.S.S., with the fine powder removed through a 30 mesh B.S.S. sieve. A sufficient number of the bottles or tubes to be tested are half filled with the same weight of desiccant *via* a wide mouth funnel (to prevent a layer of dust from the calcium chloride being deposited on the neck of the bottle

and forming a channel for transmission of moisture). A suitable number of controls is similarly prepared. The containers are then weighed and put into a closed atmosphere usually at 90 per cent. relative humidity at 38° C. and allowed to fall to 20° C. for about 8 hours out of the 24. The containers are re-weighed at intervals at first every week and then every month until sufficient data have been obtained: so that the behaviour of individual seals may be followed, the containers are all numbered. The result of a closure test recently carried out with polyethylene stoppers in moulded neck tubes using small corked bottles of similar capacity and neck dimensions as controls is given in Table I.

We have found it convenient to express the "efficiency" of the closure under test as a percentage calculated as follows:—

$$\frac{\text{Mean gain in weight of control}}{\text{Mean gain in weight of test}} \times 100.$$

All that has been previously said illustrates the fact that no one ideal material exists for the manufacture of pharmaceutical containers.

Glass has many desirable properties but is too easily broken and is not easily accurately moulded. Some of the newer plastics have many attractive properties, for example, polyethylene is very inert, is thermoplastic, almost transparent and of low density, but unfortunately most grades are deformed by boiling water and none can withstand autoclaving. In Table II a list of some of the properties[9] of four plastic materials which it is considered are of particular pharmaceutical interest is given. These have been chosen because they are obtainable unplasticised, the addition of a plasticiser introduces a factor which might be altered without the knowledge of the user. Nylon is the only one of these substances which can be sterilised by heat alone but the high moisture absorption of present forms makes it an unsatisfactory material for many pharmaceutical purposes. It seems highly probable that as more experience is gained in the field of plastics research and development materials will be obtained which are nearer to our ideal.

My thanks are due to Mr. P. A. Young for the statistical examination of the results which are embodied in Table I.

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PHARMACEUTICAL ASPECTS OF GLASS AND RUBBER

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Glass

For many years pharmacists have been "glass conscious" and aware of the necessity of controlling its quality and characteristics when glass containers are used for pharmaceutical preparations. Thus the B.P. 1898 specifies lead-free bottles for solutions of ammonium acetate and citrate and green glass for solution of potassium hydroxide, and in our records are such cases as the extraction of arsenic by potassium carbonate from its glass container and the blackening of suspended bismuth salts by specks of sulphide on the surface of the glass. In recent years, however, we have found it necessary to take an even greater interest in glass because of the gradual introduction of drugs of very high potency, and consequently of small dosage, where regard must be paid to stability, because a small change in structure during a sterilisation process or during storage may mean a considerable drop in potency. Many of these new drugs are sensitive to change of *p*H and glass can so easily supply the means for this. This more critical attitude was reflected in the B.P. 1932 which, having introduced the modern types of parenteral injections, included control tests for the limit of soluble alkali in the glass containers used and specified those medicaments and preparations which should be packed in proved-glass containers. The official tests for the alkalinity of glass were interesting and have led to considerable controversy for there were two schools of thought. Two tests were devised: (*a*) the crushed glass (or interior) test and (*b*) the surface test. The crushed glass test was final, for any glass passing that test must also pass the surface test, but not necessarily *vice versa*. On the other hand it was urged that the surface test was a "practical" test because only the surface came into contact with the medicament. The tests were restricted to containers with capacities of 0·5 ml. to 25 ml. It did not appear logical to apply *both* tests for only the relatively expensive borosilicate glass would pass both tests whilst the cheaper soft soda-lime glass would rarely pass even the surface test. It is inevitable, of course, that economics should be important and the problem of control of the quality of glass will probably vary in different countries according to the availability of the raw materials. Thus, in Britain borosilicate glass is not in good supply. The problem, however, began to resolve itself by the introduction of the so-called "surface-treated" soda-lime glass whereby it is possible to produce on its surface a resistant skin of silica which will pass a surface test but not necessarily the "crushed glass" test The glass technologists urged the adoption of this surface treated glass for containers, other than ampoules, when an alkali limit was essential. The B.P. 1953 has, in effect, done so; the "crushed" test has been deleted and a surface test retained, but without limitation to the capacity of vessels. I feel, however, that this surface-treated glass has been officially taken on trust for there are no published

data to show that the resistant skin has a satisfactory long life and will not break down and expose an alkali-yielding under surface.

I think it is desirable that we should have a durability of surface test for this glass, particularly as it will be used for large containers which, unlike ampoules, may be used over and over again.

There are also two schools of thought regarding the type of surface test. Both tests are based upon the same technique, the neutralisation of a limiting amount of hydrochloric acid. There is the method official in the Swiss Pharmacopœia which directs that the interior surface of the container be calculated and a quantity of standard acid + indicator per unit area of surface should be added. This ensures that the quality of the glass is tested and the size of the container is eliminated as a factor. On the other hand, the official B.P. test, which is similar in principle to that in the U.S.P., arranges for the container to be filled to its prescribed capacity with the standard acid + indicator solution. In this case the volume of the container is a factor in deciding the result, for if one compares the ratio of surface area to volume of standard acid, it is obvious that the small container will be subjected to a much more severe test than a large container, in fact, it could happen that with two containers made from the same glass but of different capacities, the small one may fail and the large one pass. This does appear to be illogical but the deciding argument advanced in its support is that it is a test of actual conditions to which an alkali-sensitive solution may be subjected.

At the moment we have focussed only on the possibility of alkali being yielded by glass but I would like to ask what other material is liable to be extracted. It would be well to be careful in this respect as we now deal with such sensitive medicaments and it may well be that traces of other metals may be important in the future. We have, for example, the question of glass containers for radio-active isotopes.

In addition to the alkali hazard there is also the very disturbing phenomenon of the flaking of glass from the surface of containers of citrates, tartrates and salines. We should be happier if we knew the cause and if we could have a control test which would exclude glass showing this tendency. The Pharmacopœia has no such test and can only warn that it is likely to happen. The result is generally obvious and detectable in solutions, but not with citrated blood.

It has been suggested that the interior surface of glass containers could with advantage be coated with a silicone forming a water repellant surface, thus preventing the extraction of alkali and the flaking of the glass.

Experiments carried out in The School of Pharmacy by Mr. P. J. Parr, in which silicone-treated bottles were tested against untreated controls showed that there was no protection against flaking.

In addition to the hazards of alkali and of flaking there is also the question of protection against light and the production of non-actinic glass. Pharmacopœias record increasing numbers of medicaments and preparations which are photo-sensitive but again there is little data published of the efficacy of coloured glass as a protecting agent. There is

a tendency against the use of coloured glass for injection-solutions because such glass tends to make it difficult to see the condition of the contents. I would agree that this factor is far more important than protection against light, which can easily be ensured by storage in a carton or cupboard. The same reasoning does not apply to solid photosensitive substances and it would be interesting to know if glass can give a complete protection.

Finally, there remains what might be termed the physical characteristics which we require in pharmaceutical glass and which the glass technologist must include in his considerations of quality. We must have ampoules which (*a*) will easily melt and seal, (*b*) will not splinter on opening, (*c*) contain no glass "powder." The presence of glass powder in ampoules is a hazard and can be avoided by care in manufacture. The subject is discussed in a review[1] and by Brewer and Dunning[2] who, contrary to accepted ideas, claim that the presence of such powder is not harmful. Nevertheless we must avoid it.

Rubber

Whilst it is probably true to say that we have the problems concerning pharmaceutical glass fairly well focussed it is far from being so in the case of rubber. This is because these problems are of more recent origin, coming into prominence with the advent of parenteral injections. Indeed, it is not until the 4th Addendum (1941) of the 1932 edition, that the British Pharmacopœia mentions rubber, and attempts some control of its quality and use as a closure for multiple-dose containers. Amongst the problems which this container presents is that of contact of the medicament with a rubber closure or cap, and it becomes of increasing importance, as our experience extends, to realise that rubber, like glass, may yield substances to a pharmaceutical preparation but in addition, unlike glass, it may extract substances.

It is only in recent years that we have begun to discuss these aspects of rubber. Thus[3] at the Fifth International Congress of Military Medicine and Pharmacy held in London in 1929, there was an extended discussion on glass and on rubber but the latter was viewed only from a rubber standards angle for catheters, gloves, tubing, etc., and no mention was made of its action on medicaments.

Again although rubber tubing is used in transfusion work the new B.S.I. standard for "Rubber Tubing for Hospital Use" (1882; 1952) is concerned solely with those factors such as storage and heat which may be harmful to rubber. No consideration whatever is given to the harmful effect which the rubber tubing may have on the solutions which have to pass through it or how these effects may be minimised or obviated by a specification of the quality of rubber. Such standards are of little value to the hospital pharmacist and it would appear that pharmaceutical opinion was not consulted when the standard was devised.

Rubber in the form of surgical rubber gloves may also be deemed of pharmaceutical interest as many hospital pharmacists are concerned with their purchase, storage, sterilisation and use. Yet again, the B.S.I.

standard for surgical rubber gloves (1803; 1952) is careful to list those factors which cause a deterioration of the rubber. Warning is given against antiseptics with an oil base but no regard is given to the effect of such substances as lysol which do not cause deterioration but which dissolve in the rubber creating a highly bactericidal surface.

All this emphasises the importance of bringing together the pharmacist and the rubber technologist for the mutual tabling of problems and ideas so as to get an understanding of what is wanted and how it may be obtained. How important this is is apparent when we record what we know already of the hazards of rubber, and in the following account I have attempted to create this picture.

At the outset it is important to realise what is meant by rubber, for it is quite apparent from Mr. Haworth's account and from our own experience that it is difficult to define it. Even a specified composition of the rubber-mix may give varying results. Therefore we can control it only by a general definition and control tests.

In 1937 I had occasion to examine a batch of injection of morphine hydrochloride (2·5 per cent.) containing 0·1 per cent. of chlorocresol, packed as 30 ml. quantities in bottles closed with black rubber caps which were wired on, presumably after autoclaving. The complaint was that there were black specks floating in the solution. The injection was nine months' old and had been out to the Near East. Examination easily showed that the black specks were flaked particles from the rubber, but in addition it was found that there was little or no chlorocresol in solution and consequently no bacteriostatic protection. Also the *p*H of the solution was about 2·5. The solution was sterile but the rubber had nearly perished. It was hard and would soon have cracked and admitted bacterial infection. The solution was quite colourless and had a full morphine content. Further tests showed that the rubber had extracted the chlorocresol and, being cold-cured rubber, had yielded an appreciable amount of hydrochloric acid to the solution, which had the effect of stabilising the morphine salt. Had another type of cap been used (freely available at the time) then it would have been possible for the *p*H to have risen to about 9·5 or 10·0 giving a short life to the injection.

It is this type of experience which shakes one's faith in rubber as a suitable material to bring into contact with medicaments, particularly when one contemplates the list of varied and highly active substances which are incorporated in a rubber mix. It seems too much to hope that all of them will be firmly anchored within the mass and never show their presence outside. There has not been a great deal of work done on the effects of rubber on pharmaceutical preparations and usually the workers have not defined the composition of the rubber which they have used and, therefore, it must be borne in mind when assessing the results that another type or batch of rubber might not produce the same effects.

In judging the quality of rubber for our purpose it may be convenient to consider it under the following headings.

(*a*) The physical characteristics. (*b*) The yielding of extractives to solutions or preparations. (*c*) The absorption of substances by the rubber

from the solution or preparation. (*d*) The effect of rubber on medicaments.

(*a*) *Physical Characteristics.*

During the last 15 to 20 years great advances have been made in the technology of rubber resulting in very remarkable changes in the physical characteristics of rubber, particularly with respect to ageing or perishing or oxidation. One has only to think of the old and the modern types of rubber hot-water bottle to realise this. One of the main problems in the old days was the short life of the rubber caps used for injection bottles which quickly went hard and cracked and then allowed leakage. It was quite usual to attempt to lengthen the life by varnishing or waxing the cap to protect it from the air. The waxing method was rather unpopular with the medical practitioner as it led to blocked needles. This is not necessary to-day as a modern rubber cap does not readily perish but can retain its characteristics over years.

Another character important in rubber caps is that consistency which permits the easy passage of a needle, and therefore minimises the blunting of the needle. Modern rubbers offer a big range in this property and one realises this in attempting to pierce a carbon-rubber designed for resistance to oil. When the needle is withdrawn another important characteristic of rubber should be apparent, namely elasticity causing an efficient blocking of the hole so that the cap can be repeatedly pierced without loss of protection of its contents. Elasticity and "piercibility" are not apparently synonymous. These and other characteristics of rubber in relation to rubber caps have been discussed elsewhere[4].

(*b*) *Extractives from Rubber.*

Pharmaceutical rubber, like glass or any other closure material must not alter the composition of the enclosed preparation either by reaction with it, by addition or by abstraction It is important, for example, that if water for injection is enclosed it should, after processing and on storage, still comply with all the tests for water for injection. Extracted oxidisable matter, probably protein, has been reported by Grainger[5] as coming from rubber caps and confirmed by Lloyd[6] who also states that freshly distilled water passed through a piece of rubber tubing failed to pass the official test for readily oxidisable matter. Cooper[7] confirms this and also refers to extractive which gives a sulphide reaction with iodine and sodium azide.

Anticipating the possibility of water soluble extractives, the British Pharmacopœia, 1953, specifies that rubber caps shall be boiled in several changes of distilled water.

I understand that it is possible to obtain deproteinised raw rubber and the question arises why this type of rubber should not be specified for a pharmaceutical rubber mix. Would the cost be prohibitive or would a bottle-neck in supply be created? It is also of interest to note that the protein content of rubber is related to the power of absorption of water, which is an undesirable property as far as we are concerned, particularly

when packing moisture-sensitive material. Anyone interested in this aspect might care to read an account by Taylor and Kemp[8] where the factors governing the rate of absorption of water by rubber are discussed. Different kinds of rubber have different values for water absorption. It is apparently as important in insulation problems as it is with us.

Apart from protein, one must guard against the presence in rubber of such a filler as "whiting" which could react with solutions and medicaments of low *p*H and quickly inactivate some of them. This may be of great importance in biological preparations, such as insulin and pituitary. The almost inevitable presence of zinc in rubber can be a hazard. Ragznek[9] reports the effect of temperature and *p*H on the rate of leaching of zinc salts from rubber closures in contact with acid substances. These two papers are important and interesting in that the quality of the rubber used is specified.

Rubber extractive in intravenous injections has been suspected for pyrogenic activity, but Thompson[10] exonerates it in this respect. Only one unspecified type of rubber was investigated.

In 1941[11] I pointed out that certain types of rubber caps could react with sodium metabisulphite and reduce its protective antioxidant activity. Whittet[12] suggested that caps to be used for closing containers for injections preserved with sodium metabisulphaite should be soaked in metabisulphite solution (0·2 per cent. or more). West and Whittet[13] investigating the stability of solutions of adrenaline salts when packed in vaccine bottles stated that it was essential to use caps so treated, otherwise the solutions tended to darken in colour and lose activity. This precaution has now been adopted in the British Pharmacopœia.

We have very little reported data on the inactivation of medicaments by rubber or rubber extractives. In this respect Cowan[14] reports that different kinds of rubber had different effects on penicillin solutions, some samples inactivating a considerable proportion of the antibiotic, whilst others had no observable effect. Huelsebusch *et al.*[15] report on the stability of solutions of penicillin and streptomycin when stored in rubber tubing and assayed after 6 hours and 24 hours. There was no change in the streptomycin solution. Natural crepe rubber had no effect on penicillin solutions but other types of rubber varied from no action to an adverse action.

In the field of tissue culture and in bacteriology it has long been recognised that rubber can contribute toxic substances and invalidate experimental results. Thus Parker *et al.*[16] report toxic effects of a number of different types of rubber stoppers on animal cells in tissue cultures. Pure gum-rubber stoppers and silicone covered stoppers were much less toxic. It has been reported that the presence of tetramethylthiuramdisulphide added as an accelerator made the rubber very bactericidal and upset bacterial counts in milk. The monosulphide did not. Nikethamide has been reported as reacting with rubber and for this reason the British Pharmacopœia specifies that it shall be packed in ampoules. Conversely, this reaction has been denied. This variation in action can, I understand, be possible owing to variation in the rubber-mix.

(*c*) *The Absorption of Substances by Rubber.*

That rubber will absorb or dissolve substances is well-known to the rubber technologist and in textbooks on rubber, many examples are quoted. Much work has been done on the swelling and solution of rubber. Thus Lee[17] quoted many such substances and records their action. In this list, a few such as terpineol are of pharmaceutical interest The others are probably only of academic interest, it is noteworthy that not one of them is phenolic. We, however, would be very interested in data concerning the action of phenols on rubber, for it has a pharmaceutical aspect. We know now that phenol, chlorocresol and probably other similar water- or soap-soluble phenols can be extracted from aqueous solutions by rubber, such as rubber caps. Many cases are reported. McGuire and Falk[18] showed that 0·5 per cent. of phenol was reduced to 0·3 per cent. after 237 days at 37° C. while controls with glass stoppers showed no diminution. The rate of solution is, however, much more rapid than that. Berry has shown that in certain conditions rubber caps would reduce the strength of 0·1 per cent. chlorocresol by 75 per cent. Solutions of insulin originally protected by 0·5 per cent. of phenol have been shown to be unprotected after 12 months' storage. The amount and rate of solution of phenols in a given rubber is, I presume, amongst other factors, proportional to the area of rubber exposed, time of exposure and the temperature. A saturation point must be reached.

One presumes that ultimately an equilibrium is set up between the phenol in the rubber and in the aqueous or oily solution and that it is conceivable that if the rubber has reached saturation in respect of a strong phenol solution and is then brought into contact with water or a weaker solution, some phenol may pass back from the rubber to the water. It is possible to boil the phenol out of the rubber.

It is, of course, a serious matter if the rubber cap of a multiple-dose container should extract the protecting bacteriostatic and for this reason the Pharmacopœia specifies that caps are boiled in several changes of distilled water and then either boiled under a reflux condenser for 30 minutes, or stored for not less than 48 hours in a solution containing the same bacteriostatic in the same concentration, or preferably in twice the concentration, used in preparing the injection. Not being quite sure of itself, it adds the further caution:—On prolonged storage rubber so heated is liable to continue to absorb bacteriostatic from the injection.

(*d*) *Rubber and Disinfectants.*

Because of the reaction between phenols and rubber, I tested the reaction of several disinfectants upon it. After immersing rubber bands in the various test liquids for 7 days the degree of swelling and the alteration in extensibility and in tensile strength was noted. The results are recorded in Table I.

The bands were also tested at various periods up to one year but no other changes were detected even after 12 months. Some of the disinfectants seemed to preserve the rubber for quite contrary to what I had expected, iodine showed little effect on tensile strength even after 12

months' immersion. Similar results were obtained later by using strips cut from surgical rubber gloves. The following different reactions could be noted. The rubber might show:—

(*a*) Little change in any measurement (formalin, solution and tincture of iodine or Dakin's solution).

(*b*) An increase in extension without applying force (swelling) with an increase in maximum extension and with no appreciable loss of tensile strength (lysol, cresol, liquified phenol).

(*c*) A marked increase in extension without applying force, and a great loss of tensile strength (terpineol, eucalyptus and ti-tree oils, solution of chloroxylenol. Jeyes fluid (undiluted)).

(*d*) Complete solution of the rubber (phellandrene).

Thus in the case of terpineol or a preparation containing it (solution of chloroxylenol) the rubber swelled very considerably and in that condition it lost much of its tensile strength and became "cheesy" in texture. (It

TABLE I

EFFECT OF CERTAIN SUBSTANCES ON RUBBER BANDS AT ROOM TEMPERATURE

	Length	Maximum extension	
Control rubber bands	6·4 cm.	× 8·0 times	
	After immersion for 7 days		
	Length (swelling)	Maximum extension	Tensile strength
Distilled water	6·5 cm.	× 8·0 times	No alteration
Formalin	6·8	× 8·2 "	"
Potassium laurate soap solution	6·5	× 8·1 "	"
Dakin's solution	6·5	× 8·1 "	"
Chloramine solution	6·5	× 8·1 "	"
Cetrimide 1/1000	6·5	× 8·3 "	"
Teepol solution	6·5	× 8·6 "	"
Weak solution of iodine	6·8	× 8·3 "	"
Liquified phenol	6·9 cm.	× 8·6 "	"
Cresol	7·5	× 9·4 "	"
Lysol	6·9	× 8·8 "	"
Solution of chloroxylenol	7·3	× 9·4 "	"
" " " 1/10	7·0	× 8·8 "	"
Dettol	7·3	× 9·4 "	"
Izal (undiluted)	7·2	× 8·9 "	"
Jeyes Fluid 1/10	7·5	× 9·2 "	"
" 1/20	7·2	× 8·8 "	"
" 1/40	6·9	× 8·7 "	"
Jeyes Fluid (undiluted)	10·1 cm.	—	Almost complete loss
*cyclo*Hexanol	7·2	—	"
Methyl*cyclo*hexanol	8·2	—	"
Terpineol	10·5	—	"
Eucalyptus oil	10·5	—	"
Ti-tree oil	9·4	—	"
Pine oil	9·2	—	"
Phellandrene	Dissolved	—	—

would appear wrong therefore to use solution of chloroxylenol on rubber gloves.) If, however, the rubber be then soaked in ethanol, the terpineol dissolves out, the rubber contracts to its original length and regains its original tensile strength. In a like manner phenol or cresol can be boiled out of the rubber and the band returns to its original length.

The action of iodine and hypochlorites is interesting, for apparently there is a chemical reaction at the surface of the rubber which does not

penetrate inside. It leaves a glossy film on the surface, which improves the appearance, and, I understand, increases its resistance to the absorption of water. It certainly improves rubber caps to immerse them in hypochlorite solution for about 1 hour and then boil in water. The surface is not liable to hold particles, and greasiness is removed.

The action of cresol and lysol was interesting as the rubber absorbed a considerable amount of the phenol with no obvious signs of deterioration in character. It is obvious that the surface of rubber so treated must be highly bactericidal (when moist). Indeed, this can easily be shown if a segment of such rubber be washed in sterile water and plated out in a bacterial seeded agar. On incubation an appreciable clearance zone will show. This raises the point as to whether surgical rubber gloves should ever be immersed in a solution of lysol. I remember during the war being invited to attend a meeting of surgeons to discuss the problem of the shortage of surgical rubber gloves and the unpalatable alternative of operating with bare hands. Methods of sterilisation by immersion of the gloves in solutions of lysol were discussed and quickly discarded. The opinion was freely expressed that rubber gloves after immersion in a solution of lysol became unsuitable in that on touching tissues they were liable to cause adhesions, presumably because of the high concentration of cresol at the surface. It is only fair to say that Colebrook in his treatise on the Disinfection of Skin in 1941[19] recommended lysol 2 per cent. for use on rubber gloves after placing them on the hands and stated that iodine 2 per cent. in aqueous solution with 2 per cent. of sodium iodide was the most rapidly effective but it is also somewhat damaging to the rubber. Craig *et al.*[20] suggest similar methods including the use of solution of chloroxylenol or biniodide of mercury 1 in 250. Wright[21] recommends treatment with *1 in 1000* perchloride of mercury. Stuart[22], however, deprecates the immersion of the gloves (not on the hands) in any antiseptic on the grounds that rubber deteriorates when so treated and the trace remaining may cause damage to the skin of the surgeon's hands. The opinions, therefore, cancel out. It is unlikely that iodine or hypochlorites will leave the rubber surface bactericidal as there would appear to be a chemical reaction between them and the rubber, and no free iodine would remain.

These attempts to sterilise rubber gloves with disinfectants are probably now of historical interest only for there is general agreement, I think, that rubber gloves should be wet-heat sterilised and that modern rubber can withstand autoclaving many times at 105° to 115° C. It has been agreed that a good detergent wash prior to wet-heat sterilisation contributes a great deal to the efficacy of the sterilising and therefore to the use of minimum heat treatment. It is also now well known that it is very unwise to subject rubber gloves to dry heat. One effect of lysol on rubber in hospital practice is, however, well known—that it is possible to get cresol "burns" from rubber bed sheeting that has been washed with a solution of lysol. Presumably this is due to the concentration of cresol on the rubber surface.

I think we ought to keep reminding ourselves that the results of any

experiments concerning rubber depend upon the material. I am conscious that the results I have reported with the rubber bands are strictly only applicable to those particular bands and type of rubber mix. Mr. Haworth stresses the point that even though the formula of a rubber mix is agreed and standard auxiliary substances are specified, the main constituent, raw rubber, varies just as much as any of our own "unorganised" drugs do. Even if that too be standardised, the result can still depend upon the operator in his control of the process; spotted and discoloured surfaces reacting badly in use may result from bad mixing, as well as from different physical characters. Careless control of a vulcanising temperature may result in a rubber with a sticky surface which in turn yields an oiliness to an aqueous solution.

Therefore, I would like to ask Mr. Haworth, knowing now some of our problems, if he can suggest how we could devise specifications for a pharmaceutical rubber with all the requisite physical characteristics and long life and yet be non-reactive with any medicament. The Pharmacopœia merely states that the rubber for rubber caps should be good quality heat-vulcanised rubber.

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DISCUSSION

MR. G. SYKES (Nottingham) said that from his experiments on bacteriostatics and rubber, phenol appeared to be the best of the regular preservatives while the worst was phenylmercuric nitrate. He asked Professor Berry whether he had any information on the quaternary compounds in that connection. It seemed that there was a short-term effect of immediate surface absorption, in which the surface area might be significant, and a long-term effect in which diffusion of the preservative took place throughout the rubber, in which case the weight ratio was probably the significant factor. It would be interesting to have Professor Berry's opinion on the adequacy of the B.P. treatment of rubber caps for sterile containers, and on the value of such treatment in terms of the period of storage. It would also be interesting to learn whether Mr. Haworth had ever experienced mould growth in rubber.

DR. H. DAVIS (London) referred to the question of the flaking of glass, and said that during the war he had experienced considerable trouble with sodium citrate solutions after autoclaving and had reached the conclusion that the flakes were silica. Empirical methods were adopted, and every batch of bottles was autoclaved containing sodium citrate solution, and those which flaked were discharged. He had found that glass autoclaved with a solution of sodium metabisulphite, did not flake as much as untreated glass. Was there any relationship between that result and Miss Dimbleby's comments on sulphur dioxide-treated glass? Blood products were controlled by the Therapeutic Substances Regulations which made the purely negative suggestion that citrated blood should be in a non-flaking container. It would be of advantage if a glass were available which was guaranteed not to flake with contents of that type so that a suitable container could be specified.

With regard to the absorption of phenolic disinfectants on the surface of rubberised sheets, etc., it should be strongly emphasised that there was a risk of producing dermatitis and phenol burns as a result of soaking such sheets in weak solutions of those disinfectants.

MR. T. D. WHITTET (London) said it was interesting to note that it was customary to put some sodium sulphite in pale crêpe rubber, and he wondered whether that persisted, because it might explain some results he had obtained. He had found that metabisulphite appeared to have only a surface action. Rubber caps soaked in metabisulphite were bleached almost white, but only on the surface.

MR. COOPER (Bristol) said that on storage of intravenous solutions in M.R.C. bottles with black rubber wads, a fine film of powder was produced after six months. It appeared to be protein material. He asked Mr. Haworth whether he had any views on the deproteinisation of rubber. He had treated the wads by washing with alkali, then with acid and finally boiling with distilled water. The solution obtained by boiling 100 caps in 2 l. of water was extracted with chloroform and an

oily residue was left amounting to about 14 mg. He wondered whether this oil might be carcinogenic.

MR. W. F. HARTE (Nottingham) asked whether any of the authors had experience of silicone-treated rubber to prevent soluble matter from the rubber entering the solution, or *vice versa*.

MISS V. W. BURRELL (Harrow) referred to the increased surface stability of sulphur-treated glass containers. With regard to the absorption of antiseptics by pure latex rubber bands, tests using respectively 1 per cent. phenol, 0·5 per cent. chlorbutol, 0·3 per cent. chlorocresol, and 0·002 per cent. phenylmercuric nitrate, showed that after storage at 37° C. for 1 to 3 months there was a loss in concentration of the antiseptics; phenol was still present in the highest amount, while phenylmercuric nitrate was not detectable. On using a concentrated solution of phenylmercuric nitrate in order to effect saturation, the rubber became unsightly with black specks.

PROFESSOR H. BRINDLE (Manchester) asked for information on silicone coating of the interior surface of glass bottles. From Professor Berry's preliminary experiments it would appear that there were advantages in a silicone coating. One advantage which was not mentioned was that it enabled practically 100 per cent. recovery of liquid from a bottle or ampoule. That would appear to be a considerable economic advantage where expensive injections were being used.

MR. H. S. GRAINGER (London) said he understood that many of the syringes used in hospitals were of soft soda glass, and it was well known that they deteriorated rapidly as a result of repeated boiling. It had been observed that when detergents of the sulphestol type were employed for washing syringes there was a great tendency for them to discolour when dried at 150° C. It had been suggested that the use of that type of detergent caused more rapid deterioration of the glass surface. He was using syringes of borosilicate glass of the interchangeable piston type, and so far that type had not shown the same tendency towards deterioration as soft glass.

MR. J. H. OAKLEY (London) asked Miss Dimbleby whether glass bottles normally used for pharmaceutical purposes should be allowed to weather for a period and, if so, what was a reasonable period so that when washing took place prior to use, the maximum amount of alkali was removed. Magnesium hydroxide reacted with a manganese-containing glass resulting in the formation of a layer of manganese dioxide on the surface. More attention should be paid to the bonding materials used in "compo" corks to render them less liable to mould growth, and with cut corks a more efficient means than wax was required for surface treatment. There was also a need for an economical but more durable lining for drums used for the transport of galenicals.

MR. C. E. TURNER (Stoke-on-Trent) said that in his experience dropper bottles containing solution of atropine methonitrate, although apparently perfectly sealed, showed evaporation of the product after a month. He asked whether the rubber cap was a suitable closure for that type of

preparation, and whether the solution had an effect on the rubber cap which caused evaporation.

MR. BROOKS (Nottingham) said he had found that for the majority of bottles the most suitable type of rim was a convex surface which pressed on to the liner of the cap. Mould marks on the top of a bottle causing tearing of the liners was a problem, but it was now possible to obtain bottles which had them removed. Where aluminium was employed for capping, a special hard tempered material should be used. A type of well for the liner in plastic caps could be made by cutting the thread short before it reached the top of the cap. Resin-bonded "compo" was superior to cork liners in reducing mould growth. Blackol and tinfoil were useful liner materials because of their inert nature but it was difficult to obtain a tight seal. Polythene liners were also somewhat difficult to seal, but they were useful for reactive materials and for low boiling point liquids.

DR. R. RUYSSEN (Belgium) referring to the release of material from rubber caps said there was some danger of contaminating solutions with zinc or other heavy metals. Traces of copper or zinc would catalyse the oxidation of organic substances in solution. He suggested four tests to be applied to rubber caps. There should be no change in concentration of medicinal solutions. There should be no change of the *p*H. Heavy metal contamination should not occur, and lastly fillers should not be released as shown by a turbidity test. For the pretreatment of rubber caps before sterilisation alkali should not be used but sodium phosphate solution having a *p*H of about 8. He asked Miss Dimbleby the action of sodium phosphate on glass, because pretreatment of caps would be carried out in glass containers.

DR. R. M. SAVAGE (Barnet) suggested that manufacturers could not always apply the knowledge they had in meeting consumers' requirements because the cost would be too high in relation to that of a mass produced article of inferior quality.

MR. ROSS (Liverpool) said that natural rubber, being of botanical origin, was inherently variable, and a more fruitful source of investigation for the ideal closure would be among the synthetic rubbers. The efficiency of screw caps depended almost entirely on the suitability of the wad. Resin-bonded cork had still to prove itself better than gum-bonded cork for preventing mould growth. He had seen polyethylene bonded composition corks, but they did not seem to be commercially available. Material used for stuffing tablet bottles could contain a surprising amount of moisture which might have a deleterious effect. In his experience lead-bound metal containers were the only satisfactory containers for ether.

MR. V. REED (London) asked whether there was anything in the composition of glass measures which would make some more liable to crack than others. He also asked whether differences in colour of rubber caps were related to the absorption of phenolic preservatives.

MR. MELLOCK (Chessington) asked for information with respect to containers for volatile liquids such as ether which would stand up to high altitudes.

MR. P. J. FOWLER (Bristol) said that he had experience of a syringe service and a sulphonated detergent was available for use in washing which did not cause discoloration of the syringes.

MR. A. J. DOUGLAS (Horsham) referred to Mr. Stephenson's humidity test for the efficiency of closures and to his statement that paper-filled urea-formaldehyde caps absorbed up to 4 per cent. of their weight of water and asked whether he used any controls in such cases to rule out a small source of error.

He found that tinted white flint glass bottles were more liable to flake than the pure white bottles.

DR. G. E. FOSTER (Dartford) said that if some specification for rubber were devised the rubber would have to be tested. That would necessitate obtaining a representative sample of rubber, which was more easily said than done.

MISS I. R. HARRIS (Bromley) said she had found that by storing sterile sodium citrate solutions in amber glass bottles there had been no problem due to flaking.

MR. D. STEPHENSON, in reply, said that he had no answer to the problem of coating corks with some moisture-impervious substance. The treatment of corks with a solution of a plastic was not an economical proposition. He had searched for a plastic material with a sharp melting-point; polythene had a long melting range. In his experience, whilst the majority of bottles had a domed rim, a flat rim gave a better seal. With the correct tightness on the wad and a flat rim there was a wide distance between the material and the outside atmosphere giving the likelihood of a good closure. The lack of a recess for wads in plastic caps was due to the method of moulding plastics. As to composition corks, polythene bonded cork was available and would probably meet many of the criticisms. Some forms of polyvinyl chloride might provide suitable substitutes for rubber for injection products.

Cellulose fibre used for stuffing tablet bottles needed to be dried as it could contain 12 to 15 per cent. of moisture. With containers for high altitudes as much air as possible should be excluded. Flexible containers were advisable.

MISS V. DIMBLEBY, in reply said that in washing with water or dilute acids the reagent extracted some of the sodium ions in the glass surface leaving a thin layer with more silica and less alkali than underneath. The resulting increase in resistance might not be very evident on long storage or with alkalis. Washing with detergents, especially if alkaline might attack the silica skeleton of the glass, forming hydrated silica to some extent and leading to adsorption of some colouring material.

Weathering needed careful control. The lime might be extracted, and a silica layer would be left which had not the same reflecting or transmission qualities as the glass itself so that it appeared as a white deposit

on the surface. In some experiments a number of ordinary 4 oz. medicine bottles after weathering for 9 months gave a better response in the 5 hour boiling test, with water than they did when new, but they flaked more readily. When the weathered layer attained a certain thickness, it ceased to have the same expansion as the glass behind and no longer adhered.

Sodium phosphate solutions always caused flaking in a short time. There was a similar action with sodium citrate, but in association with other workers at Sheffield interested in storing blood she had found that there was a big difference in the behaviour of different types of glass towards sodium citrate solutions on autoclaving. This work would be published in the near future.

Silicone coatings were very useful if it was essential to recover the last drop of any expensive liquid. On repeated autoclaving, however, the coatings would gradually come away from the glass. The coating was on the glass; it was not a silica layer made from the glass structure itself. Unless the glass was thoroughly clean to start with so that the coating contained no pinholes and was impermeable to moisture, flaking might occur. Sulphuring of glass surfaces undoubtedly rendered them more resistant to the action of water for a period, but work on the extraction of arsenic had shown that the treatment was no use for long storage, especially of alkaline solutions.

All methods of testing glass were empirical. Glass had no solubility; the problems arose from the decomposition of the glass surface. The method of testing should be that which simulated as nearly as possible the service conditions, but a good safety margin should be allowed by increasing the temperature, the surface area of the glass exposed to attack, or the strength of the reagent.

MR. J. HAWORTH, in reply, said that natural rubber was treated either by smoking to absorb phenols or with sodium sulphite, to prevent surface mould growth during storage in the tropics. Persistence of sodium sulphite in pale crêpe rubber was probable. He was not sure whether the reaction between metabisulphite and rubber was a purely surface action. In the first few hours of contact between metabisulphite and pieces of rubber there was a fairly rapid action, and then no further change seemed to occur, but the thickness of the rubber and the rate of penetration, associated with the degree of vulcanisation, were involved. Changes of colour of the rubber surface could be due to actual changes in colour or to changes in the refractive index as a result of water absorption; very often these effects were confused.

Deproteinised rubber was more costly. He was interested in the report on the extraction of an oily residue from rubber which, it was suggested, might be carcinogenic. Many of the softeners used were of coal tar or petroleum origin and might be carcinogenic. Carbon black, which was a very popular filler, often contained a proportion of oily material which could be extracted by solvents, and which had recently been shown to be carcinogenic.

Silicones in his experience all gave trouble. Differences in the absorption of phenols were connected with variations in the degree of vulcanisation

and with the extent of cross linkage. The colour of the rubber did not affect the absorptive properties. The best hope of controlling the evaporation of volatile solvents seemed to lie in the different types of synthetic rubber.

On the question of choosing a representative sample, he had pointed out that one of the difficulties in testing rubber was the type of test. In testing simple properties such as tension strength it was necessary to use special machinery in order to obtain reliable results and to treat the results on different samples on a statistical basis.

On the question of economics, only 0·45 per cent. of the natural rubber imported in 1952 went into the manufacture of surgical rubbers, so the manufacturers had very little say in what the plantation industry provided.

PROFESSOR H. BERRY, in reply, said that Mr. Sykes would probably find that if he changed his rubber he would obtain large differences in the rate of absorption of bacteriostatics. The B.P. played for safety. Rubber could be made highly fungicidal. If the silicone treatment were adopted it would be necessary to get used to the greasy appearance, which might be difficult to explain to a lay person. He could not give any opinion on the question about solution of atropine methonitrate.

RESEARCH PAPERS

THE STABILITY OF ADRENALINE AND NORADRENALINE IN HUMAN URINE

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It is a well-known fact that adrenaline is very readily oxidised in aqueous solution, forming a pink-coloured substance, adrenochrome. However, Euler and Hellner[1] reported that adrenaline is remarkably stable in urine. They showed that the adrenaline present in human urine is not destroyed when stored at room temperature and pH 3 to 4 for 48 hours.

Since the detection of adrenaline and noradrenaline in urine is of value in the diagnosis of phæochromocytomas and since a considerable volume of the urine used for this test is that which has been stored overnight in the bladder, at a neutral or possibly slightly alkaline *p*H, before it is collected and acidified, it was thought to be of some interest to investigate the stability of adrenaline and noradrenaline in urine at an alkaline *p*H and at body temperature. It was also hoped to determine the nature of the substance or substances inhibiting the oxidation.

Experimental Procedure

Urine samples were collected from different individuals and *l*-adrenaline or *l*-noradrenaline added to give a concentration of 10 μg/ml. The *p*H was then adjusted to 7·5 to 8·0 with sodium hydroxide and the samples incubated at 37° C. for 5 hours, unless otherwise stated. They were then either assayed immediately or acidified with concentrated hydrochloric acid and stored in the refrigerator until the next day. No allowance was made for the naturally occurring amines in the urine since Euler[1] has shown that they are present in a concentration of about 0·04 μg./ml. This is negligible in comparison with the added amount of 10 μg./ml.

Assay methods

Initially all the urine samples were extracted by the method of Euler and Luft[2] and subsequently assayed on the cat blood pressure. Later it was found that direct estimations of the unextracted urine could be made on the isolated intestine of the rabbit, using oxygenated Tyrode Ringer solution in a 60-ml. bath. Sometimes it was also possible to perform a direct assay on the cat blood pressure. Care was taken in all instances, to set up adequate control urine samples and for all solutions to be neutralised before assaying.

Results

Urine samples incubated under the above conditions showed complete protection of the adrenaline and noradrenaline added. Aqueous solutions

of the two amines, under the same conditions, showed complete loss of activity (Fig. 1). There must therefore be some substance or substances present in urine which prevent the oxidation of the amines.

The urinary constituent which seemed most likely to prevent this oxidation is ascorbic acid. Therefore the ascorbic acid content of each

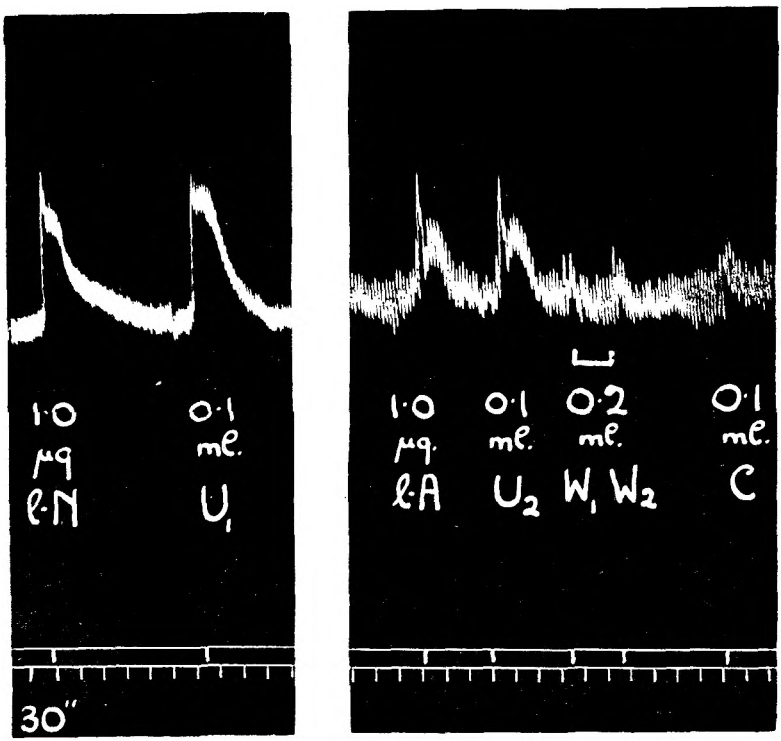

FIG. 1. Cat blood pressure. Chloralose anæsthesia.

U_1 Urine containing noradrenaline 10 µg./ml.
U_2 Urine containing adrenaline 10 µg./ml.
W_1 Water containing noradrenaline 10 µg./ml.
W_2 Water containing adrenaline 10 µg./ml.
C Control urine. All 5 samples incubated at 37° C. for 5 hours.

urine sample was estimated immediately before incubation, using 2:6-dichlorophenolindophenol reagent, and the degree of protection of adrenaline and noradrenaline compared with the protection in aqueous solutions of ascorbic acid. As can be seen from Table I, 1 mg. per cent. of ascorbic acid was never adequate to cause any protection of adrenaline or noradrenaline in water and 2 mg. per cent. only caused a certain degree of protection. However, urine containing 1 mg. per cent. of ascorbic acid caused complete protection in 6 out of 8 samples and even 0·25 mg. per cent. caused some protection (Table II). Thus, although in a few urine samples the protection could be accounted for by the amount of

ascorbic acid present, in the majority of samples there was still complete protection of the amines although the ascorbic acid content was below the amount required to protect an aqueous solution of adrenaline or noradrenaline (Fig. 2). In 2 cases the urine samples were incubated

TABLE I

PROTECTION OF *l*-ADRENALINE AND *l*-NORADRENALINE IN WATER BY ASCORBIC ACID

Ascorbic acid before incubation mg. per cent.	Percentage protection in 5 hours
1·0	0
1·0	0
1·0	0
1·0	0
1·0	0
2·0	*50*
2·0	80
2·0	10
2·0	40
2·0	5

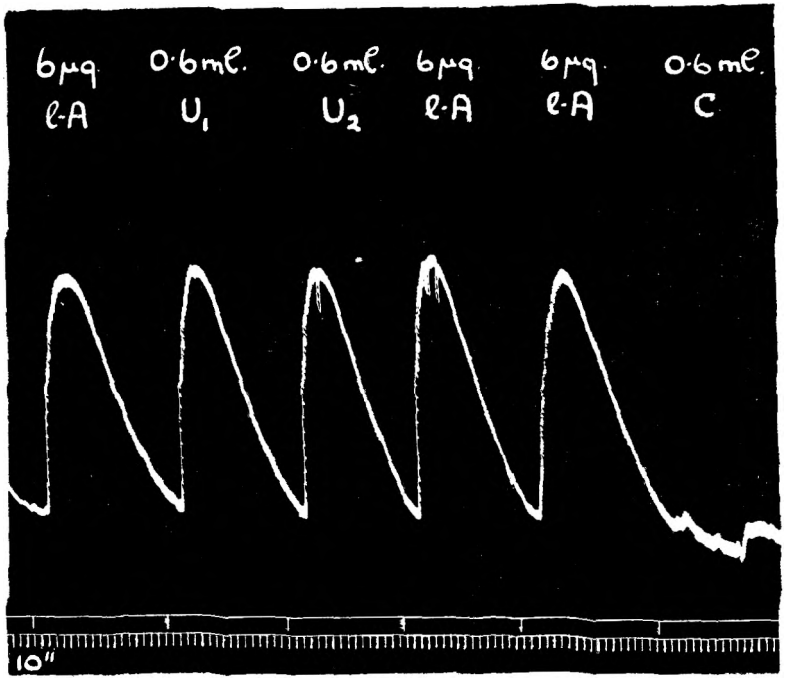

FIG. 2. Cat blood pressure. Chloralose anæsthesia.

C Control urine.
U_1 Urine containing adrenaline 10 μg./ml. and ascorbic acid 0·5 mg. per cent.
U_2 Same urine as U_1, containing adrenaline 10 μg./ml., but ascorbic acid added to give a concentration of 5·0 mg. per cent.

All 3 samples incubated at 37° C. for 5 hours.

TABLE II

PROTECTION OF *l*-ADRENALINE AND *l*-NORADRENALINE IN URINE BY ASCORBIC ACID

Ascorbic acid before incubation mg. per cent.	Percentage protection in 5 hours	Ascorbic acid before incubation mg. per cent.	Percentage protection in 5 hours
0·5	100	0·5	100
1·0	100	0·4	100
0·6	100	1·0	100
0·5	100	0·3	25
0·4	10	1·0	100
0·5	100	0·5	100
2·5	100	1·0	70
0·8	100	2·8	100
0·75	100	0·5	100
0·25	25	0·8	100
1·0	100	1·5	100
1·3	100	1·0	100
1·0	30	1·2	100
1·2	100	1·0	100

TABLE III

PROTECTION OF ADRENALINE BY ASCORBIC ACID ON PROLONGED INCUBATION

	Ascorbic acid before incubation mg. per cent.	Adrenaline present after—		
		5 hours per cent.	18 hours per cent.	24 hours per cent.
Urine I ..	0·75	100	50	12·5
Urine II ..	1·0	100	15	10
Water ..	1·5	0	—	—

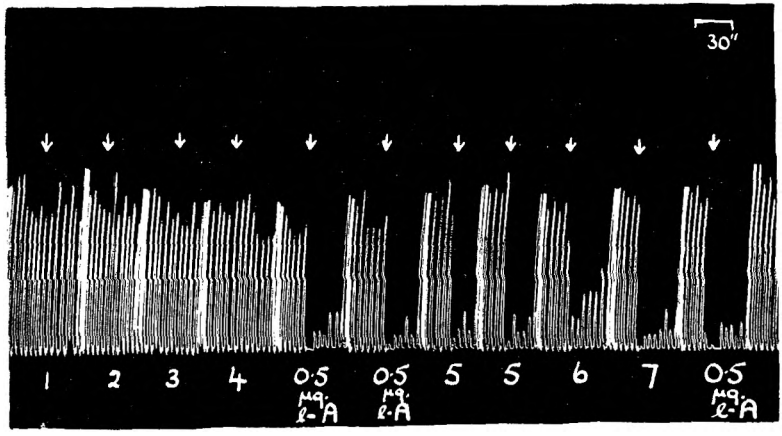

FIG. 3. Isolated intestine of the rabbit.

1. 0·2 ml. control "synthetic urine."
2. 1·0 ml. of water containing adrenaline 10 μg./ml.—no ascorbic acid.
3. 0·5 ml. of water containing adrenaline 10 μg./ml. and ascorbic acid 0·5 mg. per cent.
4. 1·0 ml. No. 3.
5. 0·05 ml "Synthetic urine" containing adrenaline 10 μg./ml. and ascorbic acid 0·5 mg. per cent.
6. 0·05 ml. "Synthetic urine" containing adrenaline 10 μg./ml.—no ascorbic acid.
7. 0·1 ml. No. 6.

All solutions incubated at 37° C. for 5 hours.

with adrenaline for 24 hours and the amount of adrenaline present was estimated at certain times during this period. The results were as shown in *Table III.*

It was therefore evident that although ascorbic acid plays some part in the stability of the amines there must be some other constituent or constituents in urine which cause this protection. The simplest way of tackling the problem was thought to be to make up a "synthetic urine" of the composition shown in Table IV, both with and without ascorbic acid and to see whether or no this protected the amines. If so, the substances causing such a protection could be determined by a process of elimination. It was found that "synthetic urine" of the composition given and containing 0·5 mg. per cent of ascorbic acid caused, when incubated at 37° C. for 5 hours, complete protection of the amines while without ascorbic acid there was a partial protection. With water, under the same conditions, there was complete destruction of adrenaline and noradrenaline (Fig. 3).

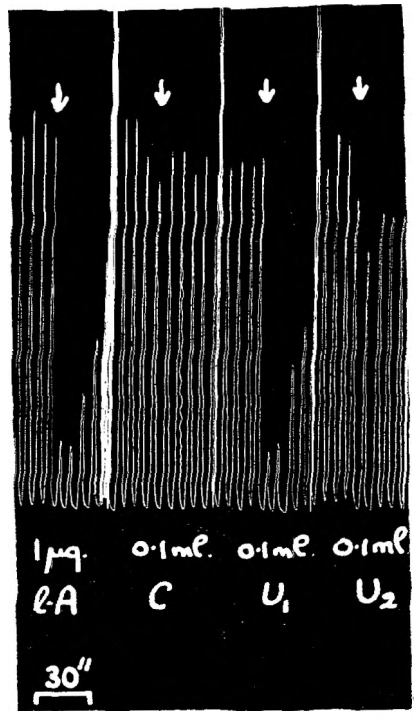

FIG. 4. Isolated intestine of the rabbit.

C Control urine.
U_1 Urine containing adrenaline 10 µg./ml. and ascorbic acid 0·25 mg. per cent.
U_2 Dephosphated urine containing adrenaline 10 µg./ml. and ascorbic acid 0·25 mg. per cent.

All urine samples incubated at 37° C. for 5 hours.

By a process of elimination it was found that only the ascorbic acid and the phosphate present caused any appreciable protection although uric acid had a little effect in delaying the destruction of the amines. When the phosphate was present in amounts approximating to those in normal urine there was protection of the amines with an ascorbic acid content as low as 0·5 mg. per cent. Confirmation of these results was given by taking urine samples of low ascorbic acid content and removing the phosphates with magnesia mixture. The dephosphated urine was then incubated in the usual way. Such urine did not cause protection of the adrenaline, whereas the same urine but containing phosphates showed complete protection (Fig. 4). Dephosphated urine, but containing more ascorbic acid, still protected the added adrenaline (Fig. 5).

There was also the possibility that the stability of ascorbic acid was not the same in urine as in water. Consequently both urine and water were incubated at *p*H 7·5 to 8·0 at 37° C. for 5 hours with different amounts of ascorbic acid added. The ascorbic acid content was deter-

TABLE IV

"SYNTHETIC URINE"

	per cent.
Urea	3·0
Uric acid	0·05
Creatinine hydrochloride	0·1
Sodium chloride	1·5
Potassium chloride	0·25
Sodium acid phosphate	0·25
Sodium sulphate	0·2
Magnesium sulphate	0·01
Calcium chloride	0·01

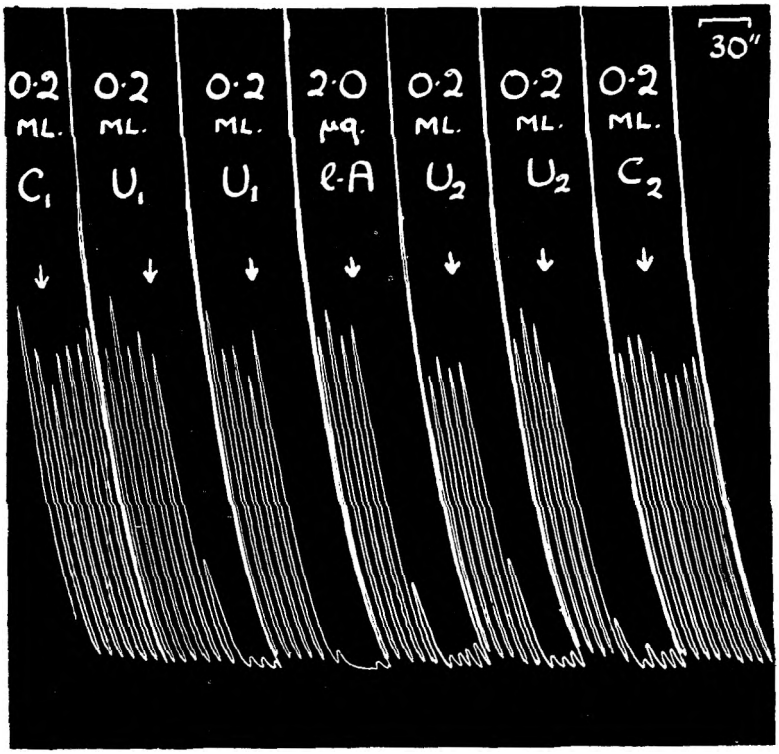

FIG. 5. Isolated intestine of the rabbit.

C_1 Control dephosphated urine.
U_1 Dephosphated urine containing adrenaline 10 µg. ml. and ascorbic acid 2·25 mg. per cent.
C_2 Control urine.
U_2 Urine containing adrenaline 10 µg. ml. and ascorbic acid 2·25 mg. per cent.
All urine samples incubated at 37° C. for 5 hours.

mined both immediately before and after incubation. It was found that with an ascorbic acid content of 0·5, 1·0 or 2·0 mg. per cent. in water there was no detectable amount (i.e. less than 0·05 mg. per cent.) left after incubation whereas with urine there was only a percentage loss. With 5 mg. per cent. there was a percentage loss in both urine and water, this being greater in water. Therefore ascorbic acid is considerably more stable in urine than in water and this partly accounts for the adrenaline stability.

Discussion

It is of some interest that adrenaline and noradrenaline are so stable in human urine. Although this can be due entirely to the ascorbic acid if it is present in a sufficient quantity, in most urine samples the protection is due to both the ascorbic acid and the phosphates present. Although each may be present in a subthreshold amount to prevent oxidation over the time interval studied, together they cause a complete protection.

It is also of interest that urine can be assayed for sympathomimetic activity directly, i.e. without previous extraction—using the isolated intestine of the rabbit. In only 1 or 2 instances did the control urine have any effect on the intestine, in the volumes used. This saves having to perform the time-consuming extraction method and is very useful in the detection of adrenaline and noradrenaline in the urine of suspected cases of phæochromocytoma. In cases where a tumour is present there is such a high concentration of amines that a satisfactory direct assay can readily be performed.

Summary

1. Both adrenaline and noradrenaline are considerably more stable in urine than in water, even at body temperature and an alkaline *p*H.

2. This stability is due to the ascorbic acid and the phosphates present in urine. It is also partly due to the greater stability of ascorbic acid in urine than in water.

3. Dephosphated urine of low ascorbic acid content does not prevent the oxidation of the amines.

4. Adrenaline and noradrenaline can be estimated in urine without previous extraction, using the isolated intestine of the rabbit.

References

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ON THE RELIABILITY OF THE METHODS USED IN THE ASSAY OF HEPARIN

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The determination of the anticoagulant activity of heparin is an extremely difficult task. The results are influenced by a number of factors, not least by the procedure of assay which is chosen. Identical results are hardly to be anticipated when such different procedures are applied as those listed below (Table I).

TABLE I

Methods used in the assay of heparin

A.	Unaltered blood.	
	Howell[9]	Fresh cat blood
	Scott and Charles[10]	Fresh cat blood
	Jaques and Charles[3]	Fresh cat blood.
	Jorpes[11] and Wilander[12]	Fresh ox blood.
	Schütz[13]	Fresh rabbit blood.
B.	Heparinised plasma.	
	Neutralization of heparin with kinase	
	Dam and Glavind[14]	Human plasma.
C.	Oxalated blood + thrombin.	
	Jaques and Charles[3]	Oxalated ox blood.
D.	Oxalated or citrated plasma + thrombin.	
	Kjems and Wagner[4]	Oxalated ox plasma.
	Studer and Winterstein[2]	Citrated ox plasma.
E.	Citrated plasma + calcium salt.	
	Reinert and Winterstein[15]	Citrated ox plasma.
	Foster[16]	Citrated ox plasma.
	Mangieri[8]	Citrated ox plasma.
	Kuizenga, Nelson and Cartland[17] (U.S.P. XIV, 1950)	Citrated sheep plasma.
F.	Oxalated plasma + calcium salt + tissue extract in excess.	
	MacIntosh[18]	Oxalated horse plasma.
G.	Sulphated whole blood + thrombokinase.	
	Adams and Smith[19] (B.P., 1953)	Ox blood "salted" with sodium sulphate.
H.	Bird plasma + tissue extract.	
	Fischer and Schmitz[20]	
	Chargaff, Bancroft and Stanley-Brown[21]	
	Astrup[22]	
	Jaques and Charles[3]	

It is to be expected that the results of the assay will vary in accordance with the method applied. Discrepancies of this kind were experienced in the assay of the different commercial brands of heparin used in anticoagulant therapy.

Our interest in this question arose when several control laboratories claimed that a brand of heparin assayed by the whole blood method (see Jalling, Jorpes and Lindén[1]) had been found with the U.S.P. method of assay to have only 80 per cent. of the declared strength. We were soon able to confirm this finding. We consequently found it necessary to

extend our studies. We were then able to show that the U.S.P. method, using citrated sheep plasma and recalcification with no addition of thrombin or thrombokinase, almost consistently gives a 10 to 15 per cent. lower figure for the anticoagulant activity than the whole blood method[1] and the thrombin method of Studer and Winterstein[2]. This applies to heparin samples varying in strength between 25 and 110 international heparin units per mg. of water-free substance. Since the two last-

TABLE II

THE METHOD USING FRESH OX BLOOD

Sample	Checked against	Date	Number of Stands	Units/mg. or ml.	
				Calculated	Found
International heparin standard	Swedish heparin standard ..	17.6.1952	8	130	130
Provisional international *heparin standard, 1942*	,,		7 (8)	130	130 (140)
Swedish heparin standard	International heparin standard	18.6.1952	18	80	85
,,	,,	30.7.1952	17	80	82
Commercial sample E 1952 of Heparin Vitrum	,,	25.7.1952	18	5000	4950
Commercial sample of Heparin Novo	Swedish heparin standard	23.7.1952	18	5000	4950
,,	,,	6.5.1952	18	5000	5325
Commercial sample of Liquemin Roche	,,	15.2.1952	11	5000	5000

mentioned methods are those most commonly used in Scandinavia and on the Continent, the discrepancy deserves attention.

Potency of the Original Swedish Heparin Standard. When assayed by the whole blood method against the international heparin standard, both commercial samples of heparin and the original Swedish heparin standard showed a correct potency (Table II).

When the Swedish heparin standard, having a potency of 81 units per mg. as assayed by the whole blood method, was assayed by the thrombin

TABLE III

ASSAY OF THE ORIGINAL SWEDISH HEPARIN STANDARD AGAINST THE INTERNATIONAL HEPARIN STANDARD

Whole blood method	Thrombin method of Studer and Winterstein	Plasma method of the U.S.P. XIV 1950
80	83	65
80	79	68
85	86	70
82	83	70
80* (25.11.51)	83	65
81† (23.7.52)	78	65
76† (6.5.52)	—	68
81	82	67

* Checked against Liquemin Roche
† Checked against Heparin Novo

method of Studer and Winterstein, 82 units/mg. were found. The U.S.P. method, however, gave only 67 units/mg. (Table III). The figures represent means of 7 or 8 assays performed on different days.

Analysis of Heparin Samples of Different Strength. We extended our studies to the analysis of 20 samples of heparin sodium varying in strength between 25 and 130 units per mg. (see Table V). All the samples were subjected to the 4 most commonly used methods of assay, i.e., the thrombin method of Studer and Winterstein[2], the fresh ox blood method of Jalling, Jorpes and Lindén[1], the U.S.P. XIV method using recalcified citrated sheep plasma, and the method of the British Pharmacopœia 1953, using salted ox plasma. As a rule, the assay was made both against the original Swedish heparin standard (I) and against the international heparin standard (II).

Methods

The Whole Blood Method Using Fresh Ox Blood[7].

The coagulation time is determined in non-paraffined test tubes of pyrex glass holding 2·5 ml. (70 × 8 mm.) with exclusion of air. 10 tubes are placed in an oak rack (30 × 4 × 2 cm.), provided with a cover of the same size. The lower side of the cover is lined with rubber, as is also the upper surface of the rack, so that all the tubes can be tightly closed at the same time. Since the exclusion of air is essential, the hinge and the metal hook closing the stand must be strongly fixed. In order to permit the stand to be closed tightly after filling, it is further equipped with a screwing device in the middle.

Each tube contains 0·2 ml. of a diluted heparin solution and a glass bead, of somewhat smaller size than the bore of the tube. 5 of the tubes contain the standard heparin, 5 the unknown. The diluted solutions of standard heparin contain, in the first tube 10 mg. of water-free substance per 32 ml. of physiological saline solution, in the second 5 mg. and so on; corresponding amounts of the unknown are taken. In summer the heparin is dissolved in 16 ml., during winter the same amount sometimes requires 64 ml.

The blood is taken directly from the vessel into a paraffined dish when the animals are slaughtered at the abattoir. One after the other, the tubes are quickly filled with blood, the cover then being tightened without allowing air to enter the tubes, and the stand is turned over several times. Mixing is obtained by means of the glass bead. Thorough mixing is essential before the stands are left. Only 2 stands can be filled with blood from one dish and in every second stand the tubes with the standard heparin are filled first.

The first reading is made at the laboratory 2 hours later. Further readings are made after 4, 8 and 24 (26) hours. Since the slowing up of the speed of the glass bead indicates the beginning of coagulation, the time for the initial coagulation is noted, as well as that of the final stage. Tubes containing air bubbles are discarded.

The temperature need not be regulated since its influence is the same on the standard and the unknown.

The evaluation of the reading is a simple empirical one described in the original publication. Samples varying 200 per cent. in strength can be compared with each other.

When 16 stands were used at the same time, the method allowed a differentiation of samples varying in strength by 5 per cent. The results were not submitted to any statistical analyses. Since the steps between the different tubes differ in strength by 100 per cent., the method is not particularly well suited for an accurate analysis. Moreover, the accuracy of an assay is largely dependent upon the skill of the person who fills the tubes with fresh blood at the abattoir.

The *Thrombin Methods.*

In discussing the different methods available for the assay of heparin, Jaques and Charles[3] in 1941 gave a definite preference to the whole blood methods, using either freshly drawn ox blood or oxalated whole blood, to which a fixed amount of thrombin had been added. The oxalated whole blood was more stable than the plasma prepared from it. Moreover, the addition of thrombin made the end-point sharper.

The idea of assaying heparin against thrombin was taken up again by Kjems and Wagner[4] in 1948. They added varying quantities of heparin (0·04 to 0·1 ml. of a solution containing 2·5 I.U./ml.) to 1 ml. of oxalated ox plasma and a constant amount of thrombin. The unknown and the standard heparin were run simultaneously and the coagulation times were plotted against concentration of heparin over the range about 20 seconds to about 60 seconds. The readings were made in a water bath at 37° C. The fresh plasma was left standing at 0° C. for at least 24 hours before it was used. Even during the time of the experiment it had to be kept at +0·5° C. The volume of the heparin solution was adjusted to 0·2 ml. Due attention was paid to the salt concentration and to the acidity of the solutions.

In its final form, the thrombin method used by Studer and Winterstein was as follows:—

Reagents.

Citrated Ox Plasma. 19 volumes of fresh ox blood were added to 1 volume of 8 per cent. w/v trisodium citrate solution. 2 hours later, plasma was separated by centrifugation for 30 minutes at 3000 r.p.m. The plasma was left standing at 0° C. for 24 hours before freezing. It could then be stored in 150 ml. portions for several weeks at −20° C. It was thawed in warm water before use, filtered through gauze and kept in ice-water during the time of the experiment.

Heparin. A 0·1 per cent. solution of heparin with 90 to 130 I.U./mg.

Thrombin. An approximately 0·1 per cent. solution of thrombin with about 60 N.I.H. units/mg. A concentration should be chosen which gives a coagulation time of about 60 seconds for the 0·6 point on the curve.

Technique.

For the 0 *point of the standard curve without heparin*, 0·5 ml. of distilled water is added to 5 ml. of thrombin solution and 0·1 ml. of this

solution is diluted in a 1 ml. tuberculin syringe to 1 ml. with distilled water prewarmed to 20° C. 0·5 ml. of this solution is again diluted in the syringe to 1 ml. with water. The content of the syringe is added to 1 ml. of the thawed plasma, warmed in a water bath at 37° C., a stop-watch being started simultaneously. The test-tube is moved lightly to and fro in the water bath for 5 to 10 seconds. A platinum needle is then moved up and down in the test-tube until the first threads of fibrin are formed, when the clotting time is read off. The process is repeated 3 to 5 times. The clotting time of the 0 point should be about 20 seconds.

For the 0·2 point of the curve, 5 ml. of the thrombin solution is pipetted into a mixture of 0·1 ml. of the standard heparin solution and 0·4 ml. of water, after which the above procedure is repeated.

The clotting times of the 0·4 and 0·6 points and, if necessary, of further points in between are determined in the same way.

For each concentration of the heparin standard the same procedure is repeated 3 to 5 times. The clotting time of the 0·6 point should fall between 60 and 70 seconds on the standard curve. Approximately the same dilutions are made of the unknown heparin solution.

Only the ascending part of the standard curve can be used.

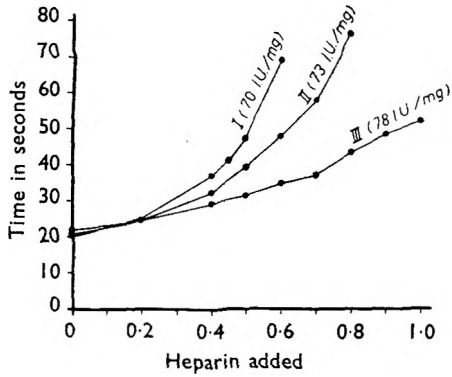

FIG. 1. Influence of storing plasma at 0° C. before freezing on the estimate of the potency of heparin.

I. Plasma frozen immediately after centrifuging.
II. Plasma stored for 24 hours before freezing.
III. Plasma stored for 48 hours before freezing.

The figures found for the anticoagulant activity of the same heparin sample are given in brackets. Concentration of the international heparin standard 0·331 mg./ml.

Comments on the Plasma Thrombin Method.

Kjems and Wagner, as well as Studer and Winterstein, pointed out that the plasma should not be frozen until it had been left for 24 hours at 0° C. In our experience, plasma prepared according to these authors tends to give too high values, as compared with the figures obtained in using plasma frozen immediately after being centrifuged. We have, therefore, preferred to freeze the plasma immediately. A new batch of fresh plasma was used every 3 weeks during 1 year, and always with reproducible figures. When thawed, the plasma can be stored for at least 4 to 5 hours in ice-water with no alteration in the standard curve. The effect of storing the plasma at 0° C. for 24 and 48 hours after centrifugation is shown in Figure 1.

The blood must be collected with the greatest care. It must be filtered through gauze before centrifugation. If small clots have been formed,

the blood is discarded. After thawing, the temperature of the plasma is never allowed to exceed + 5° C.

Our first experience[1] with the Studer and Winterstein method was disappointing. The assay of 16 different heparin preparations on as many days consistently gave about 20 per cent. higher figures than the whole blood method, irrespective of the strength of the samples, which varied between 30 and 130 units/mg. On changing over to a new batch of fresh plasma, lower and more consistent results were obtained. The original Swedish standard heparin was then found to contain 83, 79, 86, 83, 83 and 78 (mean 82) units/mg. of water-free substance as compared with the international heparin standard. The results given in Table III all refer to fresh plasma. We were unable to find any explanation for the

TABLE IV

ASSAY OF THE SWEDISH HEPARIN STANDARD AGAINST THE INTERNATIONAL HEPARIN STANDARD BY THE U.S.P. XIV METHOD
SHEEP PLASMA OF AUGUST 26, 1952

Date: September 14, 1952

Swedish heparin standard

Concentration:	12·095 mg. air-dry substance/10 ml.				Dilution 0·85 : 100						
Volume taken:	0·8	0·76	0·72	0·68	0·65	0·635	0·62	0·59	0·56		
Grade of clotting:				>0·5	>0·5	0·5	>1	>1	1		
Concentration:	12·095 mg. air-dry substance/10 ml.				Dilution 0·95 : 100						
Volume taken:	0·8	0·76	0·72	0·68	0·65	0·62	0·59	0·56			
Grade of clotting:				0·25	>0·5	>0·5	0·5	>0·75			

International heparin standard

Concentration:	5·05 mg./10 ml.		Dilution 0·9 : 100								
Volume taken:	0·8	0·77	0·74	0·71	0·68	0·66	0·64	0·62	0·60	0·58	0·56
Grade of clotting:		>0·25	0·25	>0·5	0·5	>0·75	>0·75	0·75	>1	1	1
Concentration:	5·05 mg./10 ml.		Dilution: 1 : 100								
Volume taken:	0·8	0·77	0·74	0·71	0·68	0·66	0·64	0·62	0·60	0·58	0·56
Grade of clotting:						0·25	>0·5	0·5	0·75	1	1

Calculation:

$$\frac{12{\cdot}095 \times 0{\cdot}85 \times 0{\cdot}635}{10 \times 100} \times x_1 = \frac{5{\cdot}05 \times 0{\cdot}9 \times 0{\cdot}68}{10 \times 100} \times 130; \quad \frac{12{\cdot}095 \times 0{\cdot}85 \times 0{\cdot}635}{10 \times 100} \times x_2 = \frac{5{\cdot}05 \times 1 \times 0{\cdot}62}{10 \times 100} \times 130$$

$$\frac{12{\cdot}095 \times 0{\cdot}95 \times 0{\cdot}59}{10 \times 100} \times y_1 = \frac{5{\cdot}05 \times 0{\cdot}9 \times 0{\cdot}68}{10 \times 100} \times 130; \quad \frac{12{\cdot}095 \times 0{\cdot}95 \times 0{\cdot}59}{10 \times 100} \times y_2 = \frac{5{\cdot}05 \times 1 \times 0{\cdot}62}{10 \times 100} \times 130$$

$x_1 = 62$; $x_2 = 62$; $y_1 = 59$; $y_2 = 60$ Mean: 61

difference in behaviour of the two batches of plasma. As a precautionary measure every new plasma sample should therefore be tested with two heparin samples of known strength.

As pointed out by Studer and Winterstein, the amount of thrombin remaining in excess after the addition of heparin strongly influences the shape of the curve. With a too high thrombin activity per ml. the curve will have a flatter appearance, which makes it less suitable. We found 0 points between 18 and 20 seconds most convenient to work with. If weaker thrombin solutions are used the heparin solution must be correspondingly diluted.

Studer and Winterstein recommend a heparin solution containing 100 to 130 I.U./ml. We have consistently used 30 to 40 I.U./ml. probably due to the fact, that we have used immediately frozen plasma samples (see Fig. 1).

We found the purity of the thrombin preparations less essential. We obtained equally good results when using preparations with from 10 to 70 Astrup units[5] per mg. The usual strength was 60 Astrup units per mg. and 40 to 50 mg. of thrombin was dissolved in 100 ml.

The Method of U.S.P. XIV, 1950.

The citrated sheep blood was centrifuged within 1 hour after slaughter and the plasma stored in small paraffined paper boxes at − 20° to − 25° C. After thawing the plasma and filtering through gauze it was left standing at + 2° C. for 1 hour. If a precipitate formed which did not redissolve at room temperature the plasma was discarded. The reading of the tests, a very delicate matter, was made by the same person who, in the course of a year and a half, performed readings on about 400 series of dilutions.

The technique applied is demonstrated in Table IV.

The Sulphated Whole Blood Method

With this method, aqueous heparin solutions are used to dilute whole blood, kept incoagulable by means of a high concentration of salts. The fresh ox blood is "salted" by mixing with one-fifth of its volume of 7 per cent. w/v solution of anhydrous sodium sulphate and then stored at 4° C. It is stable for 3 or 4 weeks.

In order to shorten the coagulation time a water extract of acetone-dried ox brain is added. The assay is conducted at room temperature.

Procedure. Prepare in *water* 3 dilutions of the Standard Preparation containing 1·28, 1·6 and 2·0 units/ml. and three expected equivalent dilutions of the preparation to be tested. Place 1 ml. of each dilution in 6 × ½ in. test-tubes followed by 0·2 ml. of thrombokinase extract; the amount of thrombokinase extract to be added may be varied slightly according to conditions, but should be chosen so that the longest clotting times range between 9 and 12 minutes. Add 1·0 ml. of sulphated whole blood and mix by gentle inversion, avoiding the formation of air bubbles. For each tube, record the time to the nearest 15 seconds from this addition to the formation of a firm clot which remains in the bottom of the tube when it is completely inverted. For a complete assay repeat the comparison 4 times.

The method offers many advantages. The equipment needed is very simple. The method is rapid and accurate. The data can be submitted to analysis of variance, the relationship of the log coagulation time to the log concentration of heparin being linear. Figures for the fiducial limits are easily obtained.

Discussion

As is evident from Table V, the thrombin method of Studer and Winterstein gives very small deviations between the values obtained on different days and in using the two different standard preparations. Along the whole line there is a surprisingly good agreement between the values for the same sample. No such agreement was obtained with the other methods.

The whole blood method, which we used earlier, requires much training in filling the glass tubes with fresh ox blood. The 100 per cent. difference between the concentrations, which is necessary for a clear-cut reading, is also a drawback. We have used the method because it is a whole blood method, without the introduction of any foreign factors into the system, except for the influence of the glass surface of the tubes. The technique also excludes any effect of the air, which is known to give rise to thrombokinase formation. The anticoagulant activity is determined under almost physiological conditions and the figures obtained seem to be

TABLE V

THE POTENCY OF HEPARIN SAMPLES IN I.U. AS ASSAYED ON DIFFERENT DAYS BY DIFFERENT METHODS AGAINST THE ORIGINAL SWEDISH HEPARIN STANDARD (I) AND THE INTERNATIONAL HEPARIN STANDARD (II)

Sample No.	Water free substance per cent.		Plasma + thrombin (Studer and Winterstein)		Fresh ox blood (Jalling, Jorpes and Lindén)		Recalcified plasma (U.S.P. XIV 1950)		Diluted salt plasma (B.P. 1953)	
	S	N	I	II	I	II	I	II	I	I
1	13·07	2·77	126 138	124 136		128	125 135 136	128 140 144		140 135
2	13·41	2·21	130	126 135		126	116 117	110 121	122	126
3	13·20	2·39	124 124	123 124	117	108 102 102	148 139 138	132 133 124 139	122	119 111 111
4	13·00	2·17	113 115 113 110	109 112	108	105 98 102	115 112 124	105 116 116 117	111	106 107 115
5	12·69	2·31	104 110	105 109		104 102	102 110 113	104 116	100	104 106
6	12·96	2·44	114	113		101 104 110	107	110	112	109 107
7	12·93	2·56	122	121		120	117 112	114	128	117
8	12·53	2·12	109	113		107 118	97 99 101	96 104		110
9	12·77	2·51	89 91	90	96	83 78 78	78 78	80 79		76 73 75
10	12·71	2·35	93	93		89	81 87	83	92	82 85
11	11·97	2·46	87 83	81 85		75 73	80 76	82 71	90	80 75
12	12·80	2·49	87 83	85		77 72	77	74	74	78 73 69
13	11·76	2·49	83 79	83		94 82	76 77	79		77 75
14	12·03	2·58	81			78	72 70	66		78
15	11·40	2·47	81			80	69	70		70 72
16	12·68	2·20	78	81 81		79	79		82	
17	10·03	3·21	73 73	71	85		69 66	69	69	
18	10·01	4·64	53 52	53	56		46 46		57	
19	8·39	4·09	30 27 29	29	28	29	15 17 17	15 17	24	
20	6·41	2·99	26 25	26 25		23		32	32	

In the thrombin method two concentrations of the unknown were analysed to be read off on the standard curve. In the whole blood method usually only 9 stands were taken each day. In the U.S.P. method two concentrations of the unknown were compared with two concentrations of the standard.

reliable within 5 to 10 per cent., which is the requirement in anticoagulant therapy.

We do not, however, wish to give any preference to this method, which for various reasons is inferior to the thrombin method of Kjems and Wagner and to that of Studer and Winterstein. As is shown in Table V, every second sample showed about 10 per cent. lower activity with the whole blood method as compared with the figures found with the thrombin method.

The least satisfactory results were obtained with the U.S.P. XIV method. The deviations between the values obtained on different days were quite remarkable. 10 of the 12 samples with an activity below 110 units/mg. showed 10 to 15 per cent. lower figures than with the thrombin method, and out of 8 samples with an activity above 110 units/mg., 2 presented 5 to 10 per cent. too low figures.

Our experience with the method of the British Pharmacopœia has hitherto been limited.

The Thrombin Method Versus the U.S.P. XIV Method. It is evident that recalcified sheep plasma is less suitable for the assay of ordinary heparin samples. Below 110 units/mg., it gave in our series figures 10 to 15 per cent. lower than the thrombin method and above this strength the figures are 5 to 10 per cent. either higher or lower. Different control laboratories also found well-known brands of commercial heparin less satisfactory when this method of assay was applied. We recently (June 1953) analysed a sample of a Danish heparin, which showed an activity of 3335 units/ml., as assayed against the international heparin standard with the U.S.P. method instead of the declared strength of 5000 I.U./ml. There was no reason to assume that the sample was of a low grade quality.

In their studies on treburon, a sulphated polygalacturonic acid methyl ester methyl glycoside, Mangieri, Engelberg and Randall[6] found that the U.S.P. XIV method did not show the true relative activities of treburon and heparin. Assayed by the U.S.P. method, treburon was approximately one-tenth as active as heparin. By Mangieri's recalcification method using frozen beef or sheep plasma, treburon was one-fourth as active as heparin. Also by Quick's[7] antithrombin method of 1936, using fresh rabbit plasma or frozen sheep plasma, treburon was one-fourth as active as heparin. The relative activities 4 : 1 found with these two methods *in vitro* agreed with the relative activities found *in vivo* in animal experiments. From the results of Mangieri and co-workers, the conclusion must be drawn that the U.S.P. XIV method is less sensitive in respect to the properties by means of which heparin and the heparinoids exert their anticoagulant activity in whole blood and *in vivo*.

From the theoretical point of view, the thrombin methods seem to be the most suitable. Heparin is, after all, an antithrombin more than anything else. In the first place, in the thrombin methods recalcification becomes superfluous. An important source of error is thereby eliminated. The extreme sensitivity of the coagulation mechanism to the concentration of the calcium ions is most clearly stressed by Mangieri[8], who found it necessary to titrate the amount of calcium to be added with concentrations varying in strength by 5 per cent.

Furthermore, in the non-thrombin methods the accelerating factors V and VII, proaccelerin and proconvertin, as well as the antihæmophilic globulin and the thrombokinase system, play their part in the reaction mechanism, in one way or another influencing the results. It is not surprising that the thrombin methods with the direct reaction between heparin and thrombin give the best reproducible results.

Summary

1. The anticoagulant activity of 20 samples of heparin sodium varying in strength between 25 and 130 I.U./mg. has been assayed by 4 different methods: a fresh whole blood method, a thrombin method on plasma, and the methods of the U.S.P. XIV and the B.P. 1953.

2. The U.S.P. XIV method gave 10 to 15 per cent. lower figures than the thrombin method for samples with 25 to 110 I.U./mg.

3. The whole blood method did not give as satisfactorily reproducible results as the thrombin method on plasma.

4. Commercial samples of heparin assayed with the U.S.P. XIV method tended to give lower figures for the anticoagulant activity than when assayed with the thrombin method.

5. Since different factors such as the recalcification, the proaccelerin and the proconvertin, as well as the thrombokinase system, influence the results in the non-thrombin methods, they are considered less suitable for the assay of heparin. Preference is therefore given to the thrombin methods, in which the heparin is neutralised by preformed thrombin, preferably with some whole blood method as an alternative.

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SYNERGIC EFFECTS OF AMYLOBARBITONE SODIUM AND ETHANOL

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Recently, attention was drawn to the lack of information concerning the effects of ethanol and barbiturates taken together.[1] In 1934, Carriere, Huriez and Willoquet[2] reported that ethanol antagonised the hypnotic effect and reduced the toxicity of phenobarbitone. Several subsequent investigators have been unable to confirm this work. Olszycka[3,4] found that ethanol potentiated the hypnotic effect of butobarbitone in mice and rats, and Dille and Ahlquist[5], working on rabbits, showed that it produced the same effect with pentobarbitone. Jetter and McLean[6] and Ramsey and Haag[7] investigated further the synergism between ethanol and barbiturates in laboratory animals and both groups of workers reported that the toxicity of barbiturates was increased considerably by the simultaneous administration of ethanol. Some clinical evidence also exists that ethanol potentiates the toxic effects of barbiturates. Thus, there is some confusion in the literature concerning the effects of ethanol and barbiturates given together. The pharmacological effects produced by the simultaneous administration of these two substances are obviously of considerable importance clinically and, therefore, the following experiments were carried out in the hope that the results would be of more than academic interest.

Materials

Amylobarbitone Sodium.

*iso*Amylethyl barbituric acid was used. 1 g. was dissolved in 8·85 ml. of 0·5N sodium hydroxide with the aid of gentle heat (less than 80° C. for not more than 30 minutes). The solution was diluted to 10 ml. with water and filtered. It was kept for not more than one day and diluted immediately before use with water.

Ethanol. Alcohol (90 per cent.) B.P., diluted with water, was used.

Animals. The experiments were performed using male albino mice weighing 20 to 24 g.

Methods

Two types of experiments were performed. The first was designed to determine the effect of ethanol on the acute toxicity of amylobarbitone sodium, and the second to investigate the anæsthetic effects of the two substances administered together.

Results

1. *Acute Toxicity of Amylobarbitone Sodium and Ethanol.*

300 mice were used in this experiment. Amylobarbitone sodium was administered orally as a solution containing 20 mg./ml. and ethanol

(50 per cent. v/v) was given by the same route. The MLD of each of these substances, administered alone, was determined, the number of dead animals being counted 24 hours after the administration of the drugs. Subsequently the amounts of ethanol required to produce 50 per cent. mortality in mice which had been given approximately one-quarter, one-half or three-quarters of the MLD of amylobarbitone sodium were determined. The results are shown in Table I and Figure 1.

TABLE I

DOSES OF AMYLOBARBITONE SODIUM AND ETHANOL NECESSARY TO PRODUCE 50 PER CENT. MORTALITY IN MICE

Amylobarbitone sodium		Ethanol
mg./kg.		ml./kg.
0	+	14·25
200	+	11·50
350	+	6·50
500	+	4·75
675	+	0

TABLE II

DOSES OF AMYLOBARBITONE SODIUM AND ETHANOL NECESSARY TO MAINTAIN ANÆSTHESIA IN 50 PER CENT. OF THE MICE FOR AT LEAST 1 HOUR

Amylobarbitone sodium		Ethanol
mg./kg.		ml./kg.
0	+	5·950
30	+	4·900
60	+	2·750
90	+	1·625
120	+	0

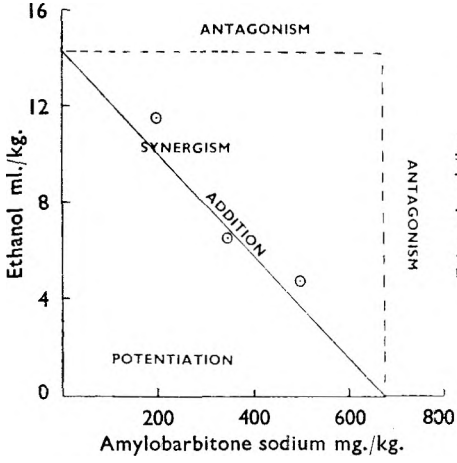

FIG. 1. Doses of amylobarbitone sodium and ethanol necessary to produce 50 per cent. mortality in mice.

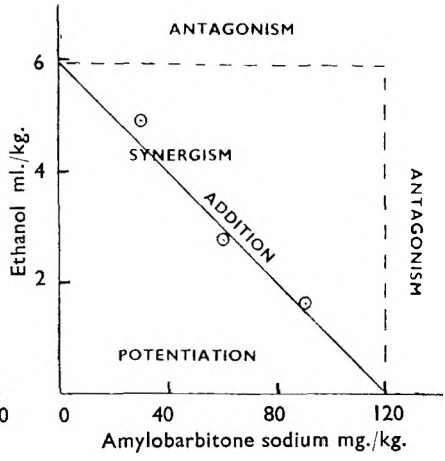

FIG. 2. Doses of amylobarbitone sodium and ethanol necessary to maintain anæsthesia in 50 per cent. of the mice for at least 1 hour.

2. *Anæsthetic Effect of Amylobarbitone Sodium and Ethanol.*

150 mice were used. The amylobarbitone sodium and ethanol were administered intraperitoneally. The median effective dose (MED) of each substance was defined as the amount necessary to maintain anæsthesia in 50 per cent. of the mice for at least 1 hour. The MED of each substance, administered alone, was determined. Groups of mice were given various doses of amylobarbitone sodium or ethanol. The animals were placed on their backs and the number in each group which failed

to right themselves within 1 hour was observed. Probits were plotted against the log-dose and the MED of amylobarbitone sodium and ethanol were estimated. Subsequent determinations were made of the amounts of ethanol to produce the median effective response when given in combination with one-quarter, one-half and three-quarters of the MED of amylobarbitone sodium. The results are shown in Table II and Figure 2.

Discussion

The results were plotted graphically to determine the nature of the combined effects of the two drugs (i.e., whether there existed synergism or antagonism) using the well-known method described by Gaddum[8]. In both figures the doses of amylobarbitone sodium were plotted as abscissæ and the doses of ethanol as ordinates. In Figure 1 a straight line was drawn to join the MLD of amylobarbitone sodium to the MLD of ethanol and in Figure 2 to join the MED of the two substances. When the results obtained with combinations of amylobarbitone sodium and ethanol were plotted on the graphs the points lay approximately on these lines. Therefore, a synergism exists between amylobarbitone sodium and ethanol. However, the combined effects are simply additive and the results of the present study provide no evidence that the toxic or anæsthetic effects of amylobarbitone sodium are potentiated by ethanol.

Several workers have investigated this problem in laboratory animals. Most investigators have reported the existence of synergic effects between barbiturates and ethanol but they have not attempted to make any quantitative studies to determine the type of this synergism. In particular, experimenters who have reported a potentiation of the effects of barbiturates by ethanol have generally not used very convincing graphical or mathematical methods to substantiate their findings.

Olszycka[3,4] measured the duration of sleep in her experiments on rats and mice. A similar method has been used in this laboratory, where an attempt was made, with mice, to determine the relationship between dose and sleeping time for amylobarbitone sodium and for combinations of the barbiturate with fixed amounts of ethanol. It was found that a linear relationship existed between the logarithm of the dose of amylobarbitone sodium and the duration of the sleep. The slope of the dose-response curve was not influenced by the presence of ethanol—a fact which provided further confirmation that the anæsthetic effect of amylobarbitone sodium is not potentiated by ethanol. However, the scatter of the observations was so wide that the method was abandoned.

In the present study, no attempt was made to follow the rates of absorption and excretion of ethanol and amylobarbitone. Since the pharmacological effects of the two drugs given together were simply additive, it was considered unlikely that the presence of one of the substances had modified the metabolism of the other. Olszycka[4] reported that the metabolism of ethanol, in rats, was not influenced by butobarbitone, and Ramsey and Haag[7] found that the distribution of ethanol and of barbiturates in body fluids was not altered when the two substances were given together.

Since the most pronounced pharmacological effect produced by both ethanol and amylobarbitone sodium is a depression of the central nervous system, it is not surprising that a synergism exists between the two substances. Jetter and McLean[6], working on rats, reported that lethal effects were produced by the administration of sublethal doses of ethanol and phenobarbitone together, and they emphasised the clinical significance of the possible effects of such a combination. Olszycka[3] found that doses of butobarbitone and ethanol, not sufficiently great to produce hypnosis when given alone, produced prolonged sleep, in mice, when administered together. The results of both these groups of workers, while indicating the existence of a synergism between these two drugs do not demonstrate that the effects of barbiturates are *potentiated* by ethanol. The present investigation showed that the type of synergism existing between amylobarbitone and ethanol is a simple additive effect. It is possible, therefore, that the administration of doses of the two drugs, not sufficient to produce toxic effects or hypnosis when administered alone, will produce very definite responses when they are given in combination. There is no doubt that the sedative effect of barbiturates would be further increased by ethanol. However, the results of experiments on laboratory animals provide no evidence that acute toxic effects would follow the ingestion of small doses of barbiturates and ethanol together.

Summary

1. Amylobarbitone and ethanol were found to act synergistically in producing toxic and anæsthetic effects in mice.

2. Graphical treatment of the results showed that the effects of the two drugs were simply additive and there was no evidence that ethanol potentiates the acute toxicity or anæsthetic effect of amylobarbitone sodium.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Protoveratrine A and B and Germitetrine B from *Veratrum album*. H. A. Nash and R. M. Brooker. (*J. Amer. chem. Soc.*, 1953, **75**, 1942.) Protoveratrine from *Veratrum album* was found to be a mixture of two alkaloids, protoveratrine A and B. They were separated by a Craig countercurrent distribution procedure using a chloroform–water–acetic acid system. Protoveratrines A and B are remarkably alike in many of their properties. Protoveratrine A was found to conform to the accepted structure of protoveratrine except that it yielded two instead of one mole of acetic acid on hydrolysis. Protoveratrine B yielded protoverine, 2-methylbutyric acid, 2:3-dihydroxy-2-methylbutyric acid and two moles of acetic acid on hydrolysis, corresponding to an empirical formula of $C_{41}H_{63}O_{15}N$. Analytical data and equivalent weight determinations support this formula. After removal of "protoveratrine" from the total alkaloids, considerable hypotensive activity remained in the residual "amorphous alkaloid" fraction. Application of paper chromatographic methods to the examination of the fraction showed the presence of at least 15 alkaloids. By means of countercurrent distribution and fractional crystallisation, an alkaloid named germitetrine B was isolated. It yielded germine, 2-methylbutyric acid, 2:3-dihydroxy-2-methylbutyric acid and two moles of acetic acid on alkaline hydrolysis. A. H. B.

***Veratrum eschscholtzii* Gray, Alkaloids of.** M. W. Klohs, M. D. Draper, F. Keller, M. Malesh and F. J. Petracek. (*J. Amer. chem. Soc.*, 1953, **75**, 2133.) *iso*Rubijervosine, a new glucosidic alkaloid, was isolated from the hitherto uninvestigated species, *Veratrum eschscholtzii* Gray, as well as the known glycosides, pseudojervine and veratrosine. *iso*Rubijervosine was obtained from the hypotensively active amorphous bases by the use of chromatography and fractional crystallisation as fine needles, m.pt. 279° to 280° C., $[\alpha]_D^{24° C.} - 20 \pm 2$ (*C* 1·45 in pyridine). The formulation $C_{33}H_{53}O_7N$ for the alkaloid was derived by analysis of the base and its pentacetyl derivative. On acid hydrolysis, *iso*rubijervosine yielded the aglycone *iso*rubijervine and D-glucose, and from this and other evidence its structure was established as 3-(β)-D-glucosyl-Δ^5-solanidene-18-ol. A. H. B.

ANALYTICAL

Barbiturates, Argentimetric Determination of. P. Chavanne and H. Marie. (*Ann. pharm. franç.*, 1953, **11**, 91.) The method is based on that of Danielsson (*Svensk farm. Tidskr.*, 1951, **55**, 125) but avoids the use of potassium metaborate which is difficult to obtain: 0·6 g. of the barbiturate is dissolved in 7 ml. of N ethanolic potash, and the solution is diluted with 33 ml. of water and treated with 0·45 g. of boric acid. After warming to dissolve the boric acid, and cooling, the mixture is titrated with 0·1 N silver nitrate in presence of 1·5 ml. of 10 per cent. potassium chromate solution as indicator. The end-point is indicated when the solution assumes permanently a colour different from that of a comparison solution containing 2 g. of precipitated calcium carbonate,

1·5 ml. of potassium chromate solution and 55 ml. of water. One molecule of silver nitrate corresponds to 2 molecules of barbiturate. The method has been applied to barbitone, allobarbitone and butobarbitone. It may also be used for hexobarbitone, but in this case there is no precipitate and the comparison solution does not contain calcium carbonate. For phenobarbitone, a different *p*H value is necessary and the amount of boric acid is therefore increased to 1·20 g. G. M.

Bismuth, Direct Titration of with Complexone. K. E. Grönkvist. (*Farm. Revy*, 1953, **52**, 305.) A method is proposed for the determination of bismuth in compounds and preparations occurring in pharmacy. The procedure is based on the titration at a *p*H of 2·5 to 4·0 with 0·05M complexone III (the disodium salt of ethylenediamine tetra-acetic acid) using the yellow-coloured complex between bismuth and thiourea as indicator. The general method consists of neutralising the colourless solution of bismuth (corresponding to 0·10 to 0·20 g. Bi) in nitric or hydrochloric acid with 5M sodium hydroxide added drop by drop until the first permanent turbidity is obtained and then adjusting the volume to about 30 ml. with water. Thiourea (6 g.) is added and solution effected by gentle heat on a water bath. After the addition of 30 ml. of water, 3 g. of potassium hydrogen phthalate and one drop of a gentian violet solution, the bismuth is titrated by means of complexone III 0·05M to a violet colour. The solutions to be determined must be colourless, and the pre-treatments of the pharmaceutical coloured substances to obtain the bismuth in colourless solution are described. The results by the proposed method are compared with results obtained by official methods of assay. Most metal ions in large amounts interfere with the determination but NH_4, K, Na, Ca, Ba, Sr and Mg in 5 times the amount of bismuth did not interfere. A. H. B.

Calomel, Assay of, in Preparations Containing Vegetable Drugs. L. Domange and L. Seguin. (*Ann. pharm. franç.*, 1953, **11**, 193.) Calomel may be determined by reaction with an excess of standard iodine solution, followed by back-titration with thiosulphate, but the method gives high results in the presence of cascara, tansy, santonica, etc., which react with iodine. It is preferable to destroy the organic matter before carrying out the determination, care being taken to avoid loss of calomel by volatilisation. The following method is recommended. Place the sample, containing 0·06 to 0·12 g. of calomel and preferably not more than 0·25 g. of vegetable matter in a conical flask, add 10 ml. of sodium persulphate, 100 ml. of water and 20 ml. of sodium hydroxide solution, shake frequently during 1 hour, cover with a small funnel and heat for 2 hours on a boiling water bath. This destroys organic matter and produces a yellow precipitate. Cool, add 10 ml. of sodium hydroxide solution and 10 ml. of formaldehyde solution and allow to stand for 15 hours to reduce the precipitate to mercury. Decant the supernatant liquid through a filter, wash with a dilute solution of sodium hydroxide and place the filter in the flask. Add 2 ml. of acetic acid (50 per cent.) and 20 ml. of 0·1N iodine, and, when all the mercury has dissolved, titrate the excess of iodine with sodium thiosulphate in the presence of starch mucilage. Preparations containing a large proportion of sucrose or lactose should be washed with water to remove the sugars before assay. Suppositories should be prepared for assay by removal of the oil of theobroma with ether, but pills may be assayed without prior treatment. G. B.

Glycerol, Periodate Determination of. L. Hartman. (*J. appl. Chem.*, 1953, **3**, 308.) A study of the determination of glycerol by the potassium periodate method has been carried out with the object of developing a rapid and exact analytical procedure. It was found that the time of oxidation recommended in the existing methods could be substantially reduced from 1 hour or more to 5 minutes without affecting the accuracy of the determination. Results and conditions are given for the oxidation procedures based on the titration of the formic acid produced and on the estimation of the residual periodate. The use of potassium dimesoperiodate (dipotassium periodate) instead of the much less soluble metaperiodate (monopotassium periodate) made it possible to increase the size of the glycerol sample and thus to achieve a greater reproducibility of results; the salt in solution can be prepared by dissolving potassium periodate in an equivalent amount of aqueous potassium hydroxide. R. E. S.

Hexoses, Determination of. B. Klein and M. Weissman. (*Analyt. Chem.*, 1953, **25**, 771.) A new colour reaction is described for the identification and determination of hexoses in the presence of pentoses, based upon the action of chromotropic acid in 15M sulphuric acid. The reaction depends on the conversion of hexoses to 5-hydroxymethylfurfural followed by the splitting of the methylol group to form formaldehyde which reacts with chromotropic acid. Under these circumstances, pentoses which form furfural, incapable of splitting off formaldehyde, do not react, but the common hexoses—glucose, galactose, mannose and fructose—react and give a characteristic violet colour, the intensity varying linearly with the concentration; the common disaccharides —lactose, sucrose and maltose—also react. The pentoses arabinose, xylose, and rhamnose failed to react under the conditions of the experiment. No quantitative stoichiometric relationship was apparent between the amount of hexose used and the formaldehyde produced. Pentoses did not interfere with either the quantitative or the qualitative reaction; 1 ml. of a solution containing 0·1 mg. of glucose with increasing amounts of arabinose produced the same optical density in the reaction and a quantitative glucose determination was unaffected. R. E. S.

***iso*Nicotinyl Hydrazide, Analysis of.** A. Anastasi, E. Macarelli and L. Novacic. (*Mikrochemie*, 1952, **40**, 113.) Alkaline solutions of *iso*nicotinyl hydrazide may be assayed polarographically. A solution, of 0·1 to 1 millimols/l., is prepared by dilution with Britton-Robinson buffer (*p*H 9) containing 0·01 per cent. of gelatin, and examined in the range $-0{\cdot}8$ to $-1{\cdot}8$ volts. A corresponding sodium acetate-hydroxide buffer may also be used, in which case the half-wave potential is $-1{\cdot}33$ in place of $-1{\cdot}2$ (saturated calomel electrode). The presence of *iso*nicotinic acid, in the case of acetate buffer, is indicated by a reduced potential step proportional to the amount of hydrolysis of the hydrazide. Free hydrazine cannot be detected polarographically. The amount of hydrazine produced by decomposition of a solution can be determined by the increase in the titre of the solution with nitrous acid, in accordance with the following equations:

$$RCO{\cdot}NH{\cdot}NH_2 + HNO_2 = RCON_3 + 2H_2O$$

$$H_2N{\cdot}NH_2 + 2HNO_2 = 3\,N + 3H_2O + NO$$ G. M.

***iso*Nicotinyl Hydrazide, Electrometric Determination of.** A. Anastasi, E. Mecarelli and L. Novacic. (*Mikrochemie*, 1952, **40**, 53.) The following methods are given. 1. Addition of excess of cerium sulphate, heating for 1 hour on the water bath, and back titration with ferrous sulphate. The results show a variation of about 2 per cent. 2. Titration with nitrous acid, with formation of $RCON_3$. The end-point may be determined with starch iodide paper, but better electrometrically. The method may be applied directly to tablets, injections and syrups. The end-point may be obtained from the potential curve of a platinum electrode against a saturated calomel electrode, by observation of the polarisation current between two platinum electrodes (applied voltage about 15 mV) or by observation of the current between a platinum and a tungsten electrode. 3. Titration of the hydrazide group in glacial acetic acid with perchloric acid, using crystal violet or, better, an electrometric method. 4. Titration of the *iso*nicotinic acid group with sodium methylate in diethylamine solution. The end-point is determined with thymol blue, or electrometrically. For the latter an antimony-glass electrode pair is used. G. M.

***iso*Nicotinyl Hydrazide, Volumetric Determination of.** H. Harting. (*J. Amer. pharm. Ass. Ass. Sci. Ed.*, 1953, **42**, 323.) A simple and rapid method for the determination of isoniazid depends upon the evolution of the hydrazine nitrogen in the presence of an excess of oxidising agent such as a mixture of potassium ferricyanide and potassium hydroxide. The nitrogen evolved is measured by displacement in a burette and its weight calculated. Each g. of nitrogen is equivalent to 4·893 g. of isoniazid. 10 ml. of a 20 per cent. solution of potassium ferricyanide and 10 ml. of a 20 per cent. solution of potassium hydroxide is sufficient to oxidise 50 mg. of isoniazid dissolved in 20 ml. of water. The method is applicable to tablets, mixtures and biological fluids, but cannot be used in the presence of thiacetazone which yields nitrogen under the same conditions.

G. B.

Phenyl Thiohydantoins, Paper Strip Identification of. J. Sjöquist. (*Acta chem. scand.*, 1953, **7**, 447.) In Edman's method (*Acta chem. scand.*, 1950, **4**, 283) for determining the amino-acid sequence in peptides, phenyl thiohydantoins are formed. A paper chromatographic procedure for the direct identification of these latter compounds is described. The descending technique was used, and solvent of heptane–pyridine and heptane–butanol with formic acid applied. The R_F values of a number of phenyl thiohydantoins prepared from naturally occurring amino-acids are recorded. A. H. B.

Platyphylline, New Reaction for. V. F. Kramarenko. (*Aptechnoe Delo*, 1953, **2**, No. 2, 52.) The present identity test for platyphylline tartrate in the Soviet Pharmacopœia VIII is based on the tartrate radical. The following reaction which is based on the presence of a carboxylic ester group in the molecule of platyphylline base is proposed. Place a small quantity of the substance in a small test-tube; add 1 drop of saturated ethanolic solution of hydroxylamine hydrochloride and 1 drop of saturated ethanolic potassium hydroxide. Heat to boiling and, after cooling, add two drops of ethanolic hydrogen chloride. Add a few pieces of marble to remove excess of hydrochloric acid and when the evolution of carbon dioxide has ceased add one drop of hydrogen peroxide and, after a minute, 1 drop of 5 per cent. ferric chloride solution. A transient blue-violet colour is observed. E. H.

Potassium, Colorimetric Determination of, with Dipicrylamine. R. Faber and T. P. Dirkse. (*Analyt. Chem.*, 1953, **25**, 808.) The method of Amdur (*Industr. Engng Chem.* (*Anal.*), 1940, **12**, 731) was examined in an attempt to determine the extent to which zinc and other ions interfered in the estimation. It was evident that the presence of even small amounts of ammonium ion caused considerable errors in the potassium determination. The effect of *p*H was studied and it was found that a *p*H of 3 or 3·5 was the lower limit for accurate results, the upper *p*H limit being about 11. In the presence of zinc it was found that only when the weight of zinc in a sample was greater than the weight of potassium need the zinc be removed or reduced in amount before proceeding with the determination of potassium. R. E. S.

Riboflavine, Polarographic Determination of. W. J. Seagers. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 317.) Purified and partially purified riboflavine were assayed by dissolving 20 to 30 mg. in 5 ml. of 0·4M sodium hydroxide and adding 20 ml. of buffer solution (0·2M acetic, boric and phosphoric acids and 0·1M potassium chloride) and sufficient water to produce 50 ml. The solution (*p*H 2·8) was placed in a polarographic cell with a dropping mercury electrode, and nitrogen passed through the solution to remove dissolved oxygen. The polarograph was taken over the range of potential 0 to −1 volt. The experiment was repeated using dried riboflavine U.S.P. reference standard and the result calculated from the ratio of the diffusion currents. Solubilised riboflavine compounds were assayed by dissolving 0·5 to 0·6 g. in 100 ml. of water and adding to a 10-ml. aliquot, 10 ml. of a mixture of 1 part of 0·4N sodium hydroxide and 4 parts of buffer solution. Another 10-ml. aliquot was similarly treated, but 20 mg. of dried reference standard was added. The solutions were diluted to 50 ml. and polarographed as before. Vitamin mixtures containing riboflavine were mixed with a solution of equal quantities of 0·1M hydrochloric acid and acetone, 10 ml. of the supernatant liquid, after standing, being placed in each of 2 flasks. To one was added 5 ml. of a mixture of 4 parts of the acetone −0·1M hydrochloric acid and 1 part of 0·4M sodium hydroxide. To the other was added 5 ml of riboflavine standard solution. The solutions were deoxygenated and polarographed as before. The results agreed satisfactorily with the fluorimetric assay, taking into account the precision of the methods, ± 2 per cent. for the fluorimetric and ± 3 per cent. for the polarographic assays. G. B.

Sugars, Colorimetric Estimation of, with Benzidine. J. K. N. Jones and J. B. Pridham. (*Nature, Lond.*, 1953, **172**, 161.) A quantitative technique, using a solution of benzidine in acetic acid, has been perfected for the majority of common sugars and their methylated derivatives, with the exception of ketoses. 1 ml. of sugar solution and 5 ml. of a 0·2 per cent. (w/v) benzidine solution in glacial acetic acid were placed in a boiling-tube and heated in a boiling water bath for 15 to 60 minutes according to the sugar under test. After cooling to room temperature the orange-yellow coloration was measured photometrically (Ilford No. 601 filter), the relationship between concentration and absorption being linear. Polysaccharides could be analysed quantitatively by the new method using paper chromatography; sugars were extracted from the paper with hot methanol (90 per cent.), the methanolic sugar solution was evaporated to a syrup *in vacuo* and distilled water added, a 1-ml. aliquot of the solution being taken for analysis. An error of 3 to 5 per cent. was found in the estimation of standard sugar mixtures. R. E. S.

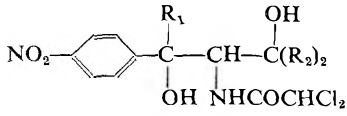
Sulphate and Organic Sulphur, Microdetermination of. L. Andersen. (*Acta chem. scand.*, 1953, **7**, 689.) A spectrophotometric method is presented for the determination of sulphate and organic sulphur on the micro- and ultramicro-scale depending on the fact that benzidine and its salts absorb strongly in the ultra-violet, $\lambda_{max.}$ at 250 mμ. The sulphate is precipitated as benzidine sulphate, is isolated, dissolved in hydrochloric acid and diluted to a certain volume, and its absorption measured in a spectrophotometer. The solubility of benzidine sulphate in water is 98 mg./l. at room temperature and the precipitation cannot, therefore, be made from a highly diluted solution. Strong acidity raises the solubility further, but it is most advantageous to work with slightly acid solutions. For ultramicro quantities the solubility can be reduced by using ethanolic solutions. For the determination of sulphur in organic material, the destruction is generally carried out by the method of Carius in a small sealed tube of about 0·5 cm. diameter; the nitric acid is evaporated, and the remaining traces of free acid carefully neutralised by means of ammonia. R. E. S.

Sulphonamide Separations based on Ion Exchange Chromatography. H. H. Hutchins and J. E. Christian. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 310). The distribution coefficients of sulphanilamide and sulphadiazine were determined for 5 cation exchange resins, by placing 10 or 25 ml. of a standardised solution of the S-35 labelled sulphonamide in hydrochloric acid solution in bottles containing various weighed amounts of the cation exchangers in the free acid form and shaking for 72 hours to bring to equilibrium. 1-ml. quantities of the solutions were analysed by Geiger-Muller counter technique. The ratio of average distribution coefficients for sulphanilamide and sulphadiazine with any of the resins was greater than 1·2, indicating the possibility of separation by chromatographic elution. The effects of flow rate, type and depth of resin, mesh size and elutriant were investigated for 6 sulphonamide derivatives. Sulphanilamide was separated from its acetyl derivative by placing 25 ml. of aqueous acidic solution on a column of Amberlite IR-120 (H) A.G., adding distilled water and collecting a 500-ml. fraction which contained the acetyl derivative, sulphanilamide being retained on the column until eluted with 1000 ml. of 4N hydrochloric acid. In a method of analysis of mixtures of sulphonamides in tablets, etc., solutions of S-35 labelled sulphadiazine and sulphanilamide of known specific activity were added to a solution prepared from tablets containing sulphanilamide and sulphadiazine. After separation on a column of Amberlite IR-120(H)A.G. the specific activity of suitable eluate fractions was determined and the content of sulphonamide calculated. This method is applicable to many types of sulphonamide mixture and to the determination of trace amounts in biological fluids. G. B.

Thioureas, Paper Chromatography of. A. Kjaer and K. Rubinstein. (*Acta chem. scand.*, 1953, **7**, 528.) A detailed description of the technique used, and the results obtained by the application of paper chromatography to the separation of a large number of thioureas is given: *N*-substituted and *NN'* and *NN*-disubstituted thioureas were investigated. The ascending technique was used and the most suitable solvent mixture proved to be chloroform/water. Exploratory experiments indicated that the application of the method to quantitative studies gave results with an accuracy of ± 5 per cent. Some related compounds, viz. thioamides, thiohydrazides, thiosemicarbazides and thiobarbituric acids were also briefly studied by the same technique. A. H. B.

ORGANIC CHEMISTRY

Chloramphenicol, Related Compounds of: Some Tertiary Alcohols. M. C. Rebstock and A. C. Moore. (*J. Amer. chem. Soc.*, 1953, **75**, 1685.) A series of compounds related to chloramphenicol were prepared in which the "1" hydrogen or the two "3" hydrogens were replaced by *p*-nitrophenyl or methyl groups. The compounds were of general formula.



and the following substances were prepared: (*a*) R_1 = *p*-nitrophenyl-, R_2 = H, (*b*) R_1 = H, R_2 = CH_3, (*c*) R_1 = H, R_2 = *p*-nitrophenyl-, (*d*) R_1 = CH_3, R_2 = H. None of the compounds was found to possess significant antibacterial, antirickettsial or antivirus properties. A. H. B.

Gentisic Acid, Infra-red Spectra of Esters of. J. F. Nash, F. W. Bope and B. V. Christensen. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 250.) Infra-red spectra were obtained by examination of solutions in chloroform, ether and nujol gel, and evaluated by comparison with the spectra of known compounds of gentisic acid. Decreased absorption in the region 3·0 to 4·2 μ occurred when the 5-hydroxyl group was modified or replaced by ether or methoxyl. Changes were also observed in the region 5·6 to 6·0 μ. Examination of the spectra of 2-diethylaminoethyl gentisate hydrochloride, 2-diethylaminoethyl 5-methoxysalicylate hydrochloride and the corresponding 3-diethylaminopropyl compounds showed the presence of 1 carbonyl group, indicating the esterification of the carboxyl group of gentisic acid. 2-Diethylaminoethyl 5-acetylgentisate hydrochloride, 2-diethylaminoethyl 5-benzoylgentisate hydrochloride and the 3-diethylaminopropyl analogues appeared to contain 2 carbonyl groups, suggesting the esterification of the carboxyl and 5-hydroxyl groups. Reaction between gentisic acid and the acid chlorides of phenylacetic, anisic, *p*-nitrobenzoic and succinic acids in the presence of alkali produced compounds with 2 carbonyl groups, esterification having occurred at the 5-hydroxyl group of gentisic acid. An exception was observed in the product of the reaction between gentisic acid and acetylsalicylic acid chloride, which appeared to have only 1 hydroxyl group. The constitution of this compound is doubtful. G. B.

Gentisic Acid, Preparation and Analyses of New Esters of. J. F. Nash, F. W. Bope and B. V. Christensen. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 207.) 8 esters of gentisic acid were prepared by reaction with the chlorides of 2-diethylaminoethanol and 3-diethylamino-1-propanol. The best yield was generally obtained by dissolving the acid in *iso*propanol, adding the chloralkamine and heating under a reflux condenser for several hours. A dry method consisted of mixing the chloralkamine with an ethanolic solution of the carboxylic acid, removing the ethanol under reduced pressure and heating the residue at 90° to 100° C. for 10 hours. 5 esters of gentisic acid were prepared by the Schotten-Baumann reaction involving the 5-hydroxyl group of gentisic acid with phenylacetic, *p*-nitrobenzoic and *o*- and *p*-methoxybenzoic acid chlorides or the mono-gentisic ester of succinic acid chloride in 10 per cent. sodium hydroxide solution. Melting points, molecular weights and elementary analyses are given for these compounds. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Bacitracin A, Nature of. G. G. F. Newton and E. P. Abraham. (*Biochem. J.*, 1953, **53**, 604.) Bacitracin A has been purified by counter-current distribution between solvents and shown to be a polypeptide, the minimum stoichiometric unit of which has a molecular weight of about 1500. The amino-acids cysteine 1, ornithine 1, lysine 1, histidine 1, aspartic acid 2, glutamic acid 1, phenylalanine 1, *iso*leucine 2, and leucine 1, have been identified. The minimum molecular weight of 1500 for each stoichiometric unit of bacitracin A hydrochloride follows from the weights of the amino-acid residues, if allowance is made for 3 molecules of hydrochloric acid; the figure is confirmed by the amide nitrogen liberated on acid hydrolysis and the spans of the titration curve. The latter indicate that the peptide contains groups which ionise in 3 distinct ranges of hydrogen ion concentration attributable to the presence of two carboxyl groups, the glyoxaline ring of histidine and a free α-amino group, and either the δ-amino group of ornithine or the ϵ-amino group of lysine. Reaction of bacitracin A with 1-fluoro-2:4-dinitrobenzene and hydrolysis of the dinitrophenyl bacitracin gave products which indicated that the peptide contains a free α-amino group belonging to a leucine or an *iso*leucine residue, a free NH in the glyoxaline ring of a histidine residue and a free δ-amino group belonging to ornithine. The ϵ-amino group of lysine is not free, and it is suggested that bacitracin A may be a cyclic polypeptide in which a carboxyl group is condensed with the ϵ-amino group of lysine. Bacitracin A is rapidly inactivated by 0·5N hydrochloric acid at 100° C., a reaction which is paralleled by the liberation of a free thiol group. This reaction is also accompanied by hydrolysis of an amide group and the disappearance of an absorption maximum at 252 mμ. Bacitracin A is similarly inactivated by treatment with 0·1N sodium hydroxide at 37° C. with liberation of 1 equivalent of ammonia. The potential thiol group is unaffected by this treatment, and there is little change in the ultra-violet absorption spectrum. Hydrogenolysis with Raney nickel converts the cysteine residue to an alanine residue with a free α-amino group. Hydrolysis of the product of hydrogenolysis yields two new substances which give a blue colour with ninhydrin; one of these substances appears to be an amino-alcohol. The possible presence of a thiazoline ring system in bacitracin A is discussed in the light of the experimental evidence; the latter is not conclusive. J. B. S.

Corticotrophin and Corticotrophin-B, Preparation of Potent Concentrates of. A. W. Bazemore, J. W. Richter, D. E. Ayer, J. Finnerty, N. G. Brink and K. Folkers. (*J. Amer. chem. Soc.*, 1953, **75**, 1949.) The investigations were carried out on whole frozen hog pituitary glands or a commercial acid–acetone extract. Procedures are described for the preparation in good yields of corticotrophin and corticotrophin-B concentrates at activity levels of approximately 80 units/mg. The process applied to whole frozen hog pituitary glands consisted of acetone defatting, extraction with a methanol–acetic acid mixture, purification with oxycellulose and pepsin digestion. Commercial acid–acetone powder was processed by oxycellulose treatment and pepsin digestion to yield similarly purified concentrates of corticotrophin-B.

A. H. B.

Corticotrophin-B, Nature of. N. G. Brink, G. E. Boxer, V. C. Jelinek, F. A. Kuehl, Jr., J. W. Richter and K. Folkers. (*J. Amer. chem. Soc.*, 1953, **75**, 1960.) The final steps in the isolation of corticotrophin-B involved the use of ion exchange resins and then countercurrent distribution to yield material of 250 to 300 units/mg. of activity. This material was believed to be corticotrophin-B in essentially pure form. It appears to be a peptide of molecular weight in the range of 5000 to 7000. It contains 14 common amino-acids in amounts corresponding to a chain of some 60 amino-acid units. The amino-acids and ammonia isolated from the hydrolysis experiments accounted for 99·5 per cent. of the total nitrogen. The preponderance of basic amino-acids reflects the known basic nature of corticotrophin-B. If a prosthetic group occurs in corticotrophin-B it must be of low molecular weight and contain so little nitrogen and exhibit so little characteristic ultra-violet or infra-red absorption that these properties would be obscured by those of the gross peptide. The substance is highly active clinically in rheumatoid arthritis. It possesses adrenal weight-increasing activity and causes melanophore expansion in frog skins. A. H. B.

Corticotrophin-B, Purification of, by Ion Exchange Techniques. J. W. Richter, D. E. Ayer, A. W. Bazemore, N. G. Brink and K. Folkers. (*J. Amer. chem. Soc.*, 1953, **75**, 1952.) Procedures are described which gave a threefold enhancement of the activity of corticotrophin-B concentrates. The active material of these concentrates was allowed to undergo exchange with a sodium-buffered column of Amberlite IRC–50. A substantial amount of inactive proteinaceous material was removed from the column by washing with aqueous pyridine, and other proteins, pyridine and sodium ions then eluted with aqueous acetic acid. The active principle was then removed from the resin with dilute hydrochloric acid, and recovered as a solid hydrochloride free from inorganic salts. It possessed an activity of 250 to 300 units/mg. It was also shown that fractionation on columns of oxycellulose led to considerable purification. The presence of a reducing agent such as sulphite or hydrogen sulphide during the above processes inhibited inactivation and made possible better separations and more highly active products. A. H. B.

Esterified Fatty Acids and Total Fatty Acids in Blood, Determination of. I. Stern and B. Shapiro. (*J. clin. Path.*, 1953, **6**, 158.) Higher fatty acid esters react at room temperature with hydroxylamine in alkaline solutions in aqueous ethanol. This reaction was examined to establish optimal conditions for an analytical procedure. The procedure adopted consisted of adding serum (0·1 to 0·3 ml.) containing 2 to 5 mEq. of fatty acid esters to 8 ml. of an ethanol–ether mixture. The mixture is brought to the boil, cooled, made up to 10 ml., filtered and 3 ml. of the filtrate measured into a 16 mm. test tube. A blank containing 3 ml. of the ethanol–ether mixture is included in every run. Then 0·5 ml. of a 2M hydroxylamine solution and 0·5 ml. of a 3·5N sodium hydroxide solution are added and mixed, and the tubes stoppered and allowed to stand for 20 minutes at room temperature. After this period, 0·6 ml. of hydrochloric acid solution is added and, after mixing, 0·5 ml. of a 0·37M ferric chloride solution dissolved in 0·1N hydrochloric acid solution is introduced. The tubes are mixed again and the colour developed is measured in a Fisher electrophotometer with an F.525^{B} filter using micro test tubes. This hydroxamic method gives results comparable with those of a gravimetric macro-method,

in which the fatty acids are extracted from 3 ml. of serum and are weighed after saponification and acidification. The method gives a high reproducibility with a standard deviation of ±4 per cent. A. H. B.

BIOCHEMICAL ANALYSIS

Barbiturates in Urine; Paper Chromatographic Identification of. L.-G. Allgén. (*Svensk. farm. Tidskr.*, 1953, **57**, 188.) Extract 50 ml. of urine with 100 ml. of redistilled chloroform; filter, and evaporate. Dissolve the residue in 0·1 to 1·0 ml. of chloroform and apply 5 μl. diluted once, twice and 4 times on a large filter paper on a line 5 cm. from 1 side as 3 spots at a distance of 3 or 4 cm. Standards consisting of a number of barbiturates are also applied along the line, one of the standards being applied alone and also mixed with urine extract (5 μl. diluted twice). The paper is shaped to a cylinder by connecting the two sides with glass needles, and development carried out as ascending chromatography in large glass cylinders with ground tops covered with glass plates, the joints being made airtight with grease. 2 or 3 chromatograms are developed, using different solvents, the following being the most suitable: *isoamyl alcohol* or *n*-hexyl alcohol saturated with concentrated ammonium hydroxide solution in saturated ammonia atmosphere, or butanol saturated with water. The solvents are poured on the bottom of the glass cylinders and the saturated ammonia atmosphere produced by placing a small beaker with concentrated ammonium hydroxide solution in the centre of the bottom, this being renewed 1 hour before each new chromatogram is started. In the first two solvents mentioned separation is obtained of the various barbiturates tested, giving R_F values between 0·2 and 0·8, whereas in butanol-water the barbiturates give R_F values of the order of 0·9 at room temperature. Butanol-water is used to distinguish barbiturates from other ultra-violet absorbing substances and from metabolic breakdown products of barbiturates. The barbiturate spots are identified on the paper by illumination with an ultra-violet lamp of maximum intensity (250 mμ). After the paper has been treated with a saturated ammonia atmosphere for a few minutes the dark barbiturate spots are easily recognised when illuminated with ultra-violet light, and may be marked with a pencil. S. L. W.

7-Dehydrocholesterol, 7-Hydroxycholesterol and Bile Acids in Serum, Estimation of. A. E. Sobel, M. Goldberg and S. R. Slater. (*Analyt. Chem.*, 1953, **25**, 629.) Methods were developed for the determination of the two steroids related to cholesterol, 7-dehydrocholesterol and 7-hydroxycholesterol, and of bile acids in serum. Preliminary experiments indicated that the 3 sterols were not present in equal amounts, cholesterol concentrations being 1000 times greater than concentrations of either the provitamin or hydroxysterol. Digitonin precipitation experiments showed that cholesterol and 7-dehydrocholesterol were readily precipitated with small amounts of water, but the hydroxysterol was quantitatively recovered as the digitonide with 54 per cent. of water and with a large excess of digitonin. The digitonides were split with pyridine, specific colour reactions being applied to the petroleum ether-extracted sterol mixture for the evaluation of individual concentrations. 7-Dehydrocholesterol was determined by ultra-violet absorption at 281·5 mμ and by the Rosenheim-Callow reaction; 7-hydroxycholesterol was determined by reaction with activated glycerol dichlorohydrin while total sterols were measured by the Liebermann-Burchard reaction. A modified fluorimetric serum bile acid procedure is given which requires 0·2 ml. of serum; results are quoted for sterol determinations in six human subjects. R. E. S.

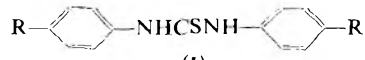
Œstrogens in Plasma, Determination of. A. H. Veldhuis. (*J. biol. Chem.*, 1953, **202**, 107.) A fluorimetric method for the determination of œstrogens in plasma is described. The method is based on the development of fluorescence when œstrogens are heated in the presence of sulphuric acid. It was found necessary to keep non-specific fluorescent materials in the reagents and solvent at a minimal concentration. The optimal time of heating the œstrone or œstradiol–sulphuric acid mixture was 4 minutes at 100° C. The œstrogens are determined in the unconjugated forms. Data on the analytical recoveries of œstrogen added to plasma are presented and the utility of the method for the determination of œstrogens in plasma demonstrated. A. H. B.

Steroid Hormones, Solubilised, Spectrophotometric Determination of. P. Ekwall, L. Sjöblom and J. Olsen. (*Acta chem. scand.*, 1953, **7**, 347.) The spectrophotometric determination of α-œstradiol, desoxycorticosterone, testosterone, testosterone propionate and cortisone-21-hemisuccinate in aqueous solutions of sodium lauryl sulphate was investigated. The absorption curves of the hormones were similar to those recorded in ethanol, but a bathochromic shift occurred except in the case of œstradiol. The absorption at the maxima increased linearly with the hormone concentration. In the concentration range investigated, the solubilities of the hormones in sodium lauryl sulphate increased linearly with the colloid concentration. A direct spectrophotometric determination of the steroid hormones on these solutions is possible. A. H. B.

CHEMOTHERAPY

Acid Hydrazides, their Derivatives and Related Compounds, Synthesis of. H. L. Yale, K. Losee, J. Martins, M. Holsing, F. M. Perry and J. Bernstein. (*J. Amer. chem. Soc.*, 1953, **75**, 1933.) The preparation of a large number of aliphatic, aromatic and heterocyclic carboxylic acid hydrazides and their derivatives and related compounds is described. Because no outstanding antituberculous activity was found among the aliphatic, alicylic and aromatic carboxylic acid hydrazides the emphasis was placed on hydrazides of heterocyclic carboxylic acids. The majority of the compounds prepared were derivatives of *iso*nicotinyl hydrazide. A. H. B.

Thiocarbanilides as Antitubercular Compounds. C. F. Huebner, J. L. Marsh, R. H. Mizzoni, R. P. Mull, D. C. Schroeder, H. A. Troxell and C. R. Scholz. (*J. Amer. chem. Soc.*, 1953, **75**, 2274.) The high antituberculous activity of 4:4′-diethoxythiocarbanilide (I; R = R′ = OC_2H_5) in mice infected with the H37RV strain prompted the synthesis and testing of over 300 thiocarbanilides and related substances. Rather specific structural features



(I)

necessary for activity were revealed. Shortening the 4-substituent to methoxy destroys activity, while lengthening the chain results in a fourfold increase to a maximum of activity in the neighbourhood of three to four carbon atoms. Increase beyond this causes activity to decline and then disappear. Replacement of alkoxy by an alkyl of equivalent length results in similar activity. Branching of the alkyl chain at the carbon adjoining the ring causes complete loss of activity. One of the 4-alkoxy groups may be replaced by halogen or dialkylamino and still retain some activity. Replacement of both of them

causes total loss of activity, while removal of one of the 4-alkoxyl groups also results in loss of activity. The 2- and 3-position isomers are inactive. A second substituent in the ring destroys activity as does substitution of methyl on the ureido nitrogen. The thiocarbanilide moiety is shown to be essential by the inactivity of the corresponding carbanilide, guanidine, guanylthiourea, dithiobiuret and the *cyclo*hexyl substituted thiourea. Some of the more active compounds gave favourable results in delayed and limited therapeutic trials in both mice and guinea-pigs. Resistant strains did not develop. The compounds have only a low toxicity. A. H. B.

Thioureas, Substituted, Antituberculous Activity of. R. L. Mayer, P. C. Eisman and E. A. Konopka. (*Proc. Soc. exp. Biol., N.Y.*, 1953, **82**, 769.) The authors have investigated the antimycobacterial and antifungal activities of more than 350 thiourea derivatives and related compounds. Many of the disubstituted thioureas, especially the thiocarbanilides, possessed *in vitro* activity and also had excellent chemotherapeutic effects in mice and guinea-pigs. The results obtained with 11 compounds of the general formula $R{\cdot}C_6H_4{\cdot}NHCSNH{\cdot}C_6H_4{\cdot}R'$ are reported. Mice were infected intravenously with 0·5 ml of a 1:10 dilution of a 7-day culture of H37Rv. Immediately after infection, groups of 10 mice were fed for 21 days on a diet containing from 0·01 to 0·1 per cent. of the test compound. After a further 15 days on a normal diet the surviving animals were killed. Guinea-pigs were infected subcutaneously with 1 ml. of a 1:200 dilution of the culture. After 21 days, the tuberculin-positive animals were given the medicated diet and this was continued for 115 days, when the surviving animals were killed. With some of the compounds examined there was a parallelism between antifungal and antimycobacterial activity suggesting a biochemical relation between the two forms of activity which could be useful in the search for new antituberculosis agents. There was no correlation between *in vitro* and *in vivo* tuberculostatic activity. *In vitro* resistance developed only slowly; no resistance developed *in vivo*. Streptomycin-resistant strains of H37Rv were sensitive to the thiocarbanilides, and isoniazid-resistant strains were sensitive to the thiourea derivatives. Certain of the compounds are suggested for clinical trial. H. T. B.

PHARMACY

GALENICAL PHARMACY

Vitamin B_{12}-Folic Acid Parenteral Solutions, Stability of. A. Taub and H. Lieberman. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 183.) Cyanocobalamin in aqueous solution has optimum stability at *p*H 4 to 6·5, but under these conditions folic acid is not sufficiently soluble to provide a suitable combined injection solution. A number of solubilisers were examined and citrate buffer, gluconic acid, glutamic acid, sodium benzoate, sodium gentisate, ethylenediamine, triethanolamine, urea, lysine, polyoxyethylene 20, sorbitan monooleate and propylene glycol were not satisfactory, but aminoacetic acid, nicotinamide, methylglucamine and glucose could be used. It was observed that folic acid could be brought into solution in a concentration of 5 mg./ml. by dissolving in water containing sodium hydroxide (*p*H 8·5) and adjusting to *p*H 6·0 with dilute hydrochloric acid, clouding of the solution being prevented by the addition of 10 per cent. of nicotinamide or aminoacetic acid. The latter caused discoloration after several weeks' storage. Solutions containing 30 μg./ml. of cyanocobalamin and 5 mg./ml. of folic acid were prepared with

glucose or methylglucamine at *p*H 6·5, but only nicotinamide prevented cloudiness developing at *p*H 6·0. These solutions showed no material loss of folic acid on storage for 3 weeks at 45° C. or 6 months at room temperature. It is concluded that solutions containing up to 30 μg./ml. of cyanocobalamin and 5 mg./ml. of folic acid with 10 per cent. of nicotinamide, at *p*H 6·0 to 6·5 remain clear and stable sufficiently long for practical purposes. G. B.

NOTES AND FORMULÆ

Chloramphenicol, Diffusion of, from Ointments. C. Trolle-Lassen. (*Arch. Pharm. Chemi*, 1953, **59**, 243.) The rate of diffusion of chloramphenicol from a number of ointment bases was tested by two methods. In the first the preparation was drawn up into a glass tube, the end of which was then closed by a film of cellophane. This tube was suspended in a test tube so that the cellophane-covered end was just below the surface of water in the test tube. After a certain period the concentration of chloramphenicol in the water was determined from the optical density of the aqueous liquid at 278 mμ. Plotting the amount of chloramphenicol in the solution against the square root of the time gave a straight line, the slope of which (α) was a measure of the diffusion. In the second method subcutaneous depots of the ointment were formed in the shoulder region of mice and, after 2 hours, the mice were killed and the concentration of chloramphenicol in the blood determined microbiologically. The results obtained were as follows:

Base	Results of tests	
	in vitro α	*in vivo* chloramphenicol μg./ml. of blood
Tragacanth gel	573	346
Oil-in-water emulsion	157	227
Water-in-oil emulsion	24·4	(14)
Soft paraffin base	0·313	88
Hydrophilic base	26·8	162

For the 3 preparations with an external aqueous phase the results of both methods give the same order of efficiency, and it would appear that the *in vitro* method represents sufficiently closely the conditions *in vivo*. For the water-in-oil emulsion and the soft paraffin base the order of efficiency as determined by the two methods is different. G. M.

Chlormerodrin (Neohydrin). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **152**, 331.) Chlormerodrin is [3-(chloromercuri)-2-methoxypropyl]urea, $ClHg{\cdot}CH_2{\cdot}CH(OCH_3){\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}NH_2$, and occurs as a stable, white, odourless powder, with a bitter, metallic taste, m.pt. 145·0° to 155·0° C., very soluble in sodium hydroxide solution, very slightly soluble in chloroform, and soluble in about 180 parts of ethanol, about 90 parts of methanol, and about 90 parts of water (a 0·5 per cent. solution has *p*H 4·3 to 5·0). It yields no immediate precipitate or colour when dissolved in alkali and treated with sodium sulphide (absence of ionisable mercury and other heavy metals). It also yields not more than 0·3 per cent. of sulphated ash and loses not more than 1·0 per cent. in weight when dried at 105° C. for 5 hours. The content of mercury, determined gravimetrically by precipitation with hydrogen sulphide in acid solution, is 53·5 to 55·7 per cent., equivalent to 98·0 to 102·0 per cent. of chlormerodrin. Chlormerodrin is administered by mouth as a mercurial diuretic. G. R. K.

PHARMACOGNOSY

Betulinic Acid in *Menyanthes trifoliata* L. A. Stabursvik. (*Acta chem. scand.*, 1953, **7**, 446.) The details of the isolation of betulinic acid from fresh rhizomes of *Menyanthes trifoliata* are recorded. A table is given showing that the acid has rather a scattered distribution throughout the plant kingdom and does not seem to be characteristic of certain families. A. H. B.

***Digitalis purpurea*, Paper Chromatography of.** K. B. Jensen. (*Acta pharm. tox. kbh.*, 1953, **9**, 99.) The paper describes the increase of separation effected by combining the previously used liquid system (chloroform–methanol–water) and the system chloroform–benzene–formamide. Details of the apparatus and technique for the one dimensional paper chromatography are given. Spot tests for the detection of the glycosides and aglycones are described which utilise the production of fluorescent and coloured spots by heating with trichloroacetic acid, trichloroacetic acid–chloramine and antimony trichloride. The Baljet reaction with alkaline picric acid, the Legal reaction with alkaline sodium nitroprusside and the Raymond reaction with alkaline *m*-dinitrobenzene were modified for chromatographic use on paper. The acid reagents gave both colour and fluorescence so that the A series and B series of substances (purpureaglycoside A, digitoxin, digitoxigenin—and purpureaglycoside B, gitoxin, gitoxigenin) and glycosides and aglycones could be distinguished. Diagrams of the chromatograms are given together with a description of the colours produced by the reagents and their sensitivity. R. E. S.

PHARMACOLOGY AND THERAPEUTICS

Aminosalicylic Acid, Effect of, on Thyroid and Adrenal. T. Wong, J. R. Hogness and R. H. Williams. (*Proc. Soc. exp. Biol., N.Y.*, 1953, **82**, 598.) The authors have investigated the effect of prolonged administration of aminosalicylic acid on thyroid and adrenal function in rats. 4 groups each of 10 rats were treated respectively with 1 per cent. of aminosalicylic acid in the diet for 2 weeks, stock diet alone, stock diet with 0·2 per cent. of propylthiouracil, and stock diet plus 1 mg. daily of corticotrophin gel (Armour) subcutaneously. After the 2-week period, a tracer dose of ^{131}I was injected intraperitoneally into each animal and food was removed. After 4 hours, blood was removed from the aorta, the thyroid glands were weighed and their radioactive iodine content determined. For the adrenal function investigation the following were determined—weight of gland, its ascorbic acid content and cholesterol content, thymic weight, liver glycogen, and circulating eosinophils. Aminosalicylic acid exerted a marked goitrogenic effect though not as great as the propylthiouracil in the quantities taken. The thyroid glands of the animals receiving aminosalicylic acid were about twice the weight of the controls, while those of the propylthiouracil treated group were about 3 times the weight of the controls. Corticotrophin had no significant effect on the gland weight. The total radioactive iodine content of the glands of the aminosalicylic acid group was only 40 per cent. of that of the controls, and there was no significant difference in this respect in the groups treated with aminosalicylic acid and with propylthiouracil. No differences in the adrenals of the 4 groups were noted, but the group treated with aminosalicylic acid showed a raised liver glycogen concentration, possibly due to a direct action of the compound on the liver. It is

suggested that aminosalicylic acid, like propylthiouracil, inhibits iodine concentration by the gland, thus lowering the production of thyroxine and stimulating the secretion of thyrotropic hormone by the anterior pituitary. This in turn results in thyroid stimulation and enlargement. H. T. B.

Ascorbic Acid, Glucose-1-phosphate and Lysergic Acid Diethylamide in Rheumatoid Arthritis. R. R. H. Lovell, J. A. Osborne, H. C. Goodman and B. Hudson. (*Lancet*, 1953, **264**, 970.) Various factors are known to influence experimental tuberculin hypersensitivity and on the basis of a possible analogy between tuberculin hypersensitivity in man and the tissue reaction in rheumatoid arthritis, the effects of some of these factors were investigated in the latter disease. On the basic analogy, ascorbic acid deficiency would enhance the inflammation and diminish the responsiveness of the disease to hormones. Sensitivity to tuberculosis in guinea-pigs is diminished by α-D-glucose-1-phosphate and lysergic acid diethylamide and the effect of these substances in man was investigated in regard to both rheumatoid arthritis and tuberculin sensitivity. Lysergic acid diethylamide has been reported to cause transient mental changes and disturbances in the autonomic nervous system in doses as small as 0·02 mg. The investigation was carried out on 10 patients with rheumatoid arthritis and 1 with polyarteritis nodosa. All were treated by rest, controlled exercises, splinting if necessary and salicylates or analgesics. Ascorbic acid was given orally. Patients treated with glucose-1-phosphate and lysergic acid diethylamide received inert preparations for up to 14 days prior to treatment. Cortisone, 200 mg. daily, was given orally in 1 case and intramuscularly in 1 case. Adrenocorticotrophic hormone, 100 mg. daily, was given by intramuscular injection. Glucose-1-phosphate was given intravenously as a solution of the dipotassium salt in normal saline in doses of 4 to 800 mg. daily. Lysergic acid diethylamide was given orally in doses of from 0·005 to 0·1 mg. daily in about 30 ml. of water before breakfast. The disease was not influenced by ascorbic acid depletion or saturation, nor did these factors influence the effect of cortisone or adrenocorticotrophic hormone. The other substances also had no effect on rheumatoid arthritis nor on the tuberculin reaction in man. It is suggested that the action of cortisone on the tuberculin reaction in man reflects, at least in part, a non-specific diminution of an inflammatory response rather than an interruption of the mechanism which initiates a hypersensitivity reaction. H. T. B.

Butylscopolammonium, Curarising Action of. E. Philippot and M. J. Dallemagne. (*Arch. int. Pharmacodyn.*, 1953, **93**, 337.) Butylscopolammonium bromide (buscopan) is a curarising agent similar in action to tubocurarine. Injected in doses between 5 and 10 mg./kg. it inhibits neuro-muscular transmission at the level of the limbs in the cat. The action is as marked for the tibial as for the soleus, and is preceeded by a transitory potentiation especially in the latter muscle. The injection of adrenaline or neostigmine frees the neuromuscular junction. The action of butylscopolammonium bromide is antagonised by decamethonium iodide, which removes the muscle block. If, subsequently the block is restored with decamethonium bromide, it can be relieved by a further injection of butylscopolammonium bromide. G. B.

Cortisone and Desoxycorticosterone (Deoxycortone), Effects of, on the Toxicity of Barbiturates. C. K. Gorby, C. A. Leonard, J. L. Ambrus and W. E. Harrisson. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 213.) Groups of mice were treated with 100 mg./kg./day of cortisone acetate or deoxycortone

acetate administered subcutaneously for 6 days. Pentobarbitone sodium or phenobarbitone sodium was then given intraperitoneally. Cortisone exerted a slight protective effect when doses of pentobarbitone sodium below the LD50 were given, and deoxycortone slightly increased the effect of doses above the LD50. Both substances increased the toxic effect of phenobarbitone sodium, and this was also observed when the hormones were injected immediately after the phenobarbitone sodium. G. B.

***NN'*-Dibenzylethylenediamine Penicillin: Oral Use in Children.** I. A. B. Cathie and J. C. W. MacFarlane. (*Brit. med. J.*, 1953, **1**, 805.) A total of 101 children of all ages were given 300,000 units of *NN'*-dibenzylethylenediamine penicillin (penidural) by mouth and their blood levels estimated 3 to 4 hours after administration for reliability of absorption. In addition a series of 17 adult volunteers were investigated with the same preparation. Reliable absorption was shown in all of the 118 cases. In several cases hourly penicillin levels were estimated and a cumulative effect was seen following a second oral dose. In the treatment of acute infections, blood levels during the first 6 hours with this oral penicillin alone are inadequate and treatment should be augmented by a single intramuscular injection of a large amount of crystalline penicillin. A rational scheme of treatment in acute infection would appear to be an initial intramuscular injection of 600,000 units of penidural and 100,000 units of crystalline penicillin, with 300,000 units of penidural orally, the treatment continuing thereafter with oral penidural alone. S. L. W.

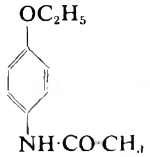
Hydralazine in the Treatment of Hypertension. J. H. Moyer. (*Arch. intern. Med.*, 1953, **91**, 419.) The results are recorded of treating hypertension for periods of 1 to 2 years with hydrallazine (1-hydrazinophthalazine, apresoline) alone and with hydrallazine either before or after ganglionic blockade with hexamathonium. 54 patients received hydrallazine alone in progressively increasing doses. Initially 25 mg. was given orally with each meal and at bedtime. After a few days the amount of drug being taken was increased until a significant reduction in blood pressure occurred or side reactions prohibited further increase in the dose. Patients treated with hydrallazine and hexamethonium included 20 who had previously received hexamethonium alone without adequate regulation of blood pressure and 32 who had previously received hydrallazine alone, but had failed to obtain significant reduction in blood pressure without prohibitive side reactions. Although 35 per cent. of the patients on hydrallazine alone obtained a significant reduction in blood pressure after 3 months' treatment, only 9 per cent. continued to obtain adequate control after treatment for 1 to 2 years. Of 20 patients in whom treatment with hexamethonium alone was a failure and 32 in whom treatment with hydrallazine alone was a failure, treatment with the two drugs combined gave satisfactory results in 75 per cent. Patients with malignant hypertension greatly improved on combined therapy. Hexamethonium is by far the more potent hypertensive agent, but hydrallazine minimises wide fluctuations in blood pressure. The side effects which most often necessitated discontinuing hydrallazine were tachycardia and palpitations or gastrointestinal disturbances. Headache was common but was usually relieved spontaneously after several weeks. Except for paræsthesias and hyperæsthesias the side effects decreased in intensity with time and some degree of tolerance with loss of therapeutic response also occurred. H. T. B.

Levorphan, Dextrorphan, Racemorphan (Dromoran), Absorption and Excretion of. A. L. Fisher and J. P. Long. (*J. Pharmacol.*, 1953, **107**, 241.) Although these drugs have a close similarity of structure to morphine and have similar analgesic properties, the excretion patterns are shown to be only qualitatively similar to those of morphine. A comparison of excretion by non-tolerant and tolerant dogs indicates similar excretions. The highest percentage of excreted conjugate by normal dogs was found to be about the average excretion of conjugate in tolerant dogs. With morphine, tolerant dogs excreted less conjugate than normal dogs, while excretion of unconjugated morphine remained about the same. The excretions of levorphan and dextrorphan, the *l*- and *d*-isomers, and of a mixture of the two were found to be similar to the excretion of racemorphan hydrobromide, the racemic compound. Following intravenous infusion, the drug was found to disappear very rapidly from the plasma, only small amounts being found even 1 minute following the injection. The rate of absorption from the gastrointestinal tract of fasting cats was found to be very rapid when the concentration was high, absorption being about 75 per cent. complete after 2 hours. It is partially excreted as the glucuronide and possibly in some other conjugated form. A method for the determination in plasma, urine and gastrointestinal contents, using a modification of the methyl orange technique for basic amines, is described. S. L. W.

Levorphan Tartrate for Relief of Postoperative Pain. R. D. Hunt and F. F. Foldes. (*New Engl. J. Med.*, 1953, **248**, 803.) Animal experiments and observations on patients addicted to drugs have shown that dextrorphan (*d*-dromoran, 3-hydroxy-*N*-methylmorphinan) has no morphine-like effects, and that the analgesic potency of the racemic form resides wholly in the *l*-isomer. An investigation was therefore undertaken to determine whether levorphan could be substituted for the racemic form of the drug (racemorphan) in clinical medicine. In a series of 311 surgical patients a dose of 3 mg. of levorphan tartrate was administered for the relief of postoperative pain, and complete relief was obtained in 77·2 per cent. of cases. The average duration of analgesia was 5½ hours. The results are compared with those obtained from a similar group of surgical patients who received the theoretical equivalent of racemorphan hydrobromide (5 mg.). A higher percentage of patients derived complete analgesia from the first dose of 3 mg. of levorphan than from the first dose of 5 mg. of racemorphan, but results from subsequent doses were approximately equal. The incidence of side effects was no greater than with the racemic compound, and clinically the two drugs would appear to be interchangeable. 3 mg. of levorphan tartrate compared favourably with 10 mg. of morphine sulphate in analgesic potency. H. T. B.

Narcotics and Sedatives, Influence of on Blood Coagulation. D. I. Macht. (*Arch. int. Pharmacodyn.*, 1953, **93**, 325.) Drugs were administered to rabbits and the change in clotting time determined at intervals. Morphine, ethylmorphine and diamorphine produced a marked acceleration of clotting, whereas codeine, papaverine, dihydromorphinone and dihydrocodeinone did not. Freshly prepared solutions of cocaine had no effect on the clotting time, but solutions which had been allowed to stand for several days were found to shorten it. Ether, chloroform and a number of barbiturates did not affect the clotting time. Procaine, even in small doses markedly accelerated clotting. When diamorphine or procaine were given repeatedly or in large quantities, an entire change in the blood clotting mechanism took place and the blood remained fluid for several hours. *N*-allylnormorphine and pethidine showed a thromboplastic effect, but methadone did not. G. B.

Phenacetin, Thymacetin and an Isomer, 4-Ethoxy-2-*iso*propyl-5-methylacetanilide, Comparative Study of. J. R. Lewis. (*Arch. int. Pharmacodyn.*, 1953, **93**, 450.) *These chemically related compounds were examined for analgesic potency* by intraperitoneal injection of 10 per cent. suspensions in gelatin solution into rats (radiant heat stimulus test) for sedative and antipyretic action in the rat, for the production of methæmoglobinæmia by oral administration in the cat, and for toxicity when administered to mice by stomach tube.



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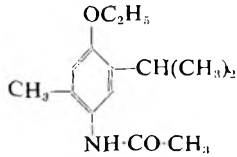
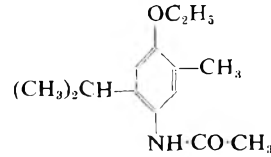


Thymacetin



4-Ethoxy-2-*iso*propyl-5-methylacetanilide

Thymacetin was shown to have greater analgesic, antipyretic and sedative activity than phenacetin. It was less toxic and did not produce methæmoglobinæmia. 4-Ethoxy-2-*iso*propyl-5-methylacetanilide was considerably less active. In a limited clinical trial, thymacetin produced good analgesic effects, but there was a high incidence of side effects, mainly nausea. G. B.

Primidone—a New Anticonvulsant Drug. J. Yule Bogue and H. C. Carrington. (*Brit. J. Pharmacol.*, 1953, **8**, 230.) Primidone (mysoline, 5-ethyl-5-phenylhexahydropyrimidine-4:6-dione), a new anticonvulsant drug, chemically related to phenobarbitone, has been tested for anticonvulsant activity against electroshock and leptazol seizures in rats. Against electrically induced seizures an *oral dose of 5 mg./kg. was more effective than the same dose of* phenobarbitone, and its activity compared favourably with other convulsant drugs. Against leptazol-induced convulsions 20 mg./kg. protected 60 per cent. of the rats, compared with 10 mg./kg. of phenobarbitone. The acute and chronic toxicities in the rat, mouse and monkey were remarkably low; and it was much less toxic than phenobarbitone. G. F. S.

Propyliodone (*n*-Propyl-3:5-diiodo-4-pyridone-*N*-acetate). E. G. Tomich, B. Basil and B. Davis. (*Brit. J. Pharmacol.*, 1953, **8**, 166.) The effects of aqueous and oily suspensions of this compound, a bronchographic agent with a rapid lung clearance, have been studied in the lungs of rabbits. Intratracheal injections of aqueous propyliodone, its aqueous vehicle (consisting of a mixture of sodium carboxymethylcellulose), and an oily suspension, caused congestion of the lungs maximum at 3 days and absent after 2 weeks. Bronchographic studies showed the absence of alveolar filling and the disappearance of the contrast media within 3 days, making serial bronchography a possibility. With iodised poppy-seed oil marked congestion developed after 16 hours and persisted for 1 week when opaque oil was still present. Metabolic studies with propyliodone labelled with ^{131}I in man have shown the compound to be completely hydrolysed and eliminated as an iodine metabolite 3:5-diiodo-4-pyridone-*N*-acetate ion. 50 per cent. of the iodine was eliminated after bronchography in 72 hours. G. F. S.

Pyrimethamine for Prophylaxis Against *Plasmodium falciparum*. G. Covell, P. G. Shute and M. Maryon. (*Brit. med. J.*, 1953, **1**, 1081.) An investigation was carried out to discover whether one weekly dose of 25 mg. of pyrimethamine is sufficient to protect non-immune subjects from an attack of falciparum malaria when repeatedly exposed to mosquito infection. Blood from an African child suffering from the disease was inoculated intramuscularly into a patient suffering from neurosyphilis, and sub-inoculations were made into two other neurosyphilitic patients. All developed overt malarial attacks but only one produced gametocytes in the peripheral blood in sufficient numbers to infect a batch of *Anopheles stephensi*. About 700 insects were allowed to feed on this gametocyte carrier. 14 patients, arranged in pairs, were each given a weekly dose of 25 mg. of pyrimethamine on different days, and subjected to infection by 10 to 12 of the mosquitoes at intervals of 1 to 7 days after the first dose of pyrimethamine had been given. Two of the patients suffered a rise in temperature, both due to irrelevant causes, otherwise there were no side effect and protection from malarial attack was complete. The authors consider that a weekly dose of 25 mg. of pyrimethamine is a practical and economical method of preventing malarial attack by the particular strain of *P. falciparum* used. H. T. B.

Sodium Salicylate in Rheumatic Fever. L. L. Henderson. (*Amer. J. med. Sci.*, 1953, **225**, 480.) A clinical investigation was conducted on rheumatic fever patients to ascertain the effect of adjuvant medication on the blood salicylate level. The patients were given 1·6 g. of enteric-coated sodium salicylate every 4 hours (a total of 10 g. a day) together with the adjuvant drug being studied. The adjuvant drugs employed, and the doses given with each dose of salicylate, were as follows:—sodium bicarbonate 1·6 g., sodium bicarbonate 0·65 g., magnesium trisilicate 1 g., aluminium hydroxide gel 8 to 16 ml. The respective average plasma salicylate levels (mg./100 ml.) obtained were 29·8, 35·9, 43·0 and 49·8; using sodium salicylate without any adjuvant the average figure was 42·2. While sodium bicarbonate depresses the plasma salicylate level it tends to return the carbon dioxide combining power, which is lowered by massive doses of salicylate, to normal. Magnesium trisilicate and magnesium hydroxide gel do not lower the plasma salicylate level, but neither do they raise the carbon dioxide combining power. Massive doses of salicylate seldom result in any harmful effect, but cause considerable discomfort. Most patients will tolerate the somewhat smaller doses of sodium salicylate sufficient to relieve rheumatic discomfort without any adjuvant medication. For those complaining of gastric distress magnesium trisilicate, aluminium hydroxide in tablet form or sodium bicarbonate in 0·65 g. doses will give satisfactory relief. S. L. W.

Succinic Acid and Choline, Response in Dogs to Relaxants derived from. L. W. Hall, H. Lehmann and E. Silk. (*Brit. med. J.*, 1953, **1**, 134.) Experiments in anæsthetised dogs have shown them to be more sensitive than man to the short-acting neuromuscular relaxants suxethonium and suxamethonium. Doses equivalent to one fifth of those used in man produced apnœa of about 5 times the duration. These findings could not be correlated with a low activity of pseudo-cholinesterase in dog plasma, which is the cause of an occasional prolonged effect in man. True cholinesterase in dog red cells was only one seventh of that in man, which suggests a correlation between higher sensitivity and lower true cholinesterase. Raising the pseudo-cholinesterase, by injection of a concentrated preparation (cholose), shortened the apnœa after both relaxants equivalent to that seen in man. G. F. S.

LETTER TO THE EDITOR

Antihistamine Activity in a Series of Synthetic Bis-Onium Compounds

SIR,—An analysis of the common characteristics of several series of antihistamine substances convinced Bovet[1] that all possess one or more strongly saturated tertiary or quaternary amine groups.

Recently Khanna and Dhar[2] synthesised a number of bis-onium compounds whose myoneural junction blocking properties have already been studied[3] and we have been interested to find whether these compounds have also antihistamine properties.

Both guinea-pig and rabbit ileum were used and the effect of the potential antihistamine drugs upon histamine-induced spasm in the isolated organ bath, noted. Two procedures were followed: (i) histamine was first added to the bath, followed by varying doses of the compound under study and the relaxation of the musculature noted, (ii) the compound was added first, and was followed by histamine, and any blocking of the effect of the histamine recorded. The tissue was finally washed and its response to the action of histamine noted again.

51 Polymethylene bis-onium compounds were tested for their activity. Some of these compounds showed weak antihistaminic property. Two compounds, *NN'*-bisbenzyl-bis(β-diethylaminoethoxy) ethane dichloride and *NN'*-bisbenzyl bispiperidino diethyl ether dichloride were found to be more active than others. 5 mg. of these compounds antagonised the action of 1 μg. of histamine on guinea-pig's ileum. The potency of these compounds in comparison to that of the already known active antihistamines was low, although both these compounds possess in their side chains a benzyl group which is often associated with the N atom of many known antihistaminine drugs. Possibilities for new and effective synthetic antihistaminics by further suitable modification and remodelling of the structure of these compounds are engaging our attention.

The authors are grateful to Dr. B. Mukerji, Director of this Institute, for his keen interest in this work.

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August 24, 1953.

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