RESEARCH PAPERS

THE FLOW PROPERTIES OF STARCH POWDERS AND MIXTURES

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The addition of small proportions of fine powders, particularly magnesium oxide, to maize starch, alters its flow properties in a manner which is contrary to the generally observed effects for fine powders. A measure of this effect is obtained by the angle of repose method and the disposition of the two components of the mixture is observed by the electron microscope. An explanation of this effect in terms of adhesion by Van der Waals' and valency forces is put forward.

Powders possess special physical properties in addition to those of the bulk material. One of these, of considerable interest in the field of pharmacy is the extent to which a powder will flow freely or, conversely, aggregate into a mass which flows with difficulty. The flow properties of powders vary considerably between those of dry sand which will flow smoothly and continuously through a small orifice, and those of a fibrous powder like asbestos which forms fluffy masses and will not maintain an even flow under any circumstances. Starch can be considered as a fairly sticky powder forming large loose aggregates on shaking, and flowing with difficulty, but when as little as 0·5 per cent of light magnesium oxide is added the mixture flows much more freely and tends to acquire a smooth flat surface on shaking in the same way as it had been reported that the admixture of various substances with sulphonilamide will alter the properties of the powder[1,2]. This effect is in contrast to the observation that, in general, fine powders flow less freely than coarse ones, and that the presence in a powder of a considerable proportion of fine particles reduces the ease with which it flows[3]. In order to elucidate this phenomenon the electron microscope was used to study the relation between the two components of the mixture, and of mixtures of starch with other fine powders. It was first necessary, however, to obtain a numerical measurement of the effect.

Viscosity governs the flow of a liquid but there is no such fundamental property of a dispersed solid. Instead a number of tests exist which separately give reproducible comparative results[4]. These have no specific relation to each other and are not necessarily influenced by the same factors. One test consists of measuring the rate of flow of a powder through a tube or orifice[5]. Since the effect under study is concerned with movement of the powders, a dynamic method of measurement like this would have been preferred but it was found that, while the mixture would flow quite readily, the starch on its own could not be induced to sustain a continuous and reproducible flow. However, measurement of the angle of repose gave reproducible results, which appeared to give a measure

of the effect of the added powders on the flow properties of the starch, and this method of measurement was adopted.

The powder was formed into a conical heap by dropping it through a glass funnel supported at a fixed distance above a horizontal plate. When the tip of the cone just reached the funnel the diameter of the base was measured in several directions and, knowing the height, the angle of slope of the side of the cone was calculated. Sticky powders give a steep cone with a large angle, while free-flowing powders give a wider cone having a smaller angle of repose. Though it is not clear to what physical properties this angle corresponds it is useful as an index.

The manner in which the powder falls on to the heap affects the results. When the particles arrive more or less singly their velocity is low and they do not have sufficient momentum to disturb the heap which has already

TABLE I

ANGLE OF REPOSE OF STARCH POWDER AND MIXTURES

Substance added	Per cent	Angle°*	Substance added	Per cent	Angle°*	Substance added	Per cent	Angle°*
(Control)	—	53	Zinc oxide ..	0·5	58	Mag. oxid. (heavy)	0·5	53
Mag. oxid. (light)	0·05	51	Titanium dioxide..	0·5	58	Chromium tricxide	0·5	53
,, ,, ,,	0·1	50	Sulphur	0·5	56	Calcium oxide ..	0·5	51
,, ,, ,,	0·3	40	Talc	0·5	56	Mag. trisilicate ..	0·5	47
,, ,, ,,	0·5	38	Talc	5·0	54	Alumina	0·5	46
,, ,, ,,	1·0	37	Zinc stearate ..	0·5	54	Mag. trisilicate ..	1·0	45
,, ,, ,,	3·0	42	Silica ppt. ..	0·5	54	Carbon black ..	0·5	43
,, ,, ,,	5·0	46	(Control)	—	53	Mag. oxide (light-est grade) ..	0·5	42
,, ,, ,,	100	54						

* The average of 5 determinations.

formed; they take up stable positions after rolling down the sides of the cone. When a large aggregate or a solid stream of particles falls from the funnel, however, its momentum is sufficient to cause flow to take place in the bulk of the heap, which is consequently deformed. These two effects were involved in each determination, and to obtain reproducible results the powder had to be added in as uniform a way as possible. This was achieved by attaching an electrical vibrator at 50 c.p.s. to the funnel, and adding the powder slowly from a spatula. The results then obtained were reproducible to within 5 per cent.

Since the absorption of water from the atmosphere is likely to affect the properties of the powders, all the samples were prepared from the same batch of maize starch and kept under identical conditions. A second factor which could influence the measurement is the previous packing of the sample. For example, if the mixture of starch and magnesium oxide is shaken vigorously, then a gentle horizontal movement will cause the surface to flow. If the container is then tapped on the bench this mobility is completely suppressed. However, by the time the powder has passed through the funnel any difference in the degree of packing will have been destroyed, an advantage of the method employed.

The results are given in Table I. They include mixtures of starch with different proportions of magnesium oxide and also mixtures with other fine powders. Each figure for the angle of repose is the average of five determinations.

The results for magnesium oxide of varying concentration are plotted in Figure 1. The variation of the angle has a flat minimum, at approximately 1 per cent after which a gradual rise takes place presumably approaching the value for magnesium oxide alone.

Light magnesium oxide being a very fine powder cannot be examined by optical microscopy, while starch grains are very large on the scale of the electron microscope, and are opaque to the electron beam so that direct observation gives only an outline of the grains. For this reason a replica technique was adopted to show the full surfaces of the starch grains and reveal the disposition of the fine particles in the mixtures. The starch powder or mixture was applied to a collodion film, on a specimen grid, by touching the filmed surface of the grid on to the powder surface. The grids with the specimens uppermost were then placed in a vacuum chamber and a layer of carbon was applied by passing a heavy current through a pair of carbon rods situated above them, one of which was turned down to a small diameter at the point of contact, a method of evaporation developed by Bradley[6]. It was found to be necessary to apply a thicker layer than is used for the highest resolution replicas, or the final specimens broke due to the comparatively large size of the starch grains; the evaporation proceeded until the film on the slide was dark brown by transmitted light. The specimen grids were then placed on curved strips of stainless steel mesh under which amyl acetate was run to remove the collodion film. The starch could be dissolved completely by strong hydrochloric acid. To remove the starch without displacing the carbon film, the grids were placed on to the surface of a bath of 50 per cent hydrochloric acid at a temperature of 60° so that they floated while the specimen was dissolved. After twenty minutes the grids were lifted on bent strips of steel mesh and floated on distilled water for an hour to wash thoroughly and then they were once more picked up in the same way and left to dry. The replica then consisted of a thin transparent shell following the contours of the original specimen. If the other material present was soluble in the acid it also appeared as a shell, but if not then the original particles appeared superimposed on the replica. Magnesium oxide powder was also examined as a shadowed specimen, prepared in the usual way.

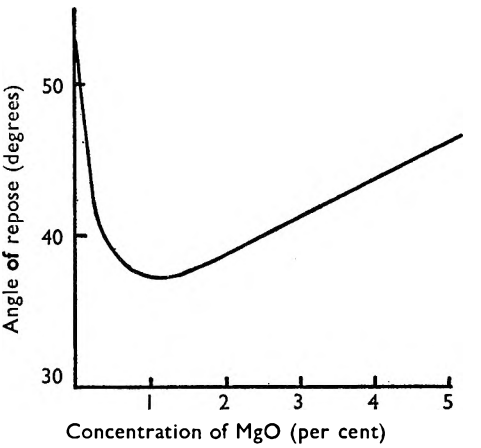

FIG. 1. Graph of the angle of repose of mixtures of starch powder and light magnesium oxide in varying proportions.

Light magnesium oxide exists as large aggregates, visible in the optical microscope, which are seen with the electron microscope, to consist of

very closely packed sheets of much smaller particles, as in Figure 2. That these are distinct particles and not just a surface texture, is demonstrated by selected area electron diffraction; a field such as that shown in Figure 2 gives rise to a pattern of continuous rings as opposed to the pattern of spots which would be given by a single crystal. The diameter of the particles is approximately 0·05 μ and, since the starch grains are 5 μ or more in diameter the small amount of magnesium oxide which is needed to produce an obvious effect is less remarkable. Thus, considering the relative densities, the mixture containing 0·5 per cent magnesium oxide contains approximately 1000 times as many particles of magnesium oxide as of starch.

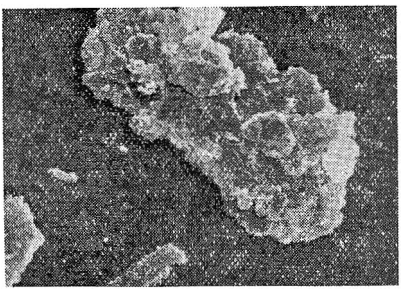

FIG. 2. Carbon replica of an aggregate of light magnesium oxide × 5,000.

Figure 3 is an electron micrograph of a replica of a whole starch grain and is typical of the sample except that the grains occur mostly in the form

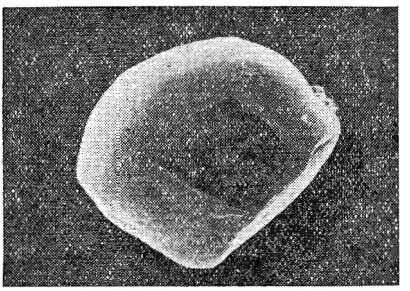

FIG. 3. Carbon replica of a typical starch grain × 6,600.

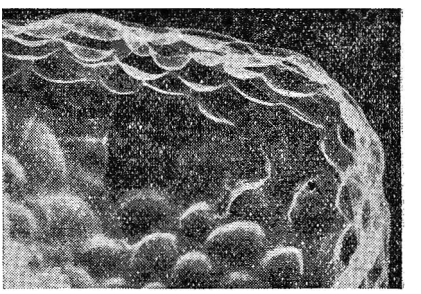

FIG. 4. Carbon replica of a part of a starch grain with an unusual surface pattern × 6,600.

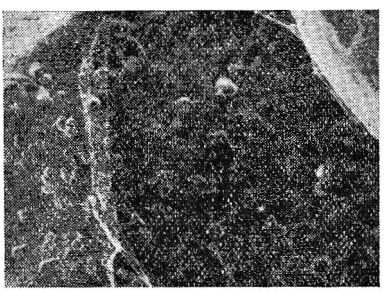

FIG. 5. Carbon replica of part of a starch grain from a mixture with 0·5 per cent light magnesium oxide showing fine particles dispersed over the surface × 20,000.

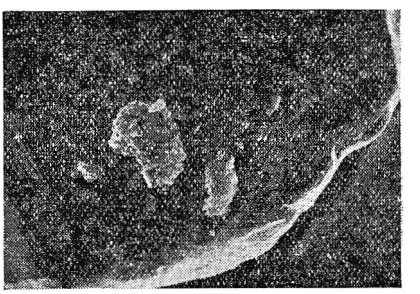

FIG. 6. Carbon replica of a starch grain from a mixture with 0·5 per cent light magnesium oxide showing the fine particles attached to the surface in aggregates × 6,600.

of aggregates. The interesting pattern on part of a starch grain shown in Figure 4 is of rarer occurrence, but is not unique. Where aggregates had existed the replica was usually shattered but the fragments were similarly smooth. Examination of a mixture, with 0·5 per cent magnesium oxide revealed the absence of any separate aggregates of magnesium oxide, and showed that the replicas of the large starch grains were covered with a regular pattern of protuberances representing the position of the magnesium oxide particles before their removal by the acid treatment (Fig. 5). Comparison of Figure 5 with the preceding micrographs thus indicates that the aggregates of the magnesium oxide particles are broken up on mixing with the starch, and that the particles are wholly adsorbed on the surfaces of the starch grains. Usually these particles were evenly spread out over the surfaces in the form of small aggregates or single particles but occasionally, as in Figure 6, larger aggregates were adsorbed.

By examining carbon replicas of other mixtures it was seen that finely divided alumina was similarly adsorbed, carbon black was partially adsorbed, a small amount from a 0·5 per cent mixture being seen separate from the starch grains, and that titanium dioxide was also strongly adsorbed. Finely divided calcium oxide was not adsorbed at all but left the grains smooth. No other powder examined produced such a uniform covering as did the magnesium oxide. A mixture with 5 per cent magnesium oxide contained a considerable amount of unadsorbed magnesium oxide in the form of aggregates.

Discussion

The effect of the magnesium oxide on the flowing properties of the starch does not appear to conform to the usual principles of solid lubrication[7]. A solid lubricant, such as talc, depends for its effect on its layer-lattice structure, with very low bond strength between the layers which thus slip easily over each other. Magnesium oxide has an unrelated cubic structure and would not be expected to act as a solid lubricant. Also the reduction of the effect which occurs when more than a certain small amount is added (Fig. 1), would not be expected to occur as a result of lubrication.

The fact that the magnesium oxide is adsorbed on to the starch provides an interpretation of the graph. Thus, the electron microscope studies show that over the range where the angle falls rapidly all the magnesium oxide is being adsorbed, and a practically continuous layer exists where the angle of repose is at its minimum. At higher concentrations the additional magnesium oxide is no longer adsorbed but persists as large aggregates mixed with the starch grains in which form it begins to increase the angle of repose once more. Thus, the dip in the curve represents a saturation effect.

Surface roughness is not a major factor governing the flow of the starch since the adsorbtion produces a rougher surface, which should give an opposite effect to that which is observed. Thus, it appears that the natural adhesion of the starch is of major importance, and that the effect of the magnesium oxide is to reduce this adhesion. It was pointed out

by Beilby[8] that small particles have a tendency to adhere to each other, or to a solid surface, which is independent of such external influences as electrostatic charging or the presence of contaminating grease films. The forces which induce this adhesion, and lead to the existence of aggregation in most fine powders will be the same as those responsible for the cohesion of crystals or amorphous bodies and can be roughly classified as polar or apolar forces. The first exist in ionic crystals and represent primary bonds of high energy, while the weaker apolar forces correspond to the secondary bonds between unionised molecules, in crystals or amorphous materials. These forces are of very short range, the Van der Waals' attractive forces, of the second type, decreasing as the seventh power of the separation of the bodies involved. But these will be effective whenever such close contact is formed that neighbouring particles are separated by a distance which is of the order of the atomic or molecular spacing.

Thus, when the starch grains are prevented from approaching each other by less than 1000 Å, by the presence of the magnesium oxide layers, the Van der Waals' forces will be greatly reduced, probably to insignificance. If it is considered that the corresponding forces between the magnesium oxide particles are less effective owing to the particles having smaller areas of true contact, then this would explain the reduced adhesion. Since these particles are crystalline, however, there is the possibility that polar forces are involved due to the existence of unsaturated valencies in the crystal faces. If the particles were oriented in such a way that faces having the same type of residual valency were always directed outwards, then this would result in a strong repulsive force between the particles which would greatly reduce the adhesion of the grains. The effect would be analogous to the peptisation of a precipitate by the mutual repulsion of adsorbed ions.

The crystals of magnesium oxide have a cubic structure of the NaCl type[9] in which magnesium and oxygen ions alternate along the principal axes. Thus, a cubic (100) face consists of a chequerboard array of the two ions and, since the ions on the surface are not surrounded by the normal number of neighbours, a corresponding pattern of unsaturated valencies exists. In this case the valence charges give a zero resultant at a little distance from the surface, the two types being equal in number. A dodecahedral or (110) face consists of alternate lines of the two types of ion and the resultant is again zero, but an octahedral (111) face contains only one type of ion and thus represents a sheet of unsaturated valencies of the same sign. The fact that the magnesium oxide particles are plate-shaped suggests that the principle faces are octahedral faces. If the starch grains have on their surface a small unsaturated valency density this would cause the magnesium oxide particles to be oriented on the surface in such a way as to present sheets of valency charge of a uniform sign to the exterior, and thus they would have the effect of greatly increasing the charge density and the mutual repulsion of the surfaces. This would also explain the great mobility of the surface layer of a mass of the powder since it is near the surface that the repulsive forces would have their

greatest effect, while further down in the body of the powder they would be overcome by the gravitational forces of the superimposed material.

Conclusion

The addition of magnesium oxide to starch does not produce a simple mixture in which the two components remain independent of each other. The strong adsorption which takes place gives a powder having properties which, in one respect at least, are quite different from those of either of the components, while other properties involving the surfaces of the particles would be expected to be altered. To this extent the process resembles a chemical reaction or, more closely, the interaction of macromolecules in solution or suspension, which is considered to involve coulombic forces[10] and in which the structural identity of the molecules is maintained. The difference is in the size of the particles involved, and the fact that the interaction occurs in the dry state.

Although the discussion has been confined to starch and magnesium oxide it has been shown that similar effects are produced by mixtures of starch with some other fine powders, and it seems likely that many other combinations of powders may give similar results.

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THE ANALGESIC ACTION OF NORMORPHINE ADMINISTERED INTRACISTERNALLY TO MICE

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The analgesic action of normorphine developed more rapidly than that of morphine and was, weight for weight, as great as that of morphine, and was as effectively antagonised by *N*-allylnormorphine when these drugs were administered intracisternally to mice. By this route, more than one molecule of *N*-allylnormorphine was required to antagonise the action of one molecule of analgesic drug.

Specific receptors, of predicted chemical and physical character, were postulated for the analgesic actions of drugs of the morphine, methadone, and pethidine series[1]. Later, Beckett, Casy, and Harper[2] brought forward a theory concerning the mode of action of morphine-type drugs which may be briefly summarised as follows. All *N*-alkylated drugs which are, by reason of their chemical structure and spatial configuration, a fit for these specific "analgesic receptor sites", become adsorbed on these sites. The formation of this drug-receptor complex does not itself produce analgesia. Analgesia occurs only if an oxidative dealkylation takes place on the receptor surface, with the production of the dealkylated (nor-) drug. Analgesia is the product of a further interaction in which the dealkylated compound takes part.

The object of the investigation has been to test two predictions which can be made from this theory. First, the analgesic action of morphine has been attributed solely to that of the normorphine formed from it by demethylation. If this is true, the analgesic action of normorphine should prove as great as that of morphine and be the more rapid in onset. The analgesic actions of these two drugs have therefore been compared. Secondly, comparison has been made of the antagonism by *N*-allylnormorphine (nalorphine) of the analgesias produced by morphine and by normorphine. According to the theory nalorphine should be attracted to the specific receptor sites for dealkylation, for its own very weak analgesic action[3,4] should be attributed to an exceedingly slow formation of normorphine from nalorphine by dealkylation. Accordingly, the action of nalorphine as an antagonist of morphine is most easily explained as one of competition between these two drugs for the specific receptors for dealkylation. If this is the true explanation of the action of nalorphine, then nalorphine should prove an antagonist of morphine but not of normorphine. This point was therefore examined.

Mice were used for these investigations, and drugs were administered by the intracisternal route to minimise the effects of any difference in the rates of destruction of morphine and normorphine in the animal body.

Methods

Male white mice, of weight range not exceeding 2 g., were distributed at random into groups of ten.

Drugs. Morphine and nalorphine (British Drug Houses Ltd.) and normorphine (by courtesy of Beckett, Casy, and Harper) were obtained and used as hydrochlorides, and were dissolved in 0·9 per cent w/v aqueous sodium chloride for injection.

Intracisternal injections. Gauge 26 intradermal needles were bent away from the bevel one quarter of an inch from the tip through an angle of approximately 20°. The head of each mouse was bent well forward, under ether anaesthesia, and the tip of the needle was passed through the intact skin, muscles, and ligaments in the midline at the back of the neck, so that the point slipped in between the occiput and the atlas vertebra. The bent part of the needle was then pushed forward, keeping it in close contact with the internal surface of the occiput, for its whole length. An injection of 0·02 ml. was made and the needle was withdrawn. Normal pain thresholds were restored in less than five minutes after the injection of 0·9 per cent sodium chloride. The distribution of such an injection of 0·1 per cent Evans Blue was observed in ten mice. Each was injected, then immediately killed by deepening the anaesthesia. The vault of the skull was at once cut away with a very sharp scalpel. The dye had covered the base of the brain and had passed into the third and fourth ventricles of all the mice. It had outlined the lateral ventricals in eight out of the ten. It had extended over the surface of the cerebral hemispheres very considerably, but to a variable extent. It had leaked back into the muscles of the neck appreciably in two cases. *Intravenous injections* were made into tail veins.

Analgesia was measured by means of a much modified Singh Grewel apparatus and procedure. The electrode system used consisted of a saline covered tinned metal plate insulated from a saline filled tinned metal trough. The mouse stood on the plate with its tail passing through a slot in an upright, which separated the electrodes, into the trough. Alternating current was supplied to the electrodes from the 12 volt tapping of a bell transformer whenever a key was depressed. The voltage was varied by means of a potentiometer, and the current flowing through the mouse on depression of the key was measured with an A.C. microammeter placed in the electrode circuit. *Pain thresholds* were measured as follows:—The key was tapped smartly four times at a current flow of 6 μ amps. This was repeated, increasing the current flow through the electrodes in increments of either 1 or 2 μ amps after each trial, until the mouse squeaked. The current value which elicited the squeak was recorded as the pain threshold. Mean pain thresholds were compared by "*t*" tests; no correction was made for coarse grouping.

Results

A Comparison of the Analgesic Actions of Morphine and Normorphine

Both the intensity and the rate of onset of the analgesic action of normorphine have been compared with those of morphine. In the first six experiments both drugs were given by intracisternal injection. In three, two groups of fifteen mice were used and pain thresholds were

determined for each mouse before, and 10, 20, and 30 minutes after a drug was given. In the other three experiments there were twelve mice in each group, and pain thresholds were measured before and 5 minutes after the drug was injected. Mice of one group were injected with morphine hydrochloride, 30 μg./kg. intracisternally, and those of the other group with normorphine hydrochloride, 30 μg./kg. similarly. The results of these experiments have been summarised in Table I. Five

TABLE I

A COMPARISON OF THE EFFECTS OF MORPHINE AND NORMORPHINE HYDROCHLORIDES, INJECTED INTRACISTERNALLY IN A DOSE OF 30 μg./kg., ON THE PAIN THRESHOLD OF MICE

Min. after injection (intracisternal)	*Pain threshold in μ amps. $\pm$ S.E. (No. of mice)	
	Morphine	Normorphine
0	10·36 $\pm$ 0·20 (45)	10·47 $\pm$ 0·19 (45)
10	12·80 $\pm$ 0·41 (45)	12·76 $\pm$ 0·29 (45)
20	13·69 $\pm$ 0·28 (45)	12·58 $\pm$ 0·31 (45)
30	14·45 $\pm$ 0·31 (45)	12·53 $\pm$ 0·35 (45)
0	10·01 $\pm$ 0·34 (45)	10·31 $\pm$ 0·27 (45)
5	12·22 $\pm$ 0·26 (45)	13·89 $\pm$ 0·29 (45)

* Pain thresholds in μ amps are expressed as means, plus or minus the standard error of the mean, followed by the number of mice within brackets.

minutes after intracisternal injection, normorphine proved more effective than morphine in raising the pain thresholds of mice ("t" calc. = 3·0, n = 66). Thereafter, the action of normorphine slowly decreased and that of morphine gradually increased. There was, therefore, no difference in the intensity of the analgesia caused by equal weights of these drugs

TABLE II

A COMPARISON OF THE EFFECTS OF MORPHINE AND NORMORPHINE HYDROCHLORIDES, INJECTED INTRAVENOUSLY, ON THE PAIN THRESHOLD OF MICE

Hydrochloride injected, mg./kg.		Min. after injection (intravenous)	*Pain threshold in μ amps. $\pm$ S.E. (No. of mice)	
Morphine	Normorphine		Morphine	Normorphine
10	50	0	10·44 $\pm$ 0·32 (20)	10·61 $\pm$ 0·42 (20)
		10	18·61 $\pm$ 0·47 (20)	15·67 $\pm$ 0·53 (20)
		20	19·42 $\pm$ 0·51 (20)	14·68 $\pm$ 0·67 (20)
10	100	0	10·14 $\pm$ 0·33 (10)	10·31 $\pm$ 0·36 (10)
		10	17·97 $\pm$ 0·55 (10)	20·78 $\pm$ 0·82 (10)
		20	19·78 $\pm$ 0·63 (10)	19·13 $\pm$ 0·64 (10)

* Pain thresholds in μ amps are expressed as means, plus or minus the standard error of the mean, followed by the number of mice within brackets.

10 minutes after their intracisternal injection, but the action of morphine became significantly the greater after 20 minutes ("t" calc. = 2·53, n = 84) and remained so.

Very different results were obtained when these two drugs were given by intravenous injection instead of intracisternally. Two experiments were made in which there were ten mice in each group, and pain thresholds

were determined for each mouse before, and 10 and 20 minutes after the intravenous injection of either morphine, or normorphine, hydrochloride. The combined results of these experiments are shown in Table II. Whereas the analgesic action of normorphine reached maximum intensity in 10 minutes, that of morphine continued to develop for 20 minutes. However, the analgesic potency of normorphine was approximately one tenth of that of morphine when the drugs were given intravenously.

The Antagonism of the Analgesic Actions of Normorphine and of Morphine by Nalorphine

The antagonism of the analgesic actions of morphine and of normorphine by nalorphine has been studied in mice using a single intracisternal injection for the administration of drugs. Each injection therefore contained either an activating drug alone, or both an activating drug and

TABLE III

MEASUREMENT OF THE ANTAGONISM OF THE ANALGESIC ACTIONS OF MORPHINE AND NORMORPHINE BY NALORPHINE IN MICE

Content of intracisternal injection, μg./kg.			Mean pain threshold in μ amps. ± S.E. (No. of mice)	
Morphine	Normorphine	Nalorphine	Before injection	20 min. after injection
10	—	—	11·0 ± 0·33 (10)	14·0 ± 0·30 (10)
10	—	10	10·8 ± 0·37 (10)	12·4 ± 0·65 (10)
—	10	—	10·8 ± 0·44 (10)	14·2 ± 0·61 (10)
—	10	10	11·2 ± 0·33 (10)	12·4 ± 0·96 (10)

the inhibitor drug, and was of standard volume, 0·02 ml. Since two activator and one inhibitor drug were studied, four groups of mice were used in each experiment. Pain thresholds were determined for the ten mice in each group before and after the intracisternal injection. The post-injection interval before the pain thresholds were measured for the second time was 20 minutes in the first six experiments. In these experiments, therefore, a constant contact period was allowed between the inhibitor drug and the receptors, but the effect of the inhibitor was measured during the development of the action of morphine and the regression of that of normorphine. The next five experiments differed from the first six in that the effects of nalorphine were determined at the time of the maximum action of the analgesic drug. The second measurement of the pain threshold was therefore made 5 minutes after the intracisternal injection of normorphine, and 30 minutes after that of morphine. The results of a single experiment of the first series are shown in Table III. These results were typical of all the experiments in which the antagonism of the actions of morphine and normorphine by nalorphine were studied. First, there was no significant difference between the initial mean pain thresholds of the different groups of mice. Secondly, these pain thresholds were significantly increased by all four treatments. Thirdly, the increase in the pain threshold which followed the intracisternal injection of the analgesic drug alone significantly exceeded that which resulted from the simultaneous injection of both the analgesic drug and nalorphine.

The percentage increases in pain threshold caused by morphine and by normorphine in the presence and absence of inhibitor have been entered, for each experiment, in Table IV. The first six experiments listed are those in which pain thresholds were measured 20 minutes after the drugs were injected intracisternally. There was great similarity in the antagonism of the analgesic actions of morphine and of normorphine in these experiments. In the next five experiments, the effects of normorphine,

TABLE IV

A COMPARISON OF THE ANTAGONISM OF THE ANALGESIC ACTIONS OF MORPHINE AND NORMORPHINE BY NALORPHINE, BY THE INTRACISTERNAL INJECTION OF THESE DRUGS INTO MICE. SEE TEXT FOR EXPLANATION

Injected drugs μg./kg.		Increase per cent in pain threshold caused by drugs			
Analgesic	Antagonist	Morphine	Morphine and nalorphine	Normorphine	Normorphine and nalorphine
10	10	27·3	14·8	31·5	10·7
		23·1	10·7	25·5	10·9
50	25	64·0	44·0	73·0	49·0
		68·2	51·6	67·6	49·6
100	500	88·7	9·8	120·5	18·1
		91·9	15·0	118·9	15·1
50	25	46·0	33·3	46·9	30·5
		42·7	26·8	47·0	32·9
50	100	53·9	15·6	55·0	24·8
		45·1	19·1	52·9	20·8
		42·7	16·7	47·1	21·9

in the presence and absence of nalorphine, were measured 5 minutes, and those of morphine 30 minutes after intracisternal injection. The fact that nalorphine again proved equally effective an antagonist of normorphine as of morphine under these circumstances indicated that the full effect of nalorphine had developed within 5 minutes of its intracisternal injection. The overall results in this Table show that at least one molecule of nalorphine is required to antagonise one molecule of either morphine or normorphine when these drugs are administered by intracisternal injection.

DISCUSSION

The analgesic action of normorphine developed more rapidly than that of morphine and was, weight for weight, as great as that of morphine when these drugs were administered by intracisternal injection. These observations are compatible with the hypothesis that *N*-alkylated drugs of the morphine type must undergo dealkylation before they can produce analgesia[2]. A decrease in the relative analgesic strength of normorphine on intravenous injection is probably to be attributed to the more rapid destruction of this secondary amine in the animal body, and to its less efficient penetration from the blood to the central nervous tissue, by comparison with morphine.

Nalorphine proved as effective an antagonist of normorphine as of morphine, and more than one molecule of the inhibitor was required completely to antagonise the action of one molecule of activator drug, when all drugs were administered by intracisternal injection. These

facts are more difficult to reconcile with the hypothesis of Beckett, Casy and Harper[2]. Previous estimates of the potency of nalorphine as an antagonist of the analgesic action of morphine have been made by the systemic route of administration. Under these conditions nalorphine antagonised many times its molecular equivalent of morphine[4].

M. M. Davis took part in this work during his tenure of an Educational Grant from the Pharmaceutical Society of Great Britain.

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ANALGESICS. PART II. SOME ARYLOXYALKYL OXAALKYLAMINES

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The synthesis is described of some structures formally related to aryloxypropanolamine in which the aryl and amine residues are joined by combinations derived from glycerol and ethylene glycol.

The syntheses of aryloxypropanolamines (I) for study as analgesics, initiated in Part I[1], is herein extended to some formally related types in which the aryl and amine residues are joined by combinations derived from glycerol and ethylene glycol.

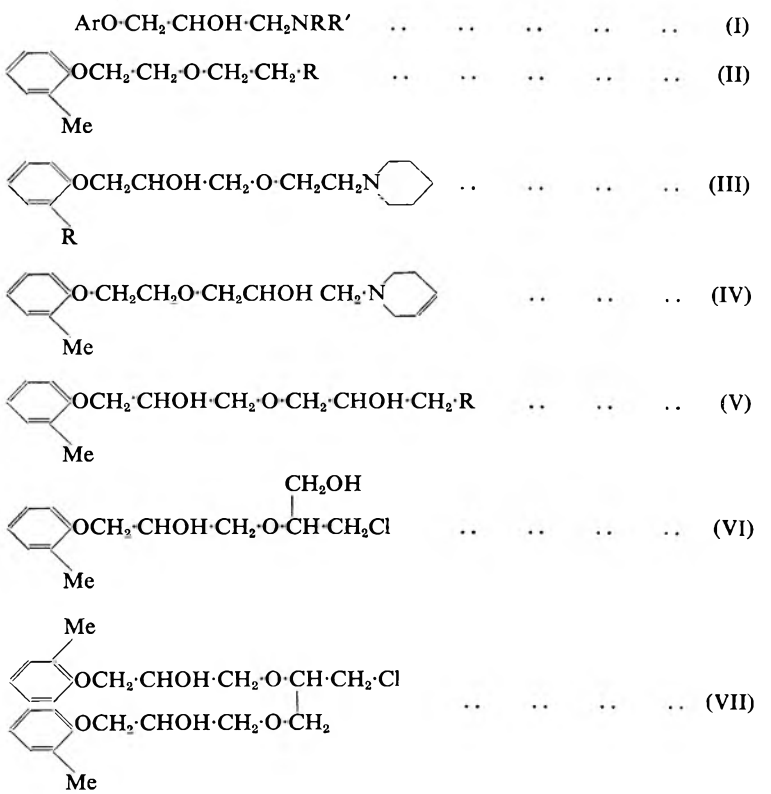
The 5-*o*-toloxy-3-oxapentylamines (II; R = NRR′) were readily prepared from 5-*o*-toloxy-3-oxapentyl chloride (II; R = Cl), in turn obtained by condensation of 2:2′-dichlorodiethyl ether with *o*-cresol. The last reaction invariably yielded some 1:5-bis-*o*-toloxy-ether (II; R = *o*-toloxy) as minor product, which was removed by fractional distillation under reduced pressure. *N*-(5-Hydroxy-6-phenoxy-3-oxahexyl)-piperidine (III; R = H) was obtained by condensing 3-phenoxy-1:2-epoxypropane with 2-hydroxyethylpiperidine in benzene solution under reflux. *N*-(5-Hydroxy-6-*o*-methoxyphenoxy-3-oxahexyl)-piperidine (III; R = OMe) was similarly prepared. The analogous *N*-(2-hydroxy-6-*o*-toloxy-4-oxahexyl)-Δ^3-piperideine (IV) was obtained by condensing 2-*o*-toloxyethanol with 2:3-epoxypropyl chloride in the presence of sodium methoxide to give 1:2-epoxy-6-*o*-toloxy-4-oxahexane, followed by reaction of the last compound with Δ^3-piperideine in benzene solution.

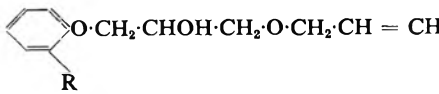
The synthesis of 2:6-dihydroxy-7-*o*-toloxy-4-oxaheptyl chloride (V; R = Cl), required for conversion to the piperidine derivative (V; R = piperidino) was next examined. Reaction of 3-*o*-toloxy-1:2-epoxypropane with 2:3-dihydroxypropyl chloride in the presence of sulphuric acid as catalyst[2] appeared to proceed normally with formation of a compound having the empirical formula of the chloride (V; R = Cl). The concomitant formation of 2-(3′-hydroxy-4′-*o*-toloxy-1-oxabutyl)-6-hydroxy-7-*o*-toloxy-4-oxaheptyl chloride (VII) as by-product, however, threw doubt upon the purity of the main product, which could well have contained some of the isomeric compound (VI). We therefore developed an unambiguous route to the piperidine derivative (V; R = piperidino). To this end 3-*o*-toloxy-1:2-epoxypropane was condensed with allyl alcohol in the presence of an acid catalyst to yield 6-hydroxy-7-*o*-toloxy-4-oxahept-1-ene (VIII; R = Me), additionally obtained by reaction between 2-hydroxy-3-*o*-toloxypropyl chloride and allyl alcohol in the presence of powdered potassium hydroxide. Epoxidation of the heptene (VIII; R = Me) with perbenzoic acid gave the epoxide (IX; R = Me) which passed smoothly into the required base (V; R = piperidino) on warming with piperidine in benzene solution. The diethylamine (V; R = NEt_2) was similarly prepared.

A variation on the above types resulted from the condensation of *o*- and *p*-alkoxyaniline and *o*- and *p*-alkoxy-*N*-alkylaniline with 2:6-dihydroxy-4-oxahexyl chloride when the novel amines (X) were obtained. 2:6-Dihydroxy-4-oxahexyl chloride was additionally condensed with morpholine, Δ^3-piperideine and pyrrolidine.

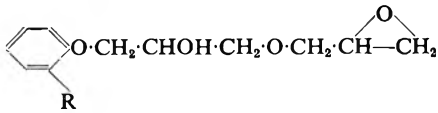
We next examined the condensation of *p*-anisidine and *p*-phenetidine with 2:6-dihydroxy-4-oxaheptyl chloride, which we had previously synthesised by the condensation of propane-1:2-diol with 2:3-epoxypropyl chloride[3]. In addition to the expected *p*-methoxy- and *p*-ethoxy-*N*-2:6-dihydroxy-4-oxaheptylaniline (XI) we obtained smaller quantities of isomeric bases formulated as *p*-methoxy- and *p*-ethoxy-*N*-2:6-dihydroxy-5-methyl-4-oxahexyl aniline (XII), which were readily separated from the isomeric compounds (XI) through their increased solubility in water. Concomitant formation of the last two compounds (XII) reveals the presence of appreciable quantities of 2:6-dihydroxy-5-methyl-4-oxahexyl chloride (XIII) in the 2:6-dihydroxy-4-oxaheptyl chloride (XIV) prepared by this route.

Finally, some unrelated derivatives based on but-2-yne were synthesised by condensing 4-phenoxy- and 4-*o*-toloxybut-2-yne chloride (XV) with diethylamine and Δ^3-piperideine to give the bases (XV*a*).

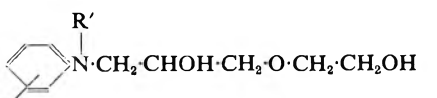




(VIII)

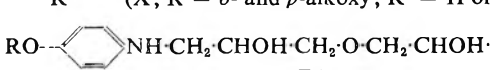


(IX)



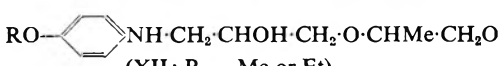
(X)

(X; R = *o*- and *p*-alkoxy; R′ = H or lower alkyl)



(XI)

(XI; R = Me or Et)



(XII)

(XII; R = Me or Et)

Cl·CH₂·CHOH·CH₂O·CHMe·CH₂OH .. .. (XIII)

Cl·CH₂·CHOH·CH₂·O·CH₂·CHOH·Me .. .. .. (XIV)

ArO·CH₂·C ≡ C·CH₂R .. .. (XV)
(Ar = Ph or *o*-tolyl, R = Cl)

(Ar = Ph or *o*-tolyl, R = NEt_2 or Δ^3-piperideino) .. .. (XV*a*)

Experimental

Melting points are uncorrected.

5-o-*Toloxy*-3-*oxapentyl chloride* (II; R = Cl). To a solution of sodium hydroxide (40 g.) in ethanol (450 ml.) and water (40 ml.) was added *o*-cresol (108 g.) followed by 2:2′-dichlorodiethyl ether (143 g.) and the mixture heated under reflux for 5 hours. Excess of ethanol was boiled off, water added, and the separated oil extracted with chloroform. The chloroform extract was washed with water, the solvent removed and the residual oil distilled at 0·3 mm., yielding:

fraction (i) b.p. 36 to 95°, 52·4 g., fraction (ii) b.p. 100 to 120°, 111·9 g., and fraction (iii) b.p. 160°, 26·9 g.

Fraction (ii) was redistilled to give 5-o-*toloxy*-3-*oxapentyl chloride*, b.p. 92° at 0·3 mm. Found: C, 62·0; H, 7·1; Cl, 16·0. $C_{11}H_{15}O_3Cl$ requires C, 61·5; H, 7·1; Cl, 16·5 per cent. Fraction (iii) on redistillation yielded 1:5-*bis*-o-*toloxy*-3-*oxapentane* (II, R = *o*-tolyl), b.p. 156° at 0·3 mm. Found: C, 75·4; H, 7·8. $C_{18}H_{22}O_3$ requires C, 75·5; H, 7·8 per cent.

N-(5-o-*Toloxy*-3-*oxapentyl*)-*piperidine* (II; R = piperidino). The foregoing chloro-compound (21·45 g.) was heated with piperidine (25·5 g.) on the steam bath for 10 hours. Excess of piperidine was removed under reduced pressure, the residue was treated with water and the oil extracted with chloroform. The extract was washed with water, the chloroform removed and the residual oil distilled at 0·3 mm. to give the product, b.p. 132°, 25·5 g. Redistillation yielded N-(5-o-*toloxy*-3-*oxapentyl*)-*piperidine*, b.p. 130° at 0·3 mm. Found: C, 72·7; H, 9·2; N, 4·7.

$C_{16}H_{25}O_2N$ requires C, 73·0; H, 9·6; N, 5·3 per cent. It formed a *picrate* which separated from a mixture of ethyl acetate and light petroleum (b.p. 40 to 60°) in yellow nodules, m.p. 77 to 78°. Found: C, 53·7; H, 5·9; N, 11·2. $C_{22}H_{28}O_9N_4$ requires C, 53·6; H, 5·7; N, 11·4 per cent.

N-(5-o-*Toloxy*-3-*oxapentyl*)-*pyrrolidine* (II; R = pyrrolidino) was obtained as an oil, b.p. 122° at 0·3 mm. Found: N, 5·4. $C_{15}H_{23}O_2N$ requires N, 5·6 per cent.

N-(5-o-*Toloxy*-3-*oxapentyl*)-Δ^3-*piperideine* (II; R = Δ^3-piperideino) was obtained as an oil, b.p. 138°/0·3 mm. Found: C, 73·9; H, 8·9; N, 4·9. $C_{16}H_{23}O_2N$ requires C, 73·5; H, 8·9; N, 5·4 per cent. It formed a *picrate* which separated from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°) in yellow needles m.p. 92 to 94°. Found: C, 53·9; H, 5·2; N, 11·2. $C_{22}H_{26}O_9N_4$ requires C, 53·9; H, 5·3; N, 11·4 per cent.

N-(5-*Hydroxy*-6-*phenoxy*-3-*oxahexyl*)-*piperidine* (III; R = H). A mixture of 3-phenoxy-1:2-epoxypropane (30 g.) and 2-hydroxyethyl piperidine (25·8 g.) in benzene (100 ml.) was heated under reflux for 20 hours. The basic fraction was extracted with N hydrochloric acid, the acid extract basified and extracted with chloroform. The chloroform extract was washed with water, the chloroform removed and the residual oil distilled at 0·1 mm. to yield N-(5-*hydroxy*-6-*phenoxy*-3-*oxahexyl*)-*piperidine*, b.p. 150°. Found: C, 68·7; H, 9·0; N, 5·3. $C_{16}H_{25}O_3N$ requires C, 68·8; H, 9·0; N, 5·1 per cent.

N-(5-*Hydroxy*-6-o-*methoxyphenoxy*-3-*oxahexyl*)-*piperidine* (III; R = OMe) was similarly obtained as an oil, b.p. 160° at 0·1 mm. Found: C, 66·2; H, 8·9; N, 4·3. $C_{17}H_{27}O_4N$ requires C, 66·0; H, 8·8; N, 4·5 per cent.

2-*Hydroxy*-6-o-*toloxy*-4-*oxahexyl chloride*. To a mixture of 2-*o*-toloxyethanol (152 g., 3 mole equivs.) and 2:3-epoxypropylchloride (31 g.) was added polyphosphoric acid (2 g.) and the mixture heated on the steam bath for 20 hours. After cooling, the residue was taken up in chloroform, washed with aqueous sodium bicarbonate and then with water. After removal of the chloroform the residue was distilled at 0·2 mm. to yield a fraction b.p. 120 to 134°; 38·0 g. This on refractionation at 0·5 mm. gave 2-*hydroxy*-6-o-*toloxy*-4-*oxahexyl chloride*, b.p. 136°. Found: C, 58·6; H, 7·1; Cl, 14·7. $C_{12}H_{17}O_3Cl$ requires C, 58·9; H, 7·0; Cl, 14·5 per cent.

1:2-*Epoxy*-6-o-*toloxy*-4-*oxahexane*. Method I: 2-*o*-Toloxyethanol (88·7 g.) was added to a solution of sodium methoxide prepared by dissolving sodium (13·4 g.) in methanol (200 ml.). The mixture was heated under reflux for several minutes and the methanol distilled off, last traces being removed by heating at 100° at 0·3 mm. for 2 hours. The residue was suspended in dry benzene (300 ml.), 1-chloro-2:3-epoxypropane (64·8 g.) added and the mixture heated under reflux for 8 hours. It was cooled, poured into water and acidified with acetic acid (5 ml.). The benzene layer was separated, washed with water, the benzene removed and the residual oil distilled under reduced pressure. The main fraction, b.p. 98 to 130° at 0·4 mm., 39·0 g., was refractionated to yield 1:2-*epoxy*-6-o-*toloxy*-4-*oxahexane* as an oil, b.p. 100° at 0·05 mm., 31·2 g. Found: C, 69·3; H, 8·2. $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·7 per cent.

An appreciable amount of 5-*hydroxy*-1:9-*bis*-o-*toloxy*-3:7-*dioxanonane* was isolated from the top fraction and obtained as a viscous oil, b.p. 210° at 0·07 mm. Found: C, 69·5; H, 7·9. $C_{21}H_{28}O_5$ requires C, 70·0; H, 7·8 per cent.

Method II: Treatment of the foregoing chlorohydrin with an equivalent of methanolic potash at 0°, followed by dilution and extraction with chloroform yielded the required epoxide.

N-(2-*Hydroxy*-6-o-*toloxy*-4-*oxahexyl*)-Δ^3-*piperideine* (IV). The foregoing epoxide (15 g.) was dissolved in benzene (20 ml.), Δ^3-piperideine (7·2 g.) added and the mixture heated under reflux for 6 hours. After removal of the benzene the residual oil was refractionated at 0·3 mm. to yield N-(2-*hydroxy*-6-o-*toloxy*-4-*oxahexyl*)-Δ^3-*piperideine* as an oil, b.p. 162°. Found: N, 4·7. $C_{17}H_{25}O_3N$ requires N, 4·8 per cent. The *hydrochloride* was a hygroscopic solid, m.p. 50 to 60°. Found: C, 60·7; H, 8·2; N, 4·4; Cl, 10·5. $C_{17}H_{26}O_3NCl$; $\frac{1}{2}H_2O$, requires C, 60·6; H, 8·1; N, 4·2; Cl, 10·5 per cent. The *picrate*, which separated from a mixture of ethyl acetate and ether, had m.p. 81 to 83°. Found: C, 52·8; H, 5·6; N, 10·7. $C_{23}H_{28}O_{10}N_4$ requires C, 53·1; H, 5·4; N, 10·8 per cent.

Condensation of 3-o-*toloxy*-1:2-*epoxypropane with* 2:3-*dihydroxypropyl chloride*. A mixture of 3-*o*-toloxy-1:2-epoxypropane (82 g.) and 2:3-dihydroxypropyl chloride (166 g., 3 moles) was treated carefully with concentrated sulphuric acid (2 ml.) added dropwise with shaking. The mixture was heated on the steam bath for 20 hours, cooled, taken up in an equal volume of chloroform, washed with dilute aqueous sodium bicarbonate and then with water. After removal of the chloroform the residual oil was distilled at 0·5 mm. to yield:—fraction (i) b.p. 80 to 145° 7 g.; fraction (ii) b.p. 190 to 205°, 64 g.; fraction (iii) b.p. 270°. Fraction (ii) on redistillation yielded a constant fraction b.p. 176° at 0·4 mm. Found: C, 57·2; H, 7·5. $C_{13}H_{19}O_4Cl$ requires C, 56·8; H, 7·0 per cent. Fraction (iii) was not distilled completely owing to decomposition of the residue. Found: C, 63·3; H, 7·4. 2-(3′-Hydroxy-4′-*o*-toloxy-1′-oxabutyl)-6-hydroxy-7-*o*-toloxy-4-oxaheptyl chloride (VII) $C_{23}H_{31}O_6Cl$ requires C, 62·9; H, 7·1 per cent.

6-*Hydroxy*-7-o-*toloxy*-4-*oxahept*-1-*ene* (VIII; R = Me). Method I: A mixture of 3-*o*-toloxy-1:2-epoxypropane (164 g.) and allyl alcohol (174 g., 3 mole) was treated carefully with concentrated sulphuric acid (2 ml.) and heated on the steam bath for 20 hours. Excess of allyl alcohol was removed at reduced pressure, the residue was taken up in chloroform, washed acid-free, the chloroform removed and the residue distilled at reduced pressure to give fraction (i) b.p. 120° at 0·25 mm. and fraction (ii) b.p. 124 to 144° at 0·25 to 1·0 mm. Distillation had to be stopped due to decomposition of the residue. Fraction (i) was redistilled at 1·2 mm. to yield 6-*hydroxy*-7-o-*toloxy*-4-*oxahept*-1-*ene* b.p. 130 to 132°. Found: C, 70·5; H, 8·3. $C_{13}H_{18}O_3$ requires C, 70·2; H, 8·2 per cent. Fraction (ii) contained a high proportion of 3-*o*-toloxypropane-1:2-diol (mephenesin) which separated on standing.

Method II: To a mixture of 2-hydroxy-3-*o*-toloxypropyl chloride (100 g.) and allyl alcohol (232 g., 8 moles), powdered potassium hydroxide

(33·6 g., 1·2 mole) was added in portions with shaking over 10 minutes. The mixture was heated on the steam bath for 8 hours, excess of allyl alcohol removed at reduced pressure, the residue cooled, diluted with water and extracted with chloroform. The chloroform extract was neutralised with acetic acid, washed with water and the chloroform removed. The residue was distilled at 0·1 mm. to yield the product (97·8 g.), b.p. 113°. Found: C, 70·4; H, 8·3 per cent.

1:2-*Epoxy*-6-*hydroxy*-7-o-*toloxy*-4-*oxaheptane* (IX; R = Me). The foregoing allyl ether (63 g.) was added to a cold solution of perbenzoic acid (39·15 g.) in benzene (995 ml.). The solution was left at 0° for 2 days and then at room temperature for 2 days. It was washed with 10 per cent sodium hydroxide, then with water. After removal of the benzene the residual oil was distilled at 0·3 mm. The main fraction, b.p. 118 to 150° (37·7 g.), was refractionated to yield the *product* as an oil, b.p. 144° at 0·4 mm. Found: C, 65·7; H, 7·6. $C_{13}H_{18}O_4$ requires C, 65·5; H, 7·6 per cent.

N-(2:6-*Dihydroxy*-7-o-*toloxy*-4-*oxaheptyl*)-*diethylamine* (V; R = NEt_2) was obtained as an oil, b.p. 174° at 0·3 mm. by condensation of the foregoing epoxide with diethylamine in benzene solution at reflux temperature for 5 hours. Found: N, 4·8. $C_{17}H_{29}O_4N$ requires N, 4·5 per cent.

N-(2:6-*Dihydroxy*-7-o-*toloxy*-4-*oxaheptyl*)-*piperidine* (V; R = piperidino) was obtained as an oil, b.p. 194° at 0·4 mm. Found: C, 66·6; H, 9·0; N, 4·3. $C_{18}H_{29}O_4N$ requires C, 66·8; H, 9·0, N, 4·3 per cent.

6-*Hydroxy*-7-*phenoxy*-4-*oxahept*-1-*ene* (VIII; R = H) was prepared from 3-phenoxy-1:2-epoxy propane and allyl alcohol as for the corresponding *o*-toloxy analogue and obtained as an oil, b.p. 110° at 0·05 mm. Found: C, 69·3; H, 7·7. $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·8 per cent.

1:2-*Epoxy*-6-*hydroxy*-7-*phenoxy*-4-*oxaheptane* (IX; R = H) was prepared by reaction of the foregoing allyl ether with perbenzoic acid. It was obtained as an oil, b.p. 140° at 0·4 mm. Found: C, 64·5; H, 7·2. $C_{12}H_{16}O_4$ requires C, 64·3; H, 7·2 per cent.

N-(2:6-*Dihydroxy*-4-*oxahexyl*)-p-*anisidine* (X; R = *p*-OMe, R′ = H). A mixture of *p*-anisidine (123 g.), 2:6-dihydroxy-4-oxahexyl chloride (77 g.) and anhydrous sodium carbonate (31·8 g.) in ethanol (500 ml.) was heated under reflux for 6 hours. After removal of most of the ethanol, the residue was diluted with water, extracted with chloroform, the extracts washed with water and the chloroform removed. The residue was distilled at 0·4 mm. yielding unchanged *p*-anisidine (70 g.) and the *product* (44 g.), b.p. 190 to 200°. The latter solidified and had m.p. 65 to 67° after crystallisation from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°). Found: C, 59·0; H, 8·2; N, 6·2. $C_{12}H_{19}O_4N$ requires C, 59·4; H, 8·1; N, 5·8 per cent.

N-*Methyl*-N-(2:6-*dihydroxy*-4-*oxahexyl*)-p-*anisidine* (X; R = *p*-OMe, R′ = Me). The foregoing amine (6 g.) was dissolved in methanol (100 ml.), methyl iodide (4·3 g.) and anhydrous sodium carbonate (2·7 g.) added and the mixture heated under reflux for 5 hours. The solvent was removed, the residue diluted with water and the oil extracted with ethyl acetate. After drying the ethyl acetate was distilled off and the residual

oil distilled at 0·1 mm. yielding the *product* as an oil, b.p. 180°. Found: C, 60·9; H, 7·9; N, 6·0. $C_{13}H_{21}O_4N$ requires C, 61·0; H, 8·2; N, 5·5 per cent.

N-*Ethyl*-N-(2:6-*dihydroxy*-4-*oxahexyl*)-p-*anisidine* (X; R = *p*-OMe, R′ = Et) was prepared as for the corresponding *N*-methyl analogue and obtained as an oil, b.p. 185° at 0·1 mm. Found: C, 61·8; H, 8·2; N, 4·8. $C_{14}H_{23}O_4N$ requires C, 62·2; H, 8·6; N, 5·2 per cent.

N-(2:6-*Dihydroxy*-4-*oxahexyl*)-p-*phenetidine* (X; R = *p*-OEt, R′ = H). To a mixture of *p*-phenetidine (82·2 g., 1·5 moles) and 2:6-dihydroxy-4-oxahexyl chloride (61·8 g.) dissolved in methanol (100 ml.) was added a solution of potassium hydroxide (22·4 g.) in methanol (100 ml.) and the mixture heated on the steam bath for 3 hours. After removal of solvent the residue was diluted with water, extracted with chloroform and the extracts washed with a small volume of brine solution. The chloroform was removed and the residual oil distilled under reduced pressure giving unchanged *p*-phenetidine together with the product (62 per cent) as an oil, b.p. 220° at 0·4 mm., which solidified rapidly. It crystallised from a mixture of ethyl acetate and light petroleum (b.p. 40 to 60°) in light yellow prisms, m.p. 70 to 72°. Found: C, 61·2; H, 8·4; N, 5·3. $C_{13}H_{21}O_4N$ requires C, 61·4; H, 8·3; N, 5·5 per cent. The *picrate* separated from ethanol in bright yellow needles, m.p. 127 to 128°. Found: C, 47·1; H, 4·9; N, 11·7. $C_{19}H_{24}O_{11}N_4$ requires C, 47·1; H, 5·0; N, 11·6 per cent.

N-*Ethyl*-N-(2:6-*dihydroxy*-4-*oxahexyl*)-p-*phenetidine* (X; R = *p*-OEt, R′ = Et). When the foregoing base was ethylated with ethyl iodide in ethanol in the presence of sodium carbonate at reflux temperature for 10 hours the *product* was obtained as an oil, b.p. 180° at 0·3 mm. Found: C, 63·6; H, 9·0; N, 4·6. $C_{15}H_{25}O_4N$ requires C, 63·6; H, 8·9; N, 4·9 per cent.

N-(2:6-*Dihydroxy*-4-*oxahexyl*)-p-n-*propoxyaniline* (X; R = *p*-OPr, R′ = H). It was prepared by condensation of *p*-*n*-propoxyaniline with 2:6-dihydroxy-4-oxahexyl chloride as described for the *p*-methoxy analogue. It was obtained in 61 per cent yield as an oil, b.p. 192 to 198° at 0·1 mm. The product solidified rapidly and crystallised from a mixture of ethyl acetate and light petroleum (b.p. 40 to 60°) in light yellow needles, m.p. 71 to 73°. Found: C, 61·9; H, 8·5; N, 4·8. $C_{14}H_{23}O_4N$ requires C, 62·2; H, 8·6; N, 5·2 per cent.

N-(2:6-*Dihydroxy*-4-*oxahexyl*)-p-n-*butoxyaniline* (X; R = *p*-OBu, R′ = H) was obtained as an oil, b.p. 195 to 200° at 0·4 mm. which solidified rapidly and crystallised from a mixture of ethyl acetate and light petroleum (b.p. 40 to 60°) in yellow needles, m.p. 69 to 71°. Found: C, 63·0; H, 8·6; N, 4·9. $C_{15}H_{25}O_4N$ requires C, 63·3; H, 8·8; N, 5·0 per cent.

N-(2:6-*Dihydroxy*-4-*oxahexyl*)-o-*phenetidine* (X; R = *o*-OEt, R′ = H) was isolated as an oil, b.p. 182 to 186°/0·4 mm. which solidified and crystallised from a mixture of ethyl acetate and light petroleum (b.p. 40 to 60°) in pale yellow needles, m.p. 57 to 58°. Found: C, 61·5; H, 8·2; N, 5·0. $C_{13}H_{21}O_4N$ requires C, 61·2; H, 8·2; N, 5·5 per cent.

N-(2:6-*Dihydroxy*-4-*oxahexyl*)-*morpholine* was obtained as an oil, b.p. 144° at 0·5 mm. Found: C, 52·0; H, 9·1; N, 7·0. $C_9H_{19}O_4N$ requires C, 52·4; H, 9·3; N, 6·8 per cent.

N-(2:6-*Dihydroxy*-4-*oxahexyl*)-Δ^3-*piperideine* formed an oil, b.p. 140° at 0·3 mm. Found: C, 59·5; H, 9·1. $C_{10}H_{19}O_3N$ requires C, 59·6; H, 9·5 per cent.

N-(2:6-*Dihydroxy*-4-*oxahexyl*)-*pyrrolidine* was an oil, b.p. 118° at 0·1 mm. Found: C, 57·2; H, 9·9. $C_9H_{19}O_3N$ requires C, 57·1; H, 10·0 per cent.

N-(2:9-*Dihydroxy*-4:7-*dioxanonyl*)-p-*phenetidine*. A mixture of 2:9-dihydroxy-4:7-dioxanonyl chloride (19·85 g.), *p*-phenetidine (27·4 g., 2 moles) and potassium hydroxide (5·6 g.) in methanol (30 ml.) was heated on the steam bath for 3 hours. Isolation in the usual manner gave the *product* as an oil (16·5 g.), which after refractionation had b.p. 220° at 0·5 mm. Found: C, 59·7; H, 8·5; N, 4·5. $C_{15}H_{25}O_5N$ requires C, 60·2; H, 8·4; N, 4·7 per cent. The *picrate* separated from ethyl acetate in bright yellow needles, m.p. 120 to 121°. Found: C, 47·8; H, 5·5; N, 10·4. $C_{21}H_{28}O_{12}N_4$ requires C, 47·7; H, 5·3; N, 10·6 per cent.

The compound (50·6 g.), previously stated by us[3] to be 2:6-dihydroxy-4-oxaheptyl chloride, was dissolved in ethanol (125 ml.) and *p*-phenetidine (82·2 g., 2 mole) added, followed by anhydrous sodium carbonate (21 g.). The mixture was heated on the steam bath for 8 hours when the solvent was removed, water added and the product extracted with chloroform. The chloroform extract was washed with water, the chloroform removed, and the residue distilled to yield:—fraction (i) b.p. 70° at 0·5 mm. (46 g.), mainly unchanged *p*-phenetidine, and (ii) b.p. 200 to 205° at 0·4 to 0·5 mm. (57 g.). Fraction (ii) crystallised on treatment with ethyl acetate and light petroleum (b.p. 40 to 60°) to yield a white solid (A), m.p. 92 to 97° (21 g.) which had m.p. 102 to 104° after repeated crystallisation from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°). It was soluble in hot water, the *product* crystallising on cooling. Found: C, 62·4; H, 8·5; N, 5·2. $C_{14}H_{23}O_4N$ requires C, 62·4; H, 8·6; N, 5·2 per cent.

The *picrate* separated from ethyl acetate in yellow fluffy needles, m.p. 130 to 132°. Found: C, 48·6; H, 5·4; N, 10·6. $C_{20}H_{26}O_{11}N_4$ requires C, 48·2; H, 5·3; N, 11·2 per cent.

The mother-liquors from solid (A) were concentrated and the gummy residue distilled at 0·8 mm. to yield a main fraction, b.p. 210°. This solidified and crystallised from a mixture of ether and light petroleum (b.p. 40 to 60°) to yield fluffy white needles, m.p. 45° (12·5 g.) (B), which after repeated crystallisation from the same solvent mixture had m.p. 54 to 56°. It was much more soluble in cold water than (A). Found: C, 62·2; H, 8·5; N, 5·1. $C_{14}H_{23}O_4N$ requires C, 62·4; H, 8·6; N, 5·2 per cent.

Because of the sharp differences in water solubilities, and relative yields, compound (A) is tentatively assigned structure (XI; R = Et) and compound (B) the structure (XII; R = Et).

In a similar experiment the mixed chlorohydrin (33·7 g.) was condensed with *p*-anisidine (49·2 g., 2 mole) in methanol (150 ml.) containing potassium hydroxide (11·2 g.). After working up as in the previous example a fraction was obtained b.p. 200 to 220° at 0·5 mm. This yielded two

isomers again, the less soluble isomer (C) crystallised from water in white fluffy needles, m.p. 92 to 93°. Found: C, 60·6; H, 8·1. $C_{13}H_{21}O_4N$ requires C, 61·1; H, 8·3 per cent. It formed a *picrate* which separated from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°) in bright yellow crystals, m.p. 134 to 136°. Found: C, 47·1; H, 5·0. $C_{19}H_{24}O_{11}N_4$ requires C, 47·1; H, 5·0 per cent.

The second more soluble *isomer* (D) had m.p. 64 to 66° after crystallisation from a mixture of ether and light petroleum (b.p. 60 to 80°). Found: C, 60·9; H, 8·2; N, 5·2. $C_{13}H_{21}O_4$ requires C, 61·1; H, 8·3; N, 5·5 per cent. Its *picrate* separated from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°) in yellow needles, m.p. 125 to 126°. Found: C, 47·4; H, 5·0; N, 11·9. $C_{19}H_{24}O_{11}N_4$ requires C, 47·1; H, 5·0; N, 11·6 per cent.

Isomer (C) is tentatively assigned structure (XI; R = Me), and isomer (D) the structure (XII; R = Me).

N-p-*Ethoxyphenylmorpholine*. A mixture of 2:2′-dichlorodiethyl ether (36 g.), *p*-phenetidine (34·5 g.) and potassium hydroxide (28 g.) in 50 per cent aqueous ethanol (200 ml.) was heated on the steam bath for 6 hours. Excess of ethanol was boiled off, the oil was taken up in chloroform, the chloroform layer washed, concentrated and the residue distilled at 0·5 mm. The product had b.p. 120° at 0·5 mm. and solidified rapidly. It crystallised from aqueous ethanol in white shining plates, m.p. 75 to 76°. Found: C, 69·6; H, 8·5. $C_{12}H_{17}O_2N$ requires C, 69·5; H, 8·3 per cent.

The bulk of the product was converted to the *hydrochloride*, which separated from a mixture of ethanol and ether in small colourless prisms (17 g.), m.p. 170 to 171°. Found: C, 59·4; H, 6·9; N, 6·2. $C_{12}H_{18}O_2NCl$ requires C, 59·1; H, 7·5; N, 5·8 per cent.

3-(3′:4′:5′-*Trimethoxyphenyl*)*aminopropan*-1:2-*diol* was obtained by condensation of 3:4:5-trimethoxyaniline (18·3 g.) with 2:3-epoxypropanol (7·4 g.) in ethanol (40 ml.) on the steam bath for 8 hours. It had b.p. 210° at 0·2 mm. Found: C, 55·7; H, 7·8; N, 5·8. $C_{12}H_{19}O_5N$ requires C, 56·0; H, 7·4; N, 5·5 per cent. The *hydrochloride* separated from a mixture of ethanol and ether in white needles with a blue-green tinge, m.p. 148 to 150°. Found: C, 49·0; H, 6·9; N, 4·8. $C_{12}H_{20}O_5NCl$ requires C, 49·0; H, 6·9; N, 4·8 per cent.

4-o-*Toloxy-but*-2-*yne chloride* (XV; Ar = *o*-tolyl). Potassium hydroxide (75 g.) was dissolved by warming in a mixture of *iso*propanol (550 ml.) and *o*-cresol (172 g.) and 1:4-dichloro-but-2-yne (172 g.) was added rapidly with stirring to the warm solution. Reaction was completed by heating the mixture on the steam bath for 30 minutes. After cooling, potassium chloride was removed, the neutral residue distilled at reduced pressure to remove excess of *iso*propanol and the *product* isolated by distillation at 0·3 mm. It formed an oil, b.p. 110°. Found: C, 67·8; H, 5·8; Cl, 18·2. $C_{11}H_{11}OCl$ requires C, 67·8; H, 5·7; Cl, 18·2 per cent.

1-Chloro-4-phenoxy-but-2-yne was prepared similarly.

1-*Diethylamino*-4-*phenoxy-but*-2-*yne hydrochloride*. A mixture of 4-phenoxy-but-2-yne chloride (24 g.) and diethylamine (36 ml.) was heated on the steam bath under reflux for 30 minutes. It was then cooled,

poured into water, made alkaline with concentrated sodium hydroxide solution and the separated oil extracted with ether. The ethereal layer was washed with water, dried over anhydrous sodium sulphate and the ether removed. The residual oil was distilled to yield the *base* as an oil, b.p. 110 to 115° at 0·2 mm.

The base was converted to the *hydrochloride* in ethereal solution and the latter was purified by crystallisation from a mixture of ethanol and ether, forming white needles of m.p. 138 to 139°. Found: C, 66·5; H, 8·1; N, 5·4; Cl, 13·6. $C_{14}H_{20}ONCl$ requires C, 66·2; H, 7·9; N, 5·5; Cl, 14·0 per cent.

1-*Diethylamino*-4-o-*toloxy*-*but*-2-*yne* had b.p. 126 to 128° at 0·6 mm. The *hydrochloride* separated from a mixture of ethanol and ether in white crystals of m.p. 116 to 117°. Found: C, 66·7; H, 8·3; N, 4·8; Cl, 13·6. $C_{15}H_{22}ONCl$ requires C, 67·2; H, 8·3; N, 5·2; Cl, 13·3 per cent.

1-Δ^3-*Piperideino*-4-o-*toloxy*-*but*-2-*yne* had b.p. 130° at 0·5 mm. The *hydrochloride* separated from a mixture of *iso*propanol and ether in white needles, m.p. 126 to 127°. Found: C, 69·0; H, 7·0; N, 4·8; Cl, 13·1. $C_{16}H_{20}ONCl$ requires C, 69·1; H, 7·3; N, 5·0; Cl, 12·8 per cent.

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ANALGESICS. PART III. SALICYLAMIDE DERIVATIVES

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The synthesis of some derivatives of 3-(*o*-aminocarbonylphenoxy)-propan-1:2-diol, 1-aryloxy-3-(*o*-aminocarbonylphenoxy)-propan-2-ol and *NN'*-bis-(3-*o*-aminocarbonylphenoxy-2-hydroxypropyl)-piperazine is described.

While the work described in Parts I and II was in progress, Way and others[1] published a paper on the pharmacology of some eighty congeners of salicylamide containing small substituent groups in the phenyl ring, on the phenolic hydroxyl group or on the amido-nitrogen. Many of these compounds showed central nervous depressant, hypnotic, antipyretic and analgesic activity. We therefore extended our studies to the preparation of some 3-(*o*-aminocarbonylphenoxy)-propan-1:2-diol derivatives formally related to salicylamide.

The preparation of 3-aminocarbonylphenoxy-1:2-epoxypropane (IV; R = H), required as an intermediate for types (I) and (III), was first examined. Condensation of salicylamide with one or two molar equivalents of 2:3-epoxypropyl chloride in aqueous alkaline solution at room temperature under the conditions used previously for the preparation of 3-aryloxy-1:2-epoxypropanes[2] (see also Part I), led to the formation of 1:3-bis-(*o*-aminocarbonylphenoxy)-propan-2-ol (III; R = R′ = $CONH_2$) as major product. The required epoxide (IV; R = H) was ultimately obtained in about 43 per cent yield by using a large excess (5 to 6 molar equivalents) of 2:3-epoxypropyl chloride in the reaction, but even then formation of the bis-compound was not completely suppressed.

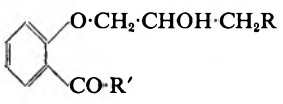
Alkaline hydrolysis of the diamide (III; R = R′ = $CONH_2$) yielded the corresponding dicarboxylic acid (III; R = R′ = CO_2H). The last compound was also prepared by reaction between sodium salicylate and 2:3-epoxypropyl chloride in aqueous alkaline solution and was purified *via* its dimethyl ester.

Condensation of the epoxide (IV; R = H) with piperidine and with piperazine yielded 3-(*o*-piperidinocarbonylphenoxy)-propane-1:2-diol (I; R = piperidino, R′ = NH_2) and *NN'*-bis-(2-hydroxy-3-*o*-aminocarbonylphenoxypropyl)-piperazine (V; R = H), respectively. Reaction of (IV; R = H) with succinimide and with phthalimide in the presence of a basic catalyst gave the succinimido- (I; R = succinimido, R′ = NH_2) and phthalimido- (I; R = phthalimido, R′ = NH_2) derivatives. The former gave 3-(*o*-carboxyphenoxy)-2-hydroxypropylamine (hydrochloride) (I; R = NH_2·HCl, R′ = OH) on hydrolysis with concentrated hydrochloric acid. The phthalimido-compound, on reaction with hydrazine hydrate, followed by careful treatment with hydrochloric acid, furnished 3-(*o*-aminocarbonylphenoxy-2-hydroxypropylamine (hydrochloride) (I; R = NH_2·HCl, R′ = NH_2).

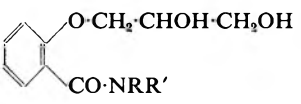
Condensation of salicyldiethylamide with excess 2:3-epoxypropyl chloride in alkaline solution yielded the epoxide (IV; R = Et) together with 3-(*o*-diethylaminocarbonylphenoxy)-propane-1:2-diol (II; R = R′ = Et). Reaction of the epoxide (IV) with piperazine gave *NN*′-bis-(3-*o*-diethylaminocarbonylphenoxy-2-hydroxypropyl)-piperazine (V; R = Et).

3-(*o*-Aminocarbonylphenoxy)-propane-1:2-diol (II; R = R′ = H) was readily obtained by condensation of salicylamide with 2:3-dihydroxypropyl chloride in aqueous alkaline solution. Its preparation from salicylamide and 2:3-epoxypropan-1-ol in the presence of a basic catalyst proved less satisfactory, though this method was useful for the preparation of the substituted amides (II; R = H, R′ = Et; R = H, R′ = Bu; R = R′ = Et and NRR′ = piperidino).

Some unsymmetrical bis-compounds (III) were prepared by condensing the appropriate epoxide (IV) with various phenols or alternatively by the reaction of 3-*o*-aryloxy-1:2-epoxypropanes with the substituted salicylamide. The condensation of methyl salicylate with 3-*o*-toloxy-1:2-epoxypropane and with 2:3-epoxypropyl chloride was also examined (see Experimental).



(I)



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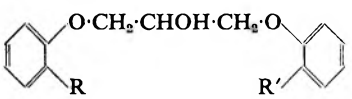

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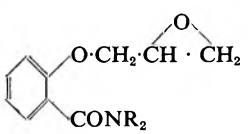

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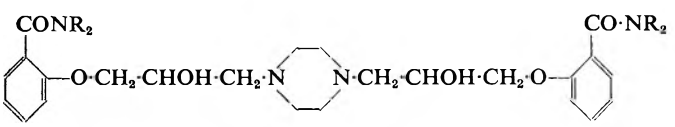

(V)

Experimental

Melting points are uncorrected.

Condensation of salicylamide with 2:3-*epoxypropyl chloride.* To a stirred suspension of salicylamide (34·5 g.) and 2:3-epoxypropyl chloride (138·8 g., 6 mole equiv.) in water (100 ml.) was added a solution of sodium hydroxide (10 g.) in water (20 ml.) and stirring continued for 24 hours. The solid which separated was collected, drained and dissolved in boiling

ethanol (200 ml.). The solution on cooling deposited white fluffy needles (11 g.) of 1:3-*bis*-(o-*aminocarbonylphenoxy*)-*propan*-2-*ol* (III; R = R′ = $CONH_2$), m.p. 213 to 215° after a further crystallisation from ethanol. Found: C, 62·1; H, 5·5; N, 8·4. $C_{17}H_{18}O_5N_2$ requires C, 61·8; H, 5·5; N, 8·5 per cent.

A second product (7·9 g., m.p. 108 to 110°) separated from the ethanolic mother liquors after concentration and cooling. Extraction of the original aqueous mother liquors with chloroform followed by recrystallisation from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°) gave a second crop (12·6 g.) of this material which on purification had m.p. 108 to 110° and proved to be 3-(o-*aminocarbonylphenoxy*)-1:2-*epoxypropane* (IV; R = H). Found: C, 61·7; H, 5·5; N, 7·1. $C_{10}H_{11}O_3N$ requires C, 62·2; H, 5·7; N, 7·3 per cent.

The foregoing diamide (6 g.) was suspended in water (100 ml.) containing sodium hydroxide (5 g.) and heated under reflux for 7 hours. Acidification with hydrochloric acid furnished 1:3-*bis*-(o-*carboxyphenoxy*)-*propan*-2-*ol* (III; R = R′ = CO_2H) which separated from ethanol in white needles, m.p. 170 to 171°. Found: C, 61·3; H, 5·0. $C_{17}H_{16}O_7$ requires C, 61·4; H, 4·9 per cent.

Condensation of sodium salicylate with 2:3-*epoxypropyl chloride in alkaline solution.* 2:3-Epoxypropyl chloride (28 g., 0·6 mole) was added to a solution of salicylic acid (69 g.) in water (200 ml.) containing sodium hydroxide (40 g., 2 moles). A little ethanol was added to make the mixture homogeneous when it was allowed to stand at room temperature for 2 days. Acidification with concentrated hydrochloric acid yielded a crude solid which could not be readily purified. It was collected, washed and dried at 100°.

Crude dry solid (93 g.) was dissolved in methanol (500 ml.) hydrogen chloride (10 g.) added and the solution heated under reflux for 4 hours to complete esterification.

The cooled reaction mixture was poured into water, extracted with chloroform, the extract washed acid-free and concentrated. The residue was heated at 100° at 0·1 mm. to remove methyl salicylate (34 g.). The high boiling residue weighed 48 g. An 18 g. portion of this was hydrolysed by heating on the steam bath with water (250 ml.) containing potassium hydroxide (9·3 g.). After cooling and acidification the solid was collected and washed with water, it had m.p. 168 to 170°, not depressed on admixture with a sample of the aforementioned dicarboxylic acid.

N-(2-*Hydroxy*-3-o-*aminocarbonylphenoxypropyl*)-*piperidine* (I; R = piperidino, R′ = NH_2). Piperidine (2·5 ml.) was added to a solution of 3-(*o*-aminocarbonylphenoxy)-1:2-epoxypropane (4·8 g.) in benzene (30 ml.) and the solution heated on the steam bath for 30 minutes. Slight dilution with light petroleum (b.p. 60 to 80°) furnished the *product* (5·5 g.) which crystallised from aqueous methanol in flat white needles, m.p. 167 to 168°. Found: C, 65·0; H, 8·0; N, 10·1. $C_{15}H_{22}O_3N_2$ requires C, 64·7; H, 8·0; N, 10·1 per cent.

The *hydrochloride dihydrate* separated from a mixture of ethanol and ethyl acetate in small deliquescent needles, m.p. 140 to 150°. Found:

C, 50·8; H, 7·2; N, 7·6. $C_{15}H_{23}O_3N_2Cl$; $2H_2O$ requires C, 51·3; H, 7·8; N, 8·0 per cent.

NN'-*bis*-(2-*Hydroxy*-3-o-*aminocarbonylphenoxypropyl*)-*piperazine* (V; R = H) was prepared by condensation of piperazine hexahydrate with 2 mole equivalents of 3-(*o*-aminocarbonylphenoxy)-1 : 2-epoxypropane in ethanolic solution. It separated from aqueous ethylene glycol in small white needles, m.p. 215 to 218°. Found: C, 60·7; H, 6·7; N, 11·8. $C_{24}H_{32}O_6N_4$ requires C, 61·0; H, 6·8; N, 11·9 per cent.

The *dihydrochloride* separated from 95 per cent ethanol in small white needles, m.p. 232 to 233°. Found: C, 52·8; H, 6·3; N, 10·4; Cl, 13·2. $C_{24}H_{34}O_6N_4Cl_2$ requires C, 52·8; H, 6·3; N, 10·3; Cl, 13·0 per cent.

N-(2-*Hydroxy*-3-o-*aminocarbonylphenoxypropyl*)-*succinimide* (I; R = succinimido, R' = H). A solution of 3-(*o*-aminocarbonylphenoxy)-1 : 2-epoxypropane (15·5 g.) and succinimide (8·8 g.) in ethanol (30 ml.) containing pyridine (5 drops) was heated under reflux for 5 hours. The product (22 g.) separated on cooling. It crystallised from ethanol in small white needles, m.p. 175 to 177°. Found: C, 57·3; H, 5·4; N, 9·6. $C_{14}H_{16}O_5N_2$ requires C, 57·5; H, 5·5; N, 9·8 per cent.

The foregoing product (18 g.) was dissolved in a mixture of ethanol 50 ml.) and concentrated hydrochloric acid (50 ml.) and heated under reflux for 12 hours. After removal of the solvent under reduced pressure, the residue was dissolved in water (50 ml.) and washed with two 50 ml. portions of ethyl acetate. The aqueous portion was again concentrated under reduced pressure and the residue crystallised from a mixture of ethanol and ethyl acetate to yield (2-*hydroxy*-3-o-*carboxyphenoxypropyl*)-*amine hydrochloride* (I; R = NH_2·HCl, R' = OH) in white needles, m.p. 150 to 154°. Found: C, 48·1; H, 5·7; N, 5·4; Cl, 14·3. $C_{10}H_{14}O_4NCl$ requires C, 48·5; H, 5·7; N, 5·7; Cl, 14·3 per cent.

N-(2-*Hydroxy*-3-o-*aminocarbonylphenoxypropyl*)-*phthalimide* (I; R = phthalimido, R' = H) was prepared as for the corresponding succinimido compound. It separated from ethanol in small white feathery crystals, m.p. 183°. Found: C, 63·3; H, 4·8; N, 8·3. $C_{18}H_{16}O_5N_2$ requires C, 63·5; H, 4·7; N, 8·2 per cent.

Treatment of the foregoing compound (23 g.) with 50 per cent hydrazine hydrate (8·5 g.) in boiling ethanol (250 ml.) for 1 hour yielded on cooling an intermediate which had m.p. 201 to 202° after collecting and washing with boiling ethanol (cf.[2]). Found: N, 15·2. $C_{18}H_{20}O_5N_4$ requires N, 15·1 per cent.

(2-*Hydroxy*-3-o-*aminocarbonylphenoxypropyl*)-*amine hydrochloride* (I; R = NH_2·HCl, R' = NH_2). The foregoing hydrazide was suspended in hot ethanol (250 ml.), concentrated hydrochloric acid (9 ml.) added and the mixture heated to boiling for a few minutes. After cooling the separated phthalyl hydrazine (10 g.) was removed. The filtrate was concentrated under reduced pressure and the solid residue crystallised from ethanol and then from a mixture of ethanol and ethyl acetate to yield small plates of 2-hydroxy-3-*o*-aminocarbonylphenoxypropylamine hydrochloride m.p. 162 to 166° (12 g.). Found: N, 11·3; Cl, 13·9. $C_{10}H_{15}O_3N_2Cl$ requires N, 11·4; Cl, 14·4 per cent.

Treatment of the foregoing hydrochloride with dilute sodium hydroxide and benzoyl chloride by the Schotten-Baumann method yielded N-(2-*hydroxy*-3-o-*aminocarbonylphenoxypropyl*)-*benzamide* (I; R = NHCOPh, R′ = NH_2) which separated from aqueous ethanol in small white needles, m.p. 162 to 163°. Found: C, 65·4; H, 6·0; N, 8·9. $C_{17}H_{18}O_4N_2$ requires C, 65·0; H, 5·8; N, 8·9 per cent.

Salicylethylamide (cf.[3]). To a cooled solution of phenyl salicylate (107 g., 0·5 mole) in benzene (100 ml.) a solution of ethylamine (45 g., 1·0 mole) in benzene (100 ml.) was added cautiously in portions. After the vigorous reaction had subsided the mixture was heated on the steam bath for 1 hour. Removal of the solvent and excess of amine, followed by distillation of the residual oil under reduced pressure yielded the *product* as an oil, b.p. 112° at 0·1 mm., which solidified and was crystallised from light petroleum (b.p. 60 to 80°) containing a little ethyl acetate, forming crystals, m.p. 62 to 63°. Found: C, 65·2; H, 6·5; N, 8·6. $C_9H_{11}O_2N$ requires C, 65·4; H, 6·7; N, 8·5 per cent.

Salicyl morpholineamide prepared by the method of Van Allan[3], crystallised from ethanol in needles, m.p. 181 to 183°. Found: C, 63·6; H, 6·3; N, 6·7. $C_{11}H_{13}O_3N$ requires C, 63·7; H, 6·3; N, 6·8 per cent.

Condensation of 2:3-*epoxypropyl chloride with salicyldiethylamide.* Salicyldiethylamide (65 g.) was dissolved in 2:3-epoxypropyl chloride (185 g., 6 mole equiv.) and a solution of sodium hydroxide (13·4 g., 1 mole equiv.) in water (200 ml.) added with stirring. Stirring at $< 20°$ was continued for 22 hours.

The oil was extracted with chloroform, the extract washed with water and concentrated under reduced pressure to remove excess of chloroform and 2:3-epoxypropyl chloride. A portion of the residue was distilled at 0·3 mm. yielding, (*a*) 3-(*diethylaminocarbonylphenoxy*)-1:2-*epoxypropane* (III; R = Et) as a pale yellow oil, b.p. 154°. Found: C, 67·1; H, 7·9; N, 5·7. $C_{14}H_{19}O_3N$ requires C, 67·4; H, 7·7; N, 5·6 per cent and (*b*) 3-(*diethylaminocarbonylphenoxy*)-*propan*-1:2-*diol* (II; R = R′ = Et), as a pale yellow viscous oil, b.p. 180°. Found: C, 63·1; H, 7·4; N, 5·6. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9; N, 5·2 per cent. A 10 g. portion of the original residue was dissolved in ethanol (10 ml.) and treated with a solution of piperazine hexahydrate (3·9 g.) in ethanol (10 ml.). After heating under reflux for 1 hour the solution was acidified with hydrochloric acid and the solvent removed under reduced pressure. The residual gum solidified slowly in a mixture of ethanol and ethyl acetate. On crystallisation from the same solvent mixture it yielded *NN′-bis-*(2-*hydroxy*-3-o-*diethylaminocarbonylphenoxypropyl*)-*piperazine dihydrochloride* (V; R = Et) in small needles, m.p. 213 to 214°. Found: C, 58·0; H, 7·8; N, 8·9; Cl, 10·7. $C_{32}H_{50}O_6N_4Cl_2$ requires C, 58·4; H, 7·7; N, 8·5; Cl, 10·8 per cent.

3-(o-*Aminocarbonylphenoxy*)-*propan*-1:2-*diol* (cf.[1]). To a stirred solution of salicylamide (68·5 g., 0·5 mole) in water (120 ml.) containing sodium hydroxide (20 g., 0·5 mole) was added 2:3-dihydroxypropyl chloride (60·5 g., 0·55 mole). Stirring at 20 to 25° was continued for 2 hours and then for 30 minutes at 50°. The *product* separated after cooling

to 0° for some hours. It crystallised from ethanol in needles, m.p. 140 to 142°. Found: C, 56·8; H, 6·4; N, 6·7. Calc. for $C_{10}H_{13}O_4N$: C, 56·9; H, 6·2; N, 6·6 per cent.

The same product was prepared by heating salicylamide (6·9 g.) with 2:3-epoxypropan-1-ol (4·5 g.) in benzene (10 ml.) containing pyridine (1 drop) for 16 hours.

3-(*Ethylaminocarbonylphenoxy*)-*propan*-1:2-*diol* (II; R = H, R′ = Et). A mixture of salicylethylamide (20·6 g.) and 2:3-epoxypropanol (9·2 g.) containing pyridine (1 drop) was heated on the steam bath for 6 hours and then at 110° for 1 hour. Direct distillation under reduced pressure yielded the *product* (18·5 g.) as a viscous oil, b.p. 215° at 0·1 mm. which solidified on standing. Found: C, 60·3; H, 7·0; N, 5·8. $C_{12}H_{17}O_4N$ requires C, 60·2; H, 7·2; N, 5·9 per cent.

3-(*n-Butylaminocarbonylphenoxy*)-*propan*-1:2-*diol* (II; R = H, R′ = *n*-Bu) separated from a mixture of ethylacetate and ether in small nodules, m.p. 85 to 87°. Found: C, 63·5; H, 7·9; N, 4·9. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9; N, 5·2 per cent.

3-(o-*Diethylaminocarbonylphenoxy*)-*propan*-1:2-*diol* (II; R = R′ = Et) was prepared by condensation of salicyldiethylamide with 2:3-epoxypropanol and obtained as a water-soluble, viscous oil, b.p. 180 to 185° at 0·3 mm. Found: C, 62·5; H, 8·0. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9 per cent. 3-(o-*Piperidinocarbonylphenoxy*)-*propan*-1:2-*diol* (II; NRR′ = piperidino) formed an orange-coloured viscous liquid, b.p. 216° at 0·3 mm. Found: C, 64·9; H, 7·4; N, 5·3. $C_{15}H_{21}O_4N$ requires C, 64·5; H, 7·6; N, 5·0 per cent.

1-(o-*Aminocarbonylphenoxy*)-3-(o-*diethylaminocarbonylphenoxy*)-*propan*-2-*ol* (III; R = $-CONH_2$, R′ = $-CONEt_2$). To a solution of 3-(*o*-aminocarbonylphenoxy)-1:2-epoxypropane (5 g.) and salicyldiethylamide (5 g.) in benzene (15 ml.) was added pyridine (2 drops) as catalyst and the solution heated on the steam bath for 3 hours. The solvent was removed and the gummy residue triturated with ethyl acetate. The solid obtained (8 g.) crystallised from a mixture of ethanol and ethyl acetate in fawn needles, m.p. 182 to 183°. Found: C, 65·1; H, 6·7; N, 7·2. $C_{21}H_{26}O_5N_2$ requires C, 65·3; H, 6·8; N, 7·3 per cent.

1-(o-*Aminocarbonylphenoxy*)-3-(o-*morpholinocarbonylphenoxy*)-*propan*-2-*ol* (III; R = $-CONH_2$, R′ = CO·N ). This was prepared by condensation of 3-(*o*-aminocarbonylphenoxy)-1:2-epoxypropane with salicoylmorpholine as described in the previous example. It crystallised from a mixture of methanol and ethyl acetate in small, hard prisms, m.p. 152 to 153°. Found: C, 62·7; H, 6·1; N, 6·8. $C_{21}H_{24}O_6N_2$ requires C, 63·0; H, 6·0; N, 7·0 per cent.

1-(o-*Aminocarbonylphenoxy*)-3-o-*toloxy-propan*-2-*ol* (III; R = $CONH_2$, R′ = Me). It was prepared by condensation of 3-(*o*-aminocarbonylphenoxy)-1:2-epoxypropane with *o*-cresol, or by the condensation of 3-*o*-toloxy-1:2-epoxypropane with salicylamide. It separated from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°) in needles,

m.p. 108 to 110°. Found: C, 68·1; H, 6·2; N, 4·5. $C_{17}H_{19}O_4N$ requires C, 67·8; H, 6·3; N, 4·7 per cent.

1-(o-*Diethylaminocarbonylphenoxy*)-3-o-*toloxy-propan*-2-*ol* (III; R = $CONEt_2$; R′ = Me) was prepared by condensation of 3-*o*-toloxy-1:2-epoxypropane with salicyldiethylamide employing pyridine as catalyst. It was obtained in high yield as a viscous oil, b.p. 210° at 0·1 mm. Found: C, 70·3; H, 7·5; N, 3·6. $C_{21}H_{27}O_4N$ requires C, 70·6; H, 7·6; N, 3·9 per cent.

1-(o-*Piperidinocarbonylphenoxy*)-3-o-*toloxypropan*-2-*ol* (III; R = CON⟨⟩, R′ = Me) was obtained as a very viscous liquid of b.p. 236° at 0·2 mm. Found: C, 71·6; H, 7·2; N, 3·6. $C_{22}H_{27}O_4N$ requires C, 71·5; H, 7·4; N, 3·8 per cent.

1-(o-*Aminocarbonylphenoxy*)-3-p-*chlorophenoxypropan*-2-*ol* crystallised from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°) in small, hard white crystals, m.p. 118 to 120°. Found: C, 59·8; H, 5·1; N, 4·4; Cl, 10·9. $C_{16}H_{16}O_4NCl$ requires C, 59·7; H, 5·0; N, 4·4; Cl, 11·0 per cent.

1-(o-*Methoxycarbonylphenoxy*)-3-o-*toloxy-propane*-2-*ol* (III; R = CO_2Me, R′ = Me). A mixture of 3-*o*-toloxy-1:2-epoxypropane (82 g.), methyl salicylate (76 g.) and pyridine (10 drops) was heated at 150° for 3 hours. After removal of unchanged material (125 g.) by distillation, the product (26 g.) was obtained as a viscous oil, b.p. 210° at 0·5 mm. Found: C, 68·7; H, 6·4. $C_{18}H_{20}O_5$ requires C, 68·3; H, 6·4 per cent.

2-*Hydroxy*-3-o-*methoxycarbonylphenoxypropyl chloride* (I; R = Cl, R′ = OMe). A mixture of methyl salicylate (152 g.) and 2:3-epoxypropyl chloride (278 g., 3 mole equiv.) containing piperidine hydrochloride (4 g.) was heated on the steam bath for 24 hours (cf.[4]). Excess of 2:3-epoxypropyl chloride, methyl salicylate and 1:3-dichloro-propan-2-ol were removed by heating on the steam bath at 1 mm. pressure. The residual oil was dissolved in an equal volume of chloroform and shaken with concentrated hydrochloric acid (20 ml.) for 1 minute with cooling. The chloroform extract was washed with water until neutral, the extract concentrated and the residual oil distilled under reduced pressure. The *product* (106·5 g.) was obtained as a fluorescent oil, b.p. 128° at 0·1 mm. Found: Cl, 14·3. $C_{11}H_{13}O_4Cl$ requires Cl, 14·5 per cent. High boiling products were formed in the reaction.

3-(o-*Methoxycarbonylphenoxy*)-*propane*-1:2-*diol* (I; R = H, R′ = OMe). A mixture of methyl salicylate (38 g.) and 2:3-epoxypropanol (18·5 g.) containing pyridine (3 drops) was heated on the steam bath for 16 hours. Distillation under reduced pressure yielded the *product* (18 g.), b.p. 160° at 0·5 mm. Found: C, 58·2; H, 5·8. $C_{11}H_{14}O_5$ requires C, 58·4; H, 6·2 per cent.

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ANALGESICS. PART IV. SOME 3-ARYLOXY-1-Δ^3-PIPERIDEINOPROPAN-2-OL DERIVATIVES

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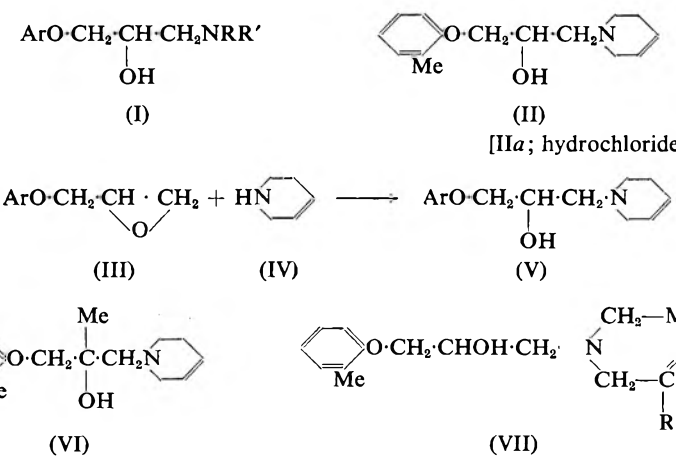
The synthesis of some 3-aryloxy-1-Δ^3-piperideinopropan-2-ol derivatives and related types is described. Their biological study has led to the selection of 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol hydrochloride, Tolpronine, for fuller evaluation as an analgesic agent.

BIOLOGICAL study of the aryloxypropanolamines (I) described in Part I (preceeding paper) showed that, in general, analgesic activity increased in passing from alkyl- and dialkylamino-derivatives (I; R = H or alkyl, R′ = alkyl) to cyclic structures in which NRR′ was piperidino, pyrrolidino or morpholino. The fortuitous observation that a still more active compound resulted from replacement of piperidino by Δ^3-piperideino in one of the more potent earlier types led to the preparation of the 3-aryloxy-1-Δ^3-piperideinopropan-2-ol derivatives (V) described herein and to the ultimate selection of 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol hydrochloride (Tolpronine)[1] (II*a*) for detailed pharmacological study.

The required 3-aryloxy-1-Δ^3-piperideinopropan-2-ol derivatives (V) were prepared by condensation of the appropriate 3-aryloxy-1:2-epoxypropane (III) with a slight excess of Δ^3-piperideine (IV) in an organic



solvent like benzene, toluene, light petroleum or ethanol. Reaction was exothermic and rapid, but was readily controlled by water cooling or by controlled addition of the glycide ether to the Δ^3-piperideine. The products were isolated by distillation under reduced pressure or directly by crystallisation. The phenyl-, *o*-chlorophenyl-, *o*-fluorophenyl-, *o*-allylphenyl-, *o*-*n*-butoxyphenyl, 2-methoxy-4-propenylphenyl- and 2-methoxy-4-allylphenyl analogues of (II) were all obtained in this way.

In addition, 2-methyl-1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol (VI), the 2-methyl homologue of the active base (II), was prepared by the condensation of 1:2-epoxy-2-methyl-3-*o*-toloxypropane with Δ^3-piperideine (IV).

Some compounds of type (VII) in which the Δ^3-piperideino-moiety characteristic of (II) is replaced by an open-chain type of structure were also prepared. These were synthesised by condensing 1:2-epoxy-3-*o*-toloxypropane with ethyl allylamine, ethyl 2-methallylamine and diallylamine, respectively. The first two compounds were also obtained from 1-ethylamino-3-*o*-toloxypropan-2-ol by reaction with allyl chloride and with 2-methallyl chloride.

Quaternation of 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol (II) with 3-*o*-chlorophenoxy-2-hydroxypropyl chloride surprisingly led to the formation of two isomeric compounds which are probably geometrical isomers of type (VIII; R = *o*-Me·C_6H_4·O·CH_2·CHOH·CH_2·, R′ = *o*-Cl·C_6H_4·O·CH_2·CHOH·CH_2·).

(VIII)

Quaternation of the base (II) with benzyl chloride led likewise to the formation of two isomers (VIII; R = *o*-Me·C_6H_4·O·CH_2·CHOH·CH_2·, R′ = Ph·CH_2·), also formed, albeit in low yield, by the condensation of *N*-benzyl-Δ^3-piperideine with 2-hydroxy-3-*o*-toloxypropyl chloride.

1-Δ^3-Piperideino-3-*o*-toloxypropan-2-ol (II) (*vide supra*) crystallised from light petroleum in white needles, m.p. 62 to 64°. It was converted into its salts with inorganic and organic acids and further transformed into its *O*-esters which were isolated as their hydrochlorides. Biological study of these derivatives led to the selection of the hydrochloride, Tolpronine (II*a*), for fuller evaluation. This salt crystallised from ethylene dichloride or from mixtures of ethanol and ether in white needles, m.p. 136 to 137°. It was readily soluble in half its weight of water to give solutions of pH about 5. These could be neutralised with sodium bicarbonate solution without precipitation of the base.

Experimental

Melting points are uncorrected.

1-Δ^3-*Piperideino*-3-o-*toloxypropan*-2-*ol* (II). (1) A mixture of 3-*o*-toloxy-1:2-epoxypropane (246 g.) and Δ^3-piperideine (137 g.; 1·1 mole equiv.) in toluene (400 ml.) was heated under reflux on the steam bath until the exothermic reaction had started when slight cooling was applied as required. The reaction was completed by heating for 30 minutes. The mixture was cooled, washed twice with water to remove excess of Δ^3-piperideine and taken to dryness under reduced pressure. The solid residue was crystallised from light petroleum (b.p. 40 to 60°) to give 1-Δ^3-*piperideino*-3-o-*toloxypropan*-2-*ol*, needles, m.p. 62 to 64°, b.p. 136° at 0·3 mm. Found: C, 72·8; H, 8·2; N, 5·9. $C_{15}H_{21}O_2N$ requires C, 72·8; H, 8·6; N, 5·7 per cent.

(2) 3-Δ^3-Piperideino-1:2-epoxypropane (13·9 g.) and *o*-cresol (10·8 g.) were heated on the steam bath for 10 hours and the product distilled directly at reduced pressure. The main fraction (15 g.) b.p. 134 to 138° at 0·3 mm. was redistilled to yield the base (II), b.p. 134° at 0·3 mm. (12·1 g.).

The *hydrochloride* separated from ethanol:ether in plates, m.p. 136 to 137°. Found: C, 63·5; H, 7·6; N, 4·6; Cl, 13·0. $C_{15}H_{22}O_2NCl$ requires C, 63·5; H, 7·8; N, 4·9; Cl, 12·5 per cent. The *picrate*, m.p. 134 to 135°, crystallised from ethyl acetate containing a trace of ethanol. Found: N, 11·8. $C_{21}H_{24}O_9N_4$ requires N, 11·8 per cent. The *hydrobromide* had m.p. 124 to 126° after crystallisation from ethyl acetate containing a trace of ethanol. Found: N, 4·4. $C_{15}H_{22}O_2NBr$ requires N, 4·3 per cent. The *benzoate*, m.p. 108 to 110°, separated from ethanol:ether. Found: N, 3·5. $C_{22}H_{27}O_4N$ requires N, 3·8 per cent. The *salicylate*, crystallised from ethanol in needles, m.p. 144 to 145°. Found: C, 68·1; H, 6·9; N, 3·4. $C_{22}H_{27}O_5N$ requires C, 68·5; H, 7·1; N, 3·6 per cent. The *acetylsalicylate*, separated from ethyl acetate:light petroleum (b.p. 60 to 80°) in needle clusters, m.p. 119°. Found: C, 67·7; H, 6·7; N, 3·3. $C_{24}H_{29}O_6N$ requires C, 67·4; H, 6·8; N, 3·3 per cent.

The 4-*hydroxy*-iso*phthalate* crystallised from ethyl acetate:ether in needles, m.p. 78 to 80°. Found: C, 63·7; H, 6·4; N, 3·1. $C_{23}H_{27}O_7N$ requires C, 64·3; H, 6·3; N, 3·3 per cent. The *acid malonate* crystallised from ethyl acetate in needles, m.p. 105 to 106°. Found: C, 61·7; H, 7·2. $C_{18}H_{25}O_6N$ requires C, 61·5; H, 7·2 per cent. The *ethyl hydrogen malonate salt* separated from ethanol:ether in prisms m.p. 136 to 137°. Found: C, 63·7; H, 7·8; N, 3·7. $C_{20}H_{29}O_6N$ requires C, 63·3; H, 7·7; N, 3·7 per cent.

1-Δ^3-*Piperideino*-2-*acetoxy*-3-o-*toloxypropane*. A solution of 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol (49·4 g.) in acetic anhydride (200 ml.) was heated under reflux for 5 hours when excess of acetic anhydride was distilled off at reduced pressure. The residue was fractionated at 0·5 mm. to yield the *product* (37·1 g.) as an oil, b.p. 126° at 0·1 mm. Found: C, 70·8; H, 7·9; N, 4·7. $C_{17}H_{23}O_3N$ requires C, 70·5; H, 8·0; N, 4·8 per cent.

1-Δ^3-*Piperideino*-2-*acetoxy*-3-o-*toloxypropane hydrochloride*. A solution of 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol (49·4 g.) in benzene (200 ml.) was treated with shaking with acetyl chloride (15·7 g.). Rapid and copious separation of solids occurred. These were collected, washed with ether and purified from ethylene dichloride, to give the *hydrochloride*, needles, m.p. 175°. Found: C, 63·1; H, 7·1; N, 3·9; Cl, 10·8. $C_{17}H_{24}O_3NCl$ requires C, 62·6; H, 7·4; N, 4·3; Cl, 10·9 per cent.

The product was readily soluble in water yielding a neutral solution. Its (10 g.) hydrolysis with 0·1N ethanolic hydrochloric acid (105 ml.) for 2 hours on the steam bath yielded after concentration 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol hydrochloride, m.p. 133 to 135° not depressed on admixture with an authentic specimen.

1-Δ^3-*Piperideino*-2-*propionoxy*-3-o-*toloxypropane hydrochloride*. A solution of 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol (49·4 g.) in benzene

(300 ml.) was treated gradually with a solution of propionyl chloride (20·4 g., 1·1 mole equiv.) in benzene (30 ml.). The mixture was heated under reflux for 2 hours. The *product* which separated on cooling was collected, washed with ether and purified by crystallisation from ethylene dichloride: ethyl acetate, forming plates, m.p. 139 to 140°. Found: C, 64·1; H, 7·8; N, 4·3. $C_{18}H_{26}O_3NCl$ requires C, 63·6; H, 7·7; N, 4·1 per cent.

1-Δ^3-*Piperideino*-2-n-*butyroxy*-3-o-*toloxypropane hydrochloride* separated from ethanol: ether in white needles, m.p. 114 to 115°. Found: C, 64·5; H, 7·8; N, 4·0; Cl, 10·2. $C_{19}H_{28}O_3NCl$ requires C, 64·5; H, 8·0; N, 4·0; Cl, 10·0 per cent.

1-Δ^3-*Piperideino*-2-iso*butyroxy*-3-o-*toloxypropane hydrochloride* crystallised from ethyl acetate containing a trace of ethanol, in needles, m.p. 122 to 124°. Found: C, 64·3; H, 7·9; N, 3·9; Cl, 10·1. $C_{19}H_{28}O_3NCl$ requires C, 64·5; H, 8·0; N, 4·0; Cl, 10·0 per cent.

Bis carbonate ester of 1-Δ^3-*piperideino*-3-o-*toloxypropan*-2-*ol*. To 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol (28·6 g.) in dry benzene (150 ml.) was added, in portions with shaking, a solution of phosgene (5·8 g.) in dry benzene (50 ml.). Reaction was completed by heating the mixture on the steam bath for 30 mins. After cooling, the solid was collected and washed with ether (24·9 g., m.p. 198 to 200° [decomp.]). Crystallisation from methanol: ether gave the *ester*, m.p. 204 to 205°. Found: C, 62·6; H, 7·3; N, 4·9. $C_{31}H_{42}O_5N_2Cl_2$ requires C, 62·7; H, 7·1; N, 4·7 per cent.

1-Δ^3-*Piperideino*-2-*phenylacetoxy*-3-o-*toloxypropane hydrochloride* crystallised from ethylene dichloride: ethyl acetate and had m.p. 129 to 130°. Found: C, 68·4; H, 7·0; N, 3·1. $C_{23}H_{28}O_3NCl$ requires C, 68·7; H, 7·0; N, 3·5 per cent.

1-Δ^3-*Piperideino*-2-*ethoxycarbonyloxy*-3-o-*toloxypropane hydrochloride* was prepared by reaction of 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol with ethyl chloroformate in benzene solution and crystallised from ethyl acetate in needles, m.p. 143 to 144°. Found: C, 60·7; H, 7·3; N, 3·9; Cl, 9·9. $C_{18}H_{26}O_4NCl$ requires C, 60·7; H, 7·4; N, 3·9; Cl, 10·0 per cent.

1-*Piperidino*-2-*ethoxycarbonyloxy*-3-o-*toloxypropane hydrochloride* needles from ethyl acetate, m.p. 131 to 133° were prepared by reaction of 1-piperidino-3-*o*-toloxypropan-2-ol with ethyl chloroformate. Found: C, 60·8; H, 7·9; N, 3·6; Cl, 9·7. $C_{18}H_{28}O_4NCl$ requires C, 60·4; H, 7·9; N, 3·9; Cl, 9·9 per cent.

1-Δ^3-*Piperideino*-3-*phenoxypropan*-2-*ol* (V; Ar = Ph) formed a pale yellow oil, b.p. 132° at 0·2 mm. Found: C, 72·0; H, 8·1; N, 5·8. $C_{14}H_{19}O_2N$ requires C, 72·0; H, 8·2; N, 6·0 per cent. The *hydrochloride* crystallised from ethanol: ether in needles, m.p. 132°. Found: C, 62·4; H, 8·0; N, 4·9; Cl, 12·9. $C_{14}H_{20}O_2NCl$ requires C, 62·3; H, 7·5; N, 5·2; Cl, 13·2 per cent.

1-Δ^3-*Piperideino*-3-o-*chlorophenoxypropan*-2-*ol* (V; Ar = *o*-Cl·C_6H_4) crystallised from light petroleum (b.p. 60 to 80°) in needles, m.p. 69 to 71°. Found: N, 4·9. $C_{14}H_{18}O_2NCl$ requires N, 5·2 per cent. The *acetylsalicylate* separated from ethanol: ether and had m.p. 97 to 98°. Found: C, 62·2; H, 5·8; N, 2·9; Cl, 7·6. $C_{23}H_{26}O_6NCl$ requires C, 61·6; H, 5·9;

N, 3·1; Cl, 7·9 per cent. The *salicylate* had m.p. 118 to 119° after crystallisation from ethyl acetate. Found: N, 3·4; Cl, 8·4. $C_{21}H_{24}O_5NCl$ requires N, 3·5; Cl, 8·8 per cent.

1-Δ^3-*Piperideino*-3-o-*fluorophenoxypropan*-2-*ol* (V; Ar = *o*-F·C_6H_4) of b.p. 132° at 0·5 mm. it had m.p. 46 to 48° after crystallisation from light petroleum (b.p. 40 to 60°). Found: C, 67·5; H, 7·2; N, 5·4. $C_{14}H_{18}O_2NF$ requires C, 66·9; H, 7·2; N, 5·6 per cent. The *hydrochloride* crystallised from ethanol:ether in needles, m.p. 115 to 117°. Found: N, 5·0. $C_{14}H_{19}O_2NClF$ requires N, 4·9 per cent.

3-o-*Butoxyphenoxy*-1:2-*epoxypropane* (III; Ar = *o*-BuO·C_6H_4) formed an oil, b.p. 100 to 102° at 0·05 mm. Found: C, 70·1; H, 8·6. $C_{13}H_{18}O_3$ requires C, 70·2; H, 8·2 per cent.

1-Δ^3-*Piperideino*-3-o-*butoxyphenoxypropan*-2-*ol* (V; Ar = *o*-BuO·C_6H_4) was prepared by condensation of the foregoing epoxide with Δ^3-piperideine in benzene solution. After crystallisation from light petroleum (b.p. 60 to 80°) the *product* formed in prisms, m.p. 46°, b.p. 146° at 0·05 mm. Found: C, 71·0; H, 9·1; N, 4·7. $C_{18}H_{27}O_3N$ requires C, 70·8; H, 8·9; N, 4·6 per cent.

3-o-*Allylphenoxy*-1:2-*epoxypropane*. Prepared by condensation of *o*-allylphenol with 2:3-epoxypropyl chloride in aqueous alkali, it formed an oil, b.p. 99 to 100° at 0·6 mm. Found: C, 75·8; H, 7·8. $C_{12}H_{14}O_2$ requires C, 75·8; H, 7·4.

2-*Hydroxy*-3-o-*allylphenoxypropyl chloride*. Isolated in low yield from the same reaction, it had b.p. 122 to 124° at 0·5 mm. Found: C, 63·6; H, 6·5; Cl, 15·4. $C_{12}H_{15}O_2Cl$ requires C, 63·6; H, 6·7; Cl, 15·6 per cent.

1-Δ^3-*Piperideino*-3-o-*allylphenoxypropan*-2-*ol* (V; Ar = *o*-$C_3H_5O·C_6H_4$) had b.p. 164 to 166° at 0·8 mm. Found: N, 5·0. $C_{17}H_{23}O_2N$ requires N, 5·1 per cent. The *hydrochloride* crystallised from ethyl acetate containing a trace of methanol in needles, m.p. 125 to 126°. Found: C, 66·1; H, 7·8; N, 4·3; Cl, 11·1. $C_{17}H_{24}O_2NCl$ requires C, 65·9; H, 7·8; N, 4·5; Cl, 11·4 per cent.

1-Δ^3-*Piperideino*-3-p-*aminophenoxypropan*-2-*ol* (V; Ar = *p*-$NH_2·C_6H_4$). To a solution of 3-*p*-acetamidophenoxy-1:2-epoxypropane (20·7 g.) in warm ethanol (40 ml.) was added Δ^3-piperideine (8·3 g.). A slight exothermic reaction occurred which was completed by heating the mixture for 1 hour. Removal of volatile material at reduced pressure left a *gum* which was hydrolysed by heating under reflux with ethanol (25 ml.) and concentrated hydrochloric acid (20 ml.) for 2 hours. Concentration at reduced pressure and treatment with ethanol gave the *hydrochloride*, buff-coloured nodules, m.p. 252 to 253° (decomp.) from methanol:ethyl acetate. Found: C, 52·0; H, 7·0; N, 8·7; Cl, 22·2. $C_{14}H_{22}O_2N_2Cl_2$ requires C, 52·3; H, 6·9; N, 8·7; Cl, 22·1 per cent.

3-(2′-*Methoxy*-4′-*propenyl*)-*phenoxy*-1:2-*epoxypropane*. 2:3-Epoxypropyl chloride (139 g., 1·5 mole) was added in one portion to a stirred solution of *iso*eugenol (164 g.) in N potassium hydroxide (1 litre) at 20° and the mixture stirred for 4 hours. A further quantity of potassium hydroxide (11·2 g.) in water (100 ml.) was then added and stirring continued for a further 4 hours. The oily layer was removed, the aqueous

layer extracted with chloroform and the combined extracts washed with water. After removal of the chloroform the residual oil was distilled at 0·6 mm. to yield fractions: (i) 136·2 g., b.p. 134°; (ii) 34·8 g., b.p. 134 to 190°; (iii) 23·5 g., b.p. 240°. The yield of the last fraction was greater than indicated but distillation was stopped owing to the high boiling point.

Fraction (i) was purified from ethyl acetate: light petroleum (b.p. 40 to 60°) to give 3-(2′-*methoxy*-4′-*propenyl*)-*phenoxy*-1 : 2-*epoxypropane*, small needles, m.p. 59 to 60°. Found: C, 71·4; H, 7·5. $C_{13}H_{16}O_3$ requires C, 70·9; H, 7·3 per cent.

Fraction (iii) crystallised from ethyl acetate to give 1 : 3-bis-(2′-*methoxy*-4′-*propenyl*)-*phenoxypropan*-2-*ol* in nodules of needles, m.p. 86 to 87°. Found: C, 71·8; H, 7·3. $C_{23}H_{28}O_5$ requires C, 71·9; H, 7·4 per cent.

Fraction (ii) was a mixture of the foregoing epoxide and the corresponding chlorohydrin. It was hydrolysed by heating under reflux with sodium carbonate (20 g.) in water (300 ml.) for 12 hours. The resulting oil was isolated with chloroform and distilled at 0·1 mm. to yield 3-(2′-*methoxy*-4′-*propenyl*)-*phenoxypropane*-1 : 2-*diol*, b.p. 164°, needles, m.p. 90 to 91° after crystallisation from ethyl acetate. Found: C, 65·3; H, 7·7. $C_{13}H_{18}O_4$ requires C, 65·5; H, 7·6 per cent.

1-Δ^3-*Piperideino*-3-(2′-*methoxy*-4′-*propenyl*)-*phenoxypropan*-2-*ol* was prepared by condensation of the foregoing epoxide with Δ^3-piperideine, b.p. 200° at 0·6 mm., needles, m.p. 71 to 72° after crystallisation from light petroleum (b.p. 60 to 80°). Found: C, 70·9; H, 8·2; N, 4·5. $C_{18}H_{25}O_3N$ requires C, 71·3; H, 8·3; N, 4·6 per cent.

3-(2′-*Methoxy*-4′-*allyl*)*phenoxy*-1 : 2-*epoxypropane*. Prepared by condensation of 2 : 3-epoxypropyl chloride with eugenol, it formed an oil, b.p. 110° at 0·05 mm., which solidified on standing. Found: C, 71·1; H, 7·3. $C_{13}H_{16}O_3$ requires C, 70·9; H, 7·3 per cent.

1-Δ^3-*Piperideino*-3-(2′-*methoxy*-4′-*allyl*)-*phenoxypropan*-2-*ol* separated from light petroleum (b.p. 40 to 60°) in needles, m.p. 51 to 52°, b.p. 182 to 184° at 0·4 mm. Found: C, 71·1; H, 8·2; N, 4·5. $C_{18}H_{25}O_3N$ requires C, 71·3; H, 8·3; N, 4·6 per cent.

1-Δ^3-*Piperideino*-2-*methyl*-3-o-*toloxypropan*-2-*ol* (VI) was obtained by condensation of 3-*o*-toloxy-2-methyl-1 : 2-epoxypropane (10 g.) with Δ^3-piperideine (5·1 g.) in light petroleum (50 ml., b.p. 60 to 80°) under reflux for 3 hours. The *product* formed an oil, b.p. 128 to 130° at 0·5 mm. Found: C, 73·1; H, 8·9; N, 5·2. $C_{16}H_{13}O_2N$ requires C, 73·5; H, 8·9; N, 5·4 per cent. The *hydrochloride* had m.p. 155 to 157° after crystallisation from ethanol : ether. Found: C, 64·5; H, 8·2; N, 4·6; Cl, 12·1. $C_{16}H_{24}O_2NCl$ requires C, 64·5; H, 8·1; N, 4·7; Cl, 11·9 per cent.

1-(N-*Allyl*-N-*ethyl*)*amino*-3-o-*toloxypropan*-2-*ol* (VII; R = H). (*a*) Condensation of 3-*o*-toloxy-1 : 2-epoxypropane with *N*-allyl-*N*-ethylamine in benzene solution yielded the *base* as an oil, b.p. 112° at 0·05 mm. Found: C, 71·8; H, 8·9; N, 5·8. $C_{15}H_{23}O_2N$ requires C, 72·2; H, 9·3; N, 5·6 per cent. The *hydrochloride* crystallised from benzene : ether in hygroscopic needles m.p. 60°. Found: C, 62·6; H, 8·5; N, 4·9. $C_{15}H_{24}O_2NCl$ requires C, 63·0; H, 8·5; N, 4·9 per cent. The *picrate*

crystallised from ethyl acetate, had m.p. 123°. Found: C, 53·1; H, 5·6; N, 12·2. $C_{21}H_{26}O_9N_4$ requires C, 52·7; H, 5·5; N, 11·7 per cent.

(*b*) To a solution of 1-ethylamino-3-*o*-toloxypropan-2-ol (41·8 g.) in ethanol (100 ml.) was added allyl chloride (23 g.) and anhydrous sodium carbonate (21·2 g.) and the mixture heated on the steam bath for 15 hours. After concentration and addition of water the residual oil was isolated with chloroform, and distilled at reduced pressure to yield the base b.p. 135° to 140° at 1·0 mm., identified by conversion to the *picrate* m.p. 123° not depressed in admixture with a sample prepared by method (*a*).

N-*Ethyl*-N-2-*methallylamine*, b.p. 102° formed a *hydrochloride* which crystallised from ethyl acetate in shining leaflets, m.p. 182 to 183°. Found: C, 53·5; H, 10·3; N, 10·0; Cl, 25·7. $C_6H_{14}NCl$ requires C, 53·1; H, 10·4; N, 10·3; Cl, 26·2 per cent.

1-(N-*Ethyl*-N-2′-*methallyl*)*amino*-3-o-*toloxypropan*-2-*ol* (VII; R = Me). (*a*) Condensation of 3-*o*-toloxy-1:2-epoxypropane with *N*-ethyl-*N*-2-methallylamine yielded the *base* as an oil b.p. 118° at 0·1 mm. Found: C, 72·5; H, 9·2; N, 5·0. $C_{16}H_{25}O_2N$ requires C, 72·9; H, 9·6; N, 5·3 per cent. The *hydrochloride* had m.p. 102 to 103° after crystallisation from ethyl acetate:light petroleum (b.p. 60 to 80°). Found: C, 63·8; H, 8·5. $C_{16}H_{26}O_2NCl$ requires C, 64·1; H, 8·8 per cent. The *picrate* separated from ethyl acetate:light petroleum (b.p. 40 to 60°) in yellow crystals, m.p. 93 to 95°. Found: C, 53·2; H, 5·5; N, 11·2. $C_{22}H_{28}O_9N_4$ requires C, 53·6; H, 5·7; N, 11·4 per cent.

(*b*) To 1-ethylamino-3-*o*-toloxypropan-2-ol (41·8 g.) and 2-methallyl chloride (19·9 g.) in ethanol (125 ml.) was added potassium hydroxide (11·2 g.) in water (10 ml.) and the mixture heated for 10 hours. The product, isolated with chloroform formed an oil, b.p. 120° at 0·5 mm., identified by conversion to the *hydrochloride* identical with that described under (*a*).

1-*Diallylamino*-3-o-*toloxypropan*-2-*ol*. Prepared by condensation of 3-*o*-toloxy-1:2-epoxypropane with diallylamine, it had b.p. 119° at 0·1 mm. Found: N, 5·4. $C_{16}H_{23}O_2N$ requires N, 5·4 per cent. The *hydrochloride* had m.p. 86 to 87° after crystallisation from a mixture of benzene:ether. Found: C, 64·5; H, 8·1; N, 4·7. $C_{16}H_{24}O_2NCl$ requires C, 64·5; H, 8·0; N, 5·1 per cent.

1-*Diallylamino*-3-o-*allylphenoxypropan*-2-*ol*. Prepared by condensation of 3-*o*-allylphenoxy-1:2-epoxypropane with diallylamine, it had b.p. 148 to 151° at 0·3 mm. Found: C, 75·0; H, 8·8; N, 4·8. $C_{18}H_{25}O_2N$ requires C, 75·2; H, 8·8; N, 4·9 per cent.

N-*bis*-(2-*Hydroxy*-3-o-*toloxypropyl*)-Δ^3-*piperideino chloride*. A mixture of 2-hydroxy-3-*o*-toloxypropyl chloride (8 g.) and 1-Δ^3-piperideino-3-*o*-toloxypropan-2-ol (10 g.) was heated on the steam bath for 20 hours. The resultant gum was dissolved in a minimum of ethanol and the solution diluted to turbidity with ethyl acetate, when the *product* separated on cooling. After crystallisation from a mixture of ethanol and ethyl acetate it had m.p. 188 to 189°. Found: C, 66·6; H, 7·4; N, 2·7. $C_{25}H_{34}O_4NCl$ requires C, 67·0; H, 7·7; N, 3·1 per cent. The *iodide* had m.p. 170° after

crystallisation from a mixture of ethanol and ether. Found: C, 55·6; H, 6·0; N, 2·8. $C_{25}H_{34}O_4NI$ requires C, 55·6; H, 6·4; N, 2·6 per cent.

N - (2 - *Hydroxy* - 3 - o - *chlorophenoxypropyl*) - N - (2 - *hydroxy* - 3 -o-*toloxypropyl*)-Δ^3-*piperideino chloride*. A mixture of 2-hydroxy-3-*o*-chlorophenoxypropyl chloride (22·1 g.) and 1-Δ^3-piperideino-3-*o*-toloxy-propan-2-ol (24·7 g.) was heated on the steam bath for 10 hours. The resultant gum was dissolved in the minimum of hot ethanol and the solution diluted to turbidity with ethyl acetate. The first crop of solid which separated (A) had m.p. (144°) 152 to 154° (12·7 g.). Concentration of the filtrate and cooling yielded a second crop of solid (B), m.p. (156°) 160 to 166° (7·5 g.). *Isomer A*, after four crystallisations from ethanol: ethyl acetate had m.p. 172 to 174°. The m.p. was not raised by a further crystallisation from ethylene dichloride in which the salt was sparingly soluble. Found: C, 61·9; H, 6·7; N, 2·9. $C_{24}H_{31}O_4NCl_2$ requires C, 61·5; H, 6·7; N, 3·0 per cent. It formed a *picrate* which separated from a small volume of ethanol in minute yellow needles, m.p. 139 to 141°. Found: C, 54·3; H, 4·9; N, 8·2. $C_{30}H_{33}O_{11}N_4Cl$ requires C, 54·5; H, 5·0; N, 8·5 per cent. *Isomer B* had m.p. 176 to 178° after three recrystallisations from ethanol:ethyl acetate and one crystallisation from ethylene dichloride. Found: C, 61·8; H, 6·7; N, 3·0. $C_{24}H_{31}O_4NCl_2$ requires C, 61·5; H, 6·7; N, 3·0 per cent. The *picrate* separated from ethanol in light yellow nodules of needles, m.p. 166 to 167° C. Found: C, 54·7; H, 4·9; N, 8·1. $C_{30}H_{33}O_{11}N_4Cl$ requires C, 54·5; H, 5·0; N, 8·5 per cent. Mixtures of isomers A and B had m.p. 152 to 160°.

N-*Benzyl*-N-(2-*hydroxy*-3-o-*toloxypropyl*)-Δ^3-*piperideino chloride*. (*a*) 1-Δ^3-Piperideino-3-*o*-toloxypropan-2-ol (49·4 g.) and benzyl chloride (25·3 g.) were warmed on the steam bath. The temperature of the mixture rose rapidly to 135° and ethanol (60 ml.) was stirred in carefully at this stage, followed by ethyl acetate (200 ml.). The mixture was left at room temperature for 24 hours when it deposited solid (A) m.p. 172 to 176° (54·7 g.). Concentration of the mother liquors yielded a solid (B) m.p. 162 to 168° (11·7 g.).

Solid (*A*) dissolved in ethylene dichloride (1200 ml.) and left at 0 to 5° overnight deposited a product (23 g.; m.p. 188 to 190°) which after crystallisation from the same solvent had m.p. 190 to 191° (isomer "A"). Found: C, 70·6; H, 7·7; N, 3·6; Cl, 9·3. $C_{22}H_{28}O_2NCl$ requires C, 70·7; H, 7·6; N, 3·8; Cl, 9·5 per cent., λmax 264 mμ (1580), 270 mμ (1947) and 277 mμ (1525) (in ethanol).

It formed an *iodide* which crystallised as the hemihydrate from ethanol in prisms, m.p. 157 to 159°. Found: C, 55·6, 55·4; H, 6·5, 6·1; N, 3·1; I, 27·5. $C_{22}H_{28}O_2NI$; $\frac{1}{2}H_2O$ requires C, 55·7; H, 6·2; N, 3·0; I, 26·8 per cent.

The original mother liquors after the removal of *isomer A* were concentrated to about 250 ml. and diluted with an equal volume of ethyl acetate. After standing at room temperature for one day, the product (m.p. 170 to 175°; 30·2 g.) was collected. Repeated crystallisation from ethylene dichloride gave *isomer B*, m.p. 171 to 173° (11·2 g.). *Solid* (*B*) crystallised twice from ethylene dichloride yielded a further crop of

isomer B m.p. 171 to 173° (4·3 g.), λmax 264 mμ (1525), 270 mμ (1898) and 277 mμ (1476) (in ethanol). Found: C, 70·4; H, 7·6; N, 3·6; Cl, 9·2. $C_{22}H_{28}O_2NCl$ requires C, 70·7; H, 7·6; N, 3·8; Cl, 9·5 per cent. The *iodide* separated from ethanol in small crystals, m.p. 176 to 177°. Found: C, 56·3; H, 6·3; N, 3·1; I, 27·2. $C_{22}H_{28}O_2NI$ requires C, 56·7; H, 6·1; N, 3·0; I, 27·3 per cent. Mixtures of isomers A and B had m.p. < 160°.

(*b*) A mixture of *N*-benzyl-Δ^3-piperideine (17·3 g.) and 2-hydroxy-3-*o*-toloxypropyl chloride (20·1 g.) was heated on the steam bath for 40 hours. The semicrystalline residue was dissolved in a small amount of ethanol and diluted with hot ethyl acetate. After allowing to stand overnight the solid was collected, m.p. 170 to 180° (7·5 g.). Fractionation from ethylene dichloride yielded *isomer A* (2·6 g., m.p. 188 to 190°) and *isomer B* (2·0 g., m.p. 171 to 173°).

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THE PHARMACOGNOSY OF THE ASPIDOSPERMA BARKS OF BRITISH GUIANA*

PART VI. THE MICROSCOPY OF THE BARK OF *Aspidosperma oblongum* A.DC. AND SUMMARY OF PARTS I–VI

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In Parts I to V of this series of papers the barks of five species of *Aspidosperma* have been described and illustrated. The present paper deals with the anatomy of a sixth bark, *Aspidosperma oblongum* A.DC, summarises the results of all six papers and provides keys for the identification of the six barks either in the whole state or in powder.

MATERIAL

The material used consisted of three samples of bark previously designated 5A, 5B and 5C collected in British Guiana in 1949, 1950 and 1954 respectively.

EXPERIMENTAL

Line drawings to illustrate the diagnostic characters of outer and inner surfaces of the bark of *Aspidosperma oblongum* and of tissue distribution as seen in smoothed transverse section are given in Figure 1, A, B and C.

Histology of the bark of A. oblongum (Figs. 1, 2, 3 and 4).

The thick cork consists of some forty to one hundred layers of rectangular to somewhat tangentially-elongated cells. The cell walls are thin and are unlignified or very slightly lignified (Fig. 1, C and D, *ck* and Fig. 3, A, *ck*). One to three layers of thin-walled and rectangular to somewhat tangentially-elongated cells form the phellogen. This produces, on its inner surface, a well-marked phelloderm as a compact tissue some eight to twenty cells in radial thickness, in which are a few thin-walled, slightly tangentially-elongated, parenchymatous cells but the majority are sclerotic and form a more or less continuous band. Individual sclereids (Fig. 1, D, *phe* and Fig. 3, A, *phe*), rectangular to somewhat isodiametric, arranged more or less in radial files with the corresponding phellogen and cork cells; the cell walls are thick and lignified, traversed by simple or branched pits with lumen small but somewhat variable in size. Cortex consists of thin-walled, starch-containing parenchymatous cells with small intercellular spaces together with sclereids arranged in groups of varying sizes. The sclerotic cells are similar to those found in the phelloderm. Somewhat tangentially-elongated latex canals (Fig. 2, A, *lat* and Fig. 3, C, *lat*), R and H = 28 to **58** to 90μ and T = 126 to **150** to 180 μ, are found associated with the sclereid groups and lying in the

* The subject matter of this communication forms part of a thesis by one of us (J.D.K.) accepted by the University of Nottingham for the degree of Doctor of Philosophy in Pharmacy.

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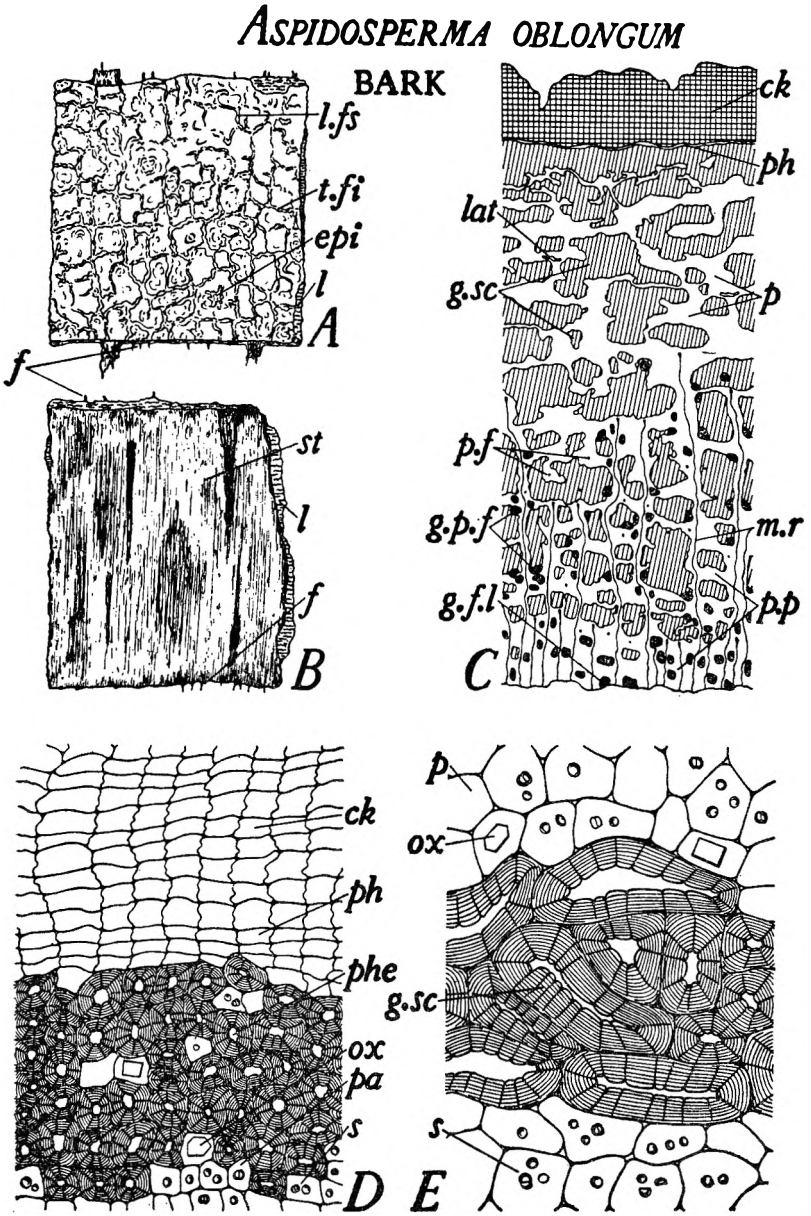

FIG. 1. *Aspidosperma oblongum* bark, macroscopical characters and T.S.:—A, outer surface × ¼; B, inner surface × ¼; C, smoothed T.S. × 12; D, cork, phloem and phelloderm; E, cortex; D and E, × 200; *ck*, cork; *epi*, epiphyte; *f*, fibre; *g.f.l*, group of phloem fibres with large lumen; *g.p.f*, group of phloem fibres with narrow lumen; *g.sc*, group of sclereids; *l*, laminations; *lat*, latex canal; *l.fs*, longitudinal furrow; *m.r*, medullary ray; *ox*, crystal of calcium oxalate; *p*, cortical parenchyma; *pa*, cortical parenchyma found associated with phellodermic sclereids; *ph*, phellogen; *phe*, phellodermic sclereids; *p.f*, isolated phloem fibre with narrow lumen; *p.p*, phloem parenchyma; *s*, starch; *st*, longitudinal striation; *t.fi*, transverse fissure.

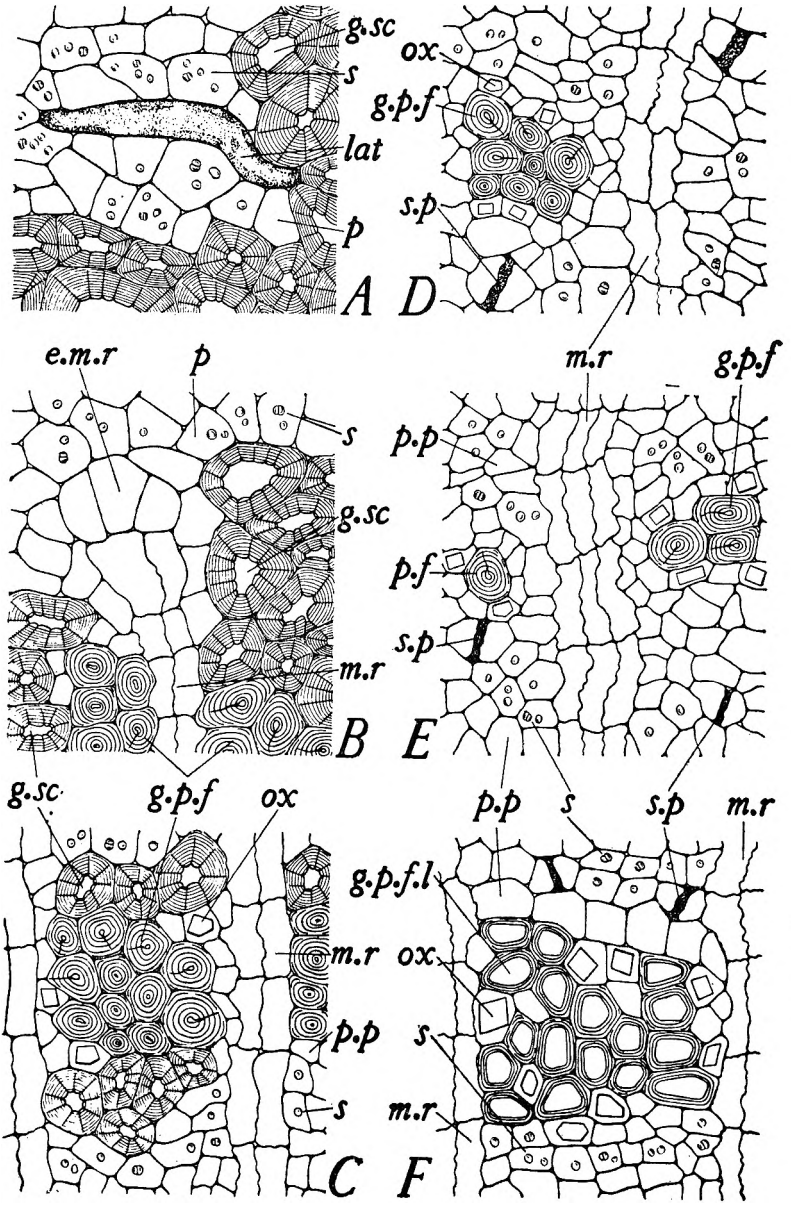

FIG. 2. *Aspidosperma oblongum* bark in T.S.:—A, cortex; B, innermost cortex and outermost phloem; C, D and E, phloem; F, innermost phloem, all × 200, *e.m.r*, end of medullary ray; *g.p.f*, group of phloem fibres with narrow lumen; *g.p.f.l*, group of phloem fibres with large lumen; *g.sc*, group of sclereids; *lat*, latex canal; *m.r*, medullary ray, *ox*, crystal of calcium oxalate; *p*, cortical parenchyma; *p.f*, isolated phloem fibre with narrow lumen; *p.p*, phloem parenchyma; *s*, starch; *s.p*, sieve plate.

cortical parenchyma; the latex, which is granular in appearance, is stained yellow with iodine solution and pinkish-red with Millon's reagent. Towards the inner region of the cortex are groups of sclereids with, at times, a few small groups of thick-walled fibres or very occasional isolated fibres; no defined endodermis and pericycle were found.

Up to about 50 per cent of the thickness of the bark is of phloem, which consists of sclereids, sieve tissue, parenchyma, fibres and medullary rays. It can be sub-divided into three unequal zones, the largest of which contains abundant sclereid groups identical with those found in the cortex and phelloderm; also present are fibres with narrow lumen, usually in groups or very rarely isolated and at times embedded in the sclereid groups. In a narrow zone towards the inner region of the phloem, groups of fibres with narrow lumen are present but sclereid groups are absent. The innermost region of the phloem contains groups of fibres with narrow lumen and a few groups of fibres with large lumen; sclereids are absent. The sieve tubes have oblique, compound sieve plates on the end walls (Figs. 2, 3 and 4, *s.p*) and are less readily distinguished in the outer part of the phloem. Phloem parenchyma is of thin-walled cells, with a few intercellular spaces, some vertical walls are reticulately thickened and exhibit compound pits. Phloem fibres, R and T = 22 to **40** to 58 μ and H = 1290 to **1880** to 2670 μ, of two types: the greater number have a narrow lumen and occur in groups of two to fourteen fibres, scattered throughout the phloem (Fig. 1, 2 and 3, *g.p.f*); very rarely isolated fibres are found in the outer region of phloem only (Fig. 1, 2, 3 and 4, *p.f*): fibres with large lumen, occurring only in the innermost region of the phloem, are in a very few groups of four to fourteen fibres (Fig. 1 and 4, *g.f.l*, Fig. 2, *g.p.f.l*). Both types of fibres are spindle-shaped with bluntly pointed ends, walls thick, lignified, stratified, traversed by a few simple pits, along which splitting may have occurred; a crystal sheath surrounds the fibres or groups of fibres, except when these are embedded in the sclereid masses. The medullary rays are very wavy, are two to three cells in width but becoming up to five cells wide (Fig. 2, B, *e.m.r*) towards the periphery of the phloem, they are 15 to 25 cells in height; individual cells are straight or somewhat wavy in outline and contain starch granules.

Cells of the cortical and phloem parenchyma contain abundant simple or 2- to 4- compound starch granules; individual granules with eccentric hilum, spherical, ovoid or plano-convex and up to 36 μ in diameter (Figs. 1, 2, 3 and 4, *s*). Calcium oxalate, in square, rectangular or obliquely rectangular prisms or small cubes up to 30 μ, occurs in crystal sheaths around the fibres and also associated with the groups of sclereids (Fig. 1, 2, 3 and 4, *ox*).

Powdered bark. The powder is light brown in colour and exhibits the characters described above. These include the cork cells which are polygonal in surface view and reddish-brown in colour, with thin, unlignified or very slightly lignified walls (Fig. 4, B, *ck*). The sclereids occur in masses, the individual cells are of various shapes and sizes, measuring 22 to **52** to 80 μ in length and 18 to **32** to 54 μ in width; they are thick-walled and lignified, the lumen is either narrow or somewhat large (Fig. 4, B,

sc). The phloem fibres are usually broken during the powdering of the bark; fragments are either isolated or in groups of two to three fibres and are of two types; the greater number of fibres have a narrow lumen and are surrounded by a calcium oxalate crystal sheath except when they are associated with sclereid groups; fewer fibres with large lumen, and either with or without crystal sheath (Fig. 4, B, *f.p* and *f.s*). Calcium oxalate crystals (Fig. 4, B, *ox*) are also present in association with a few of the groups of sclereids. Phloem parenchyma (Fig. 4, B, *p.p*) associated with the cells of the medullary rays (Fig. 4, B, *m.r*), and cortical parenchyma (Fig. 4, B, *p*) of thin-walled and tangentially-elongated cells, all contain starch granules (Fig. 4, B, *s*).

Diagnostic Characters

The diagnostic characters of the bark of *A. oblongum* are:—

1. Cork cells, pale reddish brown in colour, rectangular to somewhat tangentially-elongated, thin-walled and unlignified or very slightly lignified.
2. Phelloderm mainly lignified, of an irregular band of sclereids, eight to twenty layers in radial thickness.
3. Latex canals of cortex, very much tangentially elongated.
4. Sclereids present as groups of varying sizes in the cortex and phloem; individual cells with thick, stratified, lignified and pitted walls having narrow to somewhat large lumen.
5. Phloem fibres of two types, the majority with narrow lumen and either mostly in groups of two to fourteen fibres or very rarely isolated; fewer fibres with large lumen, always in small groups. Both types of fibres large, spindle-shaped, with thick, stratified and lignified walls, traversed by a few simple or branched pits.
6. Sieve tubes with compound sieve plates on the oblique end walls.
7. Medullary rays narrow and wavy; the cells with thin, somewhat wavy walls and containing starch granules.
8. Starch, abundantly present in cortical and phloem parenchyma, simple or 2- to 4- compound; individual grains with eccentric hilum, spherical, ovoid or plano-convex.
9. Prismatic calcium oxalate crystals in a parenchymatous sheath surrounding the fibres or groups of fibres, also at times in association with the sclereid groups.

General Summary

(*a*) *Entire Barks*

In the present series of papers the barks of six *Aspidosperma* species have been described and illustrated, namely *A. ulei*[1], *A. excelsum*[2], *A. album*[3], *A. megalocarpon*[5], *A. quebracho-blanco*[5] and *A. oblongum*, and these all show a common basic pattern of morphology and anatomy. They occur in thick and flat, curved or channelled pieces: the abundant cork is furrowed and fissured externally and bears epiphytic lichens or liverworts; the inner surface of each is longitudinally striated; the fracture is either short, granular and splintery in the outer part and fibrous in the inner part, or it is very hard. The odour of each bark is indistinct and the taste is bitter and aromatic.

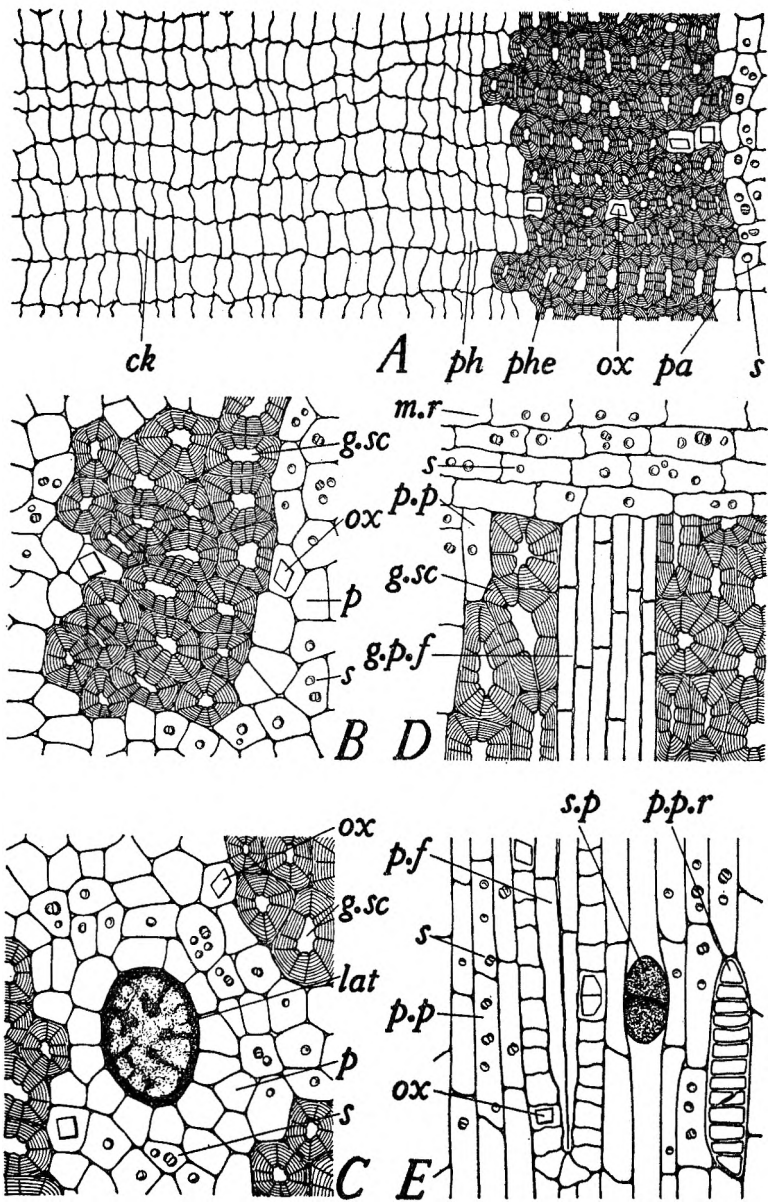

FIG. 3. *Aspidosperma oblongum* bark in L.S.:—A, cork; phellogen and phelloderm; B, and C, cortex; D and E, phloem; all × 200; *ck*, cork; *g.p.f*, group of phloem fibres with narrow lumen; *g.sc*, group of sclereids; *lat*, latex canal; *m.r*, medullary ray; *ox*, crystal of calcium oxalate; *p*, cortical parenchyma; *pa*, cortical parenchyma found associated with phellodermic sclereids; *ph*, phellogen; *phe*, phellodermic sclereids; *p.f*, isolated phloem fibre with narrow lumen; *p.p*, phloem parenchyma; *p.p.r*, phloem parenchyma with vertical walls reticulately thickened; *s*, starch; *s.p*, sieve plate.

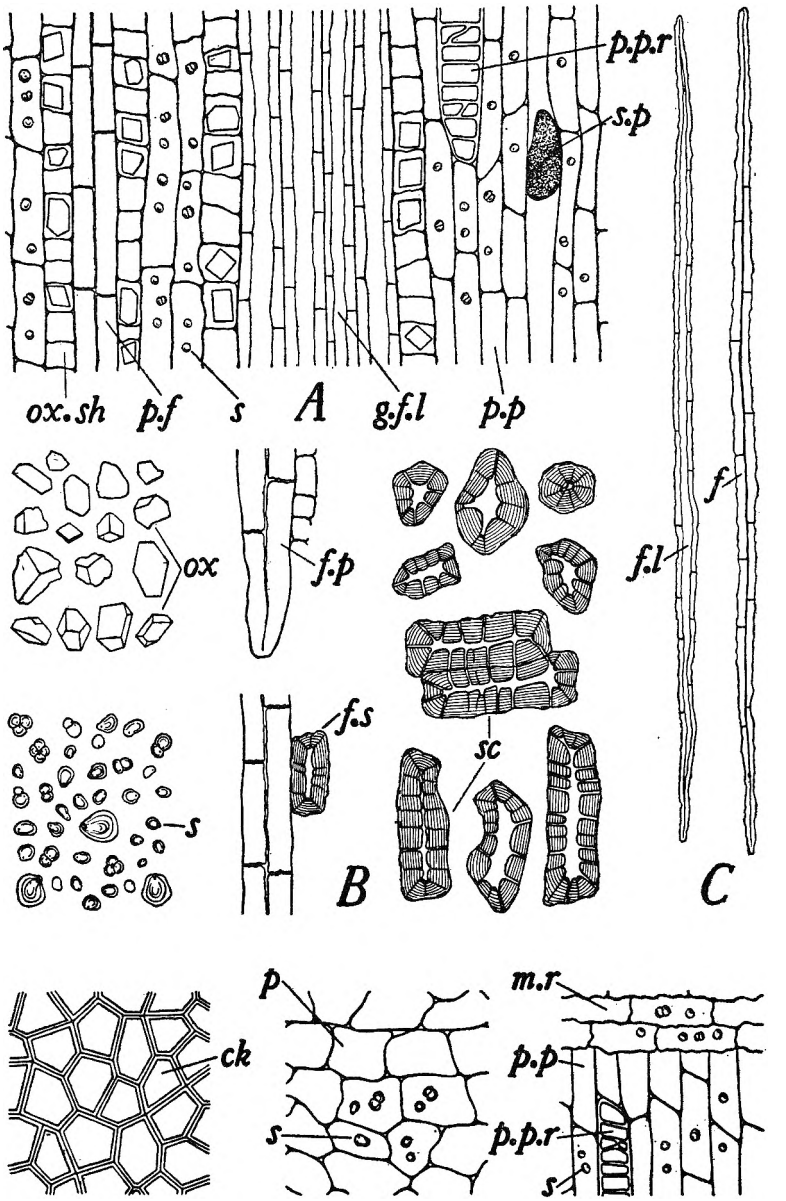

FIG. 4. *Aspidosperma oblongum* bark in L.S., powder and macerate:—A, innermost phloem × 200; B, various components as seen in powder × 200; C, fibres, isolated by maceration × 50; *ck*, cork; *f*, phloem fibre with narrow lumen; *f.l*, phloem fibre with large lumen; *f.p*, phloem fibre with narrow lumen; *f.s*, sclereidal cell attached to narrow lumened fibre; *g.f.l*, group of phloem fibres with large lumen; *m.r*, medullary ray; *ox*, crystal of calcium oxalate; *ox.sh*, calcium oxalate crystal sheath; *p*, cortical parenchyma; *p.f*, phloem fibre with narrow lumen; *p.p*, phloem parenchyma; *p.p.r*, phloem parenchyma with vertical walls reticulately-thickened; *s*, starch; *sc*, sclereids; *s.p*, sieve plate.

Histologically, each of these six barks possesses a broad zone of cork consisting of lignified or unlignified cells or both; the phelloderm and cortex contain abundant sclereids with thick, stratified and lignified walls, arranged in masses of varying sizes or in four to seven tangential bands. Latex canals are present in the cortex of four of the barks: in the other two barks these canals are absent but a small number of sclereids contain granular contents which stain in a similar manner to latex with Millon's reagent and with iodine solution. The phloem contains sieve tubes with compound sieve plates upon the oblique end walls; the medullary rays are narrow, the cells of which are either thin-walled or become sclerotic when passing through groups of sclereids. Many groups of sclereids are present in the outer part of the phloem, they are less abundant in *A. ulei* and are absent in *A. excelsum*. The scattered phloem fibres are mainly of large spindle-shaped cells with thick walls, traversed by simple or compound pits and with small lumen; some fibres with large lumen and narrow walls may also be present; each fibre or group of fibres is surrounded by a parenchymatous sheath of cells containing prismatic crystals of calcium oxalate, except when the fibres are embedded in groups of sclereids. Similar calcium oxalate crystals are associated with the sclereid groups. Starch granules, simple or 2–4 compound, are found in each of the six barks. The detailed characters by means of which these six barks may be distinguished from each other are set out in Table I.

Based on these characters the following analytical key for the identification of each bark has been drawn up.

Key to Differentiate the Six ASPIDOSPERMA *Barks:*

1. Latex canals—absent; medullary rays—sclerotic in association with the groups of sclereids — 2
   Latex canals—present; medullary rays—never sclerotic — 3
2. Cork cells—lignified; cortical sclereids—arranged in 4–7 tangential bands: *A. megalocarpon*
   Cork cells—unlignified; cortical sclereids—arranged in groups of varying sizes: *A. quebracho-blanco*
3. Cork cells—lignified; phloem fibres—always isolated: *A. excelsum*
   Cork cells—unlignified; phloem fibres—isolated and in groups — 4
4. Unlignified fibres—present in the pericyclic region; phloem fibres with large lumen—absent: *A. ulei*
   Unlignified fibres—absent; phloem fibres with large lumen—present — 5
5. Latex canals—axially elongated: *A. album*
   Latex canals—somewhat isodiametric or tangentially elongated: *A. oblongum*

(*b*) *Powdered Barks*

The colour of the powdered barks is fawn to brown, or yellow (*A. ulei*) or somewhat pink (*A. quebracho-blanco*). Diagnostic structures present

TABLE I

DIFFERENTIAL CHARACTERS OF BARKS OF SIX *Aspidosperma* SPECIES

	A. album	*A. oblongum*	*A. ulei*	*A. excelsum*	*A. megalocarpon*	*A. quebracho-blanco*
Colour of Inner Surface	Yellowish brown to almost black	As *A. album*	Characteristic yellow	As *A. album*	As *A. album*	Yellow to reddish-brown
Fracture	Short, granular, splintery in outer part, fibrous in inner part	As *A. album*	Very hard	As *A. album*	As *A. album*	As *A. album*
Cork	Cells thin-walled, unlignified or only slightly lignified	As *A. album*	As *A. album;* cells much collapsed	Most cells with inner and outer walls thickened and lignified—a few cells thin-walled and unlignified or only slightly lignified	Cells with inner and outer walls thickened and lignified. A few included groups of sclereids present	As *A. album*
Phellodermic Sclereids	One more or less continuous band of isodiametric cells	As *A. album*	As *A. album;* One to several layers of tangentially-elongated cells also present	Isodiametric cells arranged in groups of varying sizes	As *A. excelsum*	As *A. album*
Cortex: Sclereids with small lumen	One band in outer cortex otherwise in scattered groups	In groups of varying sizes	As *A. oblongum*	In four to seven bands. A few sclereids with large lumen present in inner cortex	As *A. excelsum*	As *A. oblongum*
Latex canals	Axially elongated	Somewhat tangentially-elongated	Isodiametric or somewhat tangentially-elongated	Tangentially elongated	Absent	Absent
Sclereids with granular content	Absent	Absent	Absent	Absent	Present	Present
Fibres	Absent	Absent	Unlignified or slightly lignified: embedded in sclereid groups	Absent	Absent	Absent
Phloem: Medullary Rays	Never sclerotic	As *A. album*	As *A. album*	As *A. album*	Sclerotic when in association with sclereid groups	As *A. megalocarpon*
Sclereids	In groups, except in the innermost region	As *A. album*	As *A. album* but less abundant	Absent	As *A. album*	As *A. album*
Fibres (*a*) with narrow lumen	Isolated or in small groups	In small groups or very rarely isolated	Isolated or very rarely in groups of 2 fibres	Always isolated	Isolated or very rarely in groups of 2 fibres	As *A. ulei*
(*b*) with wide lumen	Present in the innermost region in groups	As *A. album*	Absent	Present in the innermost region, isolated	Isolated, scattered throughout the phloem	As *A. excelsum*
(*c*) Sizes diameter	25—32—40μ	22—40—58μ	55—65—80μ	36—50—62μ	28—80—134μ	25—46—62μ
length	800—1750—2600μ	1200—1880—2670μ	2500—3650—5370μ	1550—2210—2800μ	600—1140—1650μ	500—850—1200μ

TABLE II

DIFFERENTIAL CHARACTERS OF POWDERED BARKS OF SIX *Aspidosperma* SPECIES

	A. album	*A. oblongum*	*A. ulei*	*A. excelsum*	*A. megalocarpon*	*A. quebracho-blanco*
Colour	Fawn to light brown	As *A. album*	Yellow	As *A. album*	As *A. album*	Orange to reddish-brown
Sclereids with small lumen	Present in groups	As *A. album*	As *A. album*	As *A. album*	As *A. album*	As *A. album*
Sclereids with large lumen	Absent	Absent	Absent	Present	Absent	Absent
Sclereids with granular contents	Absent	Absent	Absent	Absent	Present	Present
Latex canals	None visible, due to breakdown during powdering	As *A. album*	As *A. album*	Broken pieces visible	Absent	Absent
Fibres with narrow lumen	Lignified, present, see Table I	As *A. album*	As *A. album*. A few unlignified fibres also present	As *A. album*	As *A. album*	As *A. album*
Fibres with large lumen	Present	Present	Absent	Present	Present and at times associated with groups of sclereids	Present
Sclereid/Fibre Ratio	128	802	319	1008	293	105

Note: For details of cork cells and dimensions of fibres see Table I.

are fragments of cork tissue, groups of sclereids, fibres which are usually broken and either isolated or in small groups, starch grains and calcium oxalate crystals as described above for the entire barks. Latex cells are frequently much destroyed during powdering.

Certain specific differences, which depend upon tissue arrangement in the entire barks, are also destroyed by powdering. To ensure differentiation of the powdered barks of certain of these species a ratio value has been developed[4,6]. This is the number of sclereids present in unit mass of powdered bark divided by the number of fibres present in the same weight of bark. This S/F value has been found to be constant within a species and differential between barks of the six *Aspidosperma* species examined.

The differential diagnostic characters for these six barks in powder form are set out in Table II.

The following analytical key based on these characters may be used for the identification of any one of these six barks.

Key to differentiate the six powdered ASPIDOSPERMA *Barks:*

1.	Cork cells—all, or the majority lignified	2
	Cork cells—unlignified or only slightly lignified	3
2.	A few cork cells—unlignified, S/F ratio = 1008	*A. excelsum*
	Cork cells—all lignified, S/F ratio = 293	*A. megalocarpon*
3.	Granular material—present in a few sclereids, S/F ratio = 105	*A. quebracho-blanco*
	Granular material—absent from all sclereids	4
4.	Large lumened fibres—absent, S/F ratio = 319	*A. ulei*
	Large lumened fibres—present	5
5.	S/F ratio = 128	*A. album*
	S/F ratio = 802	*A. oblongum*

Woodson[7] has investigated the classification of the genus *Aspidosperma* and has arranged the 52 species in nine different series by means of their floral characters. The six barks which we have investigated in these researches belong to four of the Woodson series as follows: Series 3 *A. ulei*; Series 6 *A. oblongum*, *A. excelsum*; Series 8 *A. quebracho-blanco*; Series 9, *A. album*, *A. megalocarpon*. There is no clear parallelism between the distinguishing anatomical characters recorded in Tables I and II for these six barks, and their position in the Woodson classification.

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A COMPARATIVE *IN VITRO* EVALUATION OF A NEW BISMUTH SALT BISMUTH ALUMINATE

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Antacids commonly used to treat peptic ulcer are compared for their ability to neutralise acid and inactivate pepsin. On this basis a new bismuth salt, bismuth aluminate is compared with ten other commonly employed substances and found to be the most effective agent. All preparations are grouped according to their antacid and antipeptic properties.

The numerous methods of *in vitro* evaluation of substances indicated in the treatment of peptic ulcer are almost excessively concerned with the neutralisation of hydrochloric acid[1–4]. The effect of substances upon the proteolytic enzyme content of gastric juice which may have an important bearing upon cause of ulcer pain and the persistent nature of the lesion are usually disregarded. It is hard to justify the pre-eminence given to the role of acid and the neglect of proteolytic enzymes. Ulcer pain is usually attributed to acidity or to abnormal motility of the gastro-duodenal area. If these are the only factors of importance then it is difficult to explain the action of substances which are neither antacids nor antispasmodics. The older bismuth salts such as the carbonate and subnitrate fall into this group, and yet they are capable of relieving the pain of peptic ulcer; they can, however, inactivate pepsin[5,6] regardless of the pH of the medium. Furthermore, in support of this hypothesis, ulcers occur in the absence of an excess of acid but not in the absence of active pepsin. In the normal stomach the HCl–pepsin complex does not attack the gastric mucosa but in the presence of peptic ulcer the mucosal defences are broken down and auto-digestion of the ulcer base may be the cause of its persistent nature. An important factor in the development of ulceration in the stomach of rats the pylorus of which has been tied was a gastric juice of high peptic activity[7].

The aim of ulcer therapy is said to be to restore gastric contents to pH 2–4 for as long a period as possible. Many simple antacids exceed this requirement by raising the hydrogen ion concentration above pH 5: by so doing these substances inactivate pepsin but may also give additional stimulus to acid secretion[8]. The ideal therapeutic agent will maintain the gastric contents at pH 2–4, and at the same time inactivate pepsin.

The purpose of this report is to compare the properties of a new bismuth salt, bismuth aluminate $Bi_2(Al_2O_4)_3 10H_2O$ with a selection of those substances commonly employed in peptic ulcer therapy. Bismuth aluminate contains approximately 35 per cent bismuth by weight in the dry form. It was tested in this form and also as a cream containing 19 per cent of the salt by weight.

METHODS

Brindle[1] defined the neutralisation value of a compound as the weight required to neutralise 100 ml. of 0·05N HCl. This is determined by adding a measured quantity to 100 ml. of 0·05N HCl at 38° and continuously stirring. The amount used should be such that there will be excess of acid. After 4 hours the mixture is filtered, the filter paper washed and the residual amount of acid determined. An amount of the compound equivalent to 20 per cent excess over the neutralisation value is then added to 100 ml. of artificial gastric juice at 38° and the pH recorded throughout by electrodes immersed in the mixture which is continuously stirred. The pH increase at given times is recorded and the amount of HCl neutralised calculated by reference to a standard graph (Fig. 1).

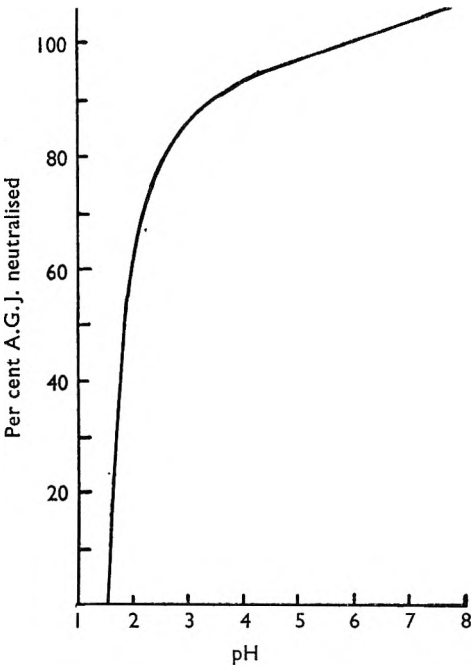

FIG. 1. Brindle's Method. The relationship of pH to the percentage of artificial gastric juice neutralised.

An experimental method was also devised in our own laboratory from the data of Armstrong and Martin[2] using the artificial gastric juice recommended by Brindle which contains 150 mg. each of pepsin, peptone and sodium chloride, and 0·05N HCl to 100 ml. adjusted to pH 1·4–1·5 at 37°. To 150 ml. of artificial gastric juice in an artificial stomach (Fig. 2) was added the dose, commonly recommended in therapy, of the preparation under test. The pH was recorded continuously and fresh artificial gastric juice allowed to drip in at 1·5 ml./minute. After each 20 minute period up to 100 minutes, 30 ml. of the mixture was removed and tested for pepsin.

Pepsin was determined by modification of the method of West, Ellis and Scott[9]. This is based upon the ability of pepsin to cause aggregation of casein particles in fresh homogenised milk. The reagents used are: acetate buffer pH 4·9 (sodium hydroxide 4·2 g., glacial acetic acid 9·2 ml., distilled water to 100·0 ml.); milk buffer mixture (equal parts of fresh homogenised milk and acetate buffer).

Before the test all reagents and the test solution are brought to 13° in a cold water bath. In a test tube are placed 2 ml. of acetate buffer, 1 ml. of distilled water and 1 ml. of test solution; to this mixture is added 1 ml. of milk buffer and a stop watch started. The test tube is shaken

and at the first appearance of aggregated particles in the film on the sides of the tube above the mixture the watch is stopped and the time noted. Each test solution is investigated at least twice and a mean precipitation time calculated. Preliminary tests indicated that if the time for the aggregation of casein particles was greater than 40 seconds, the experimental error in repeating the tests was less than 3 per cent.

Before investigating therapeutic substances, calibrating experiments to relate the time for casein precipitation to the concentration of pepsin in solutions of known strength were made. Samples of artificial gastric juice with a pepsin concentration from 25 to 300 mg./100 ml., and containing 150 mg. peptone and NaCl in 0·05 HCl were diluted 1 in 16 with distilled water and 1 ml. of diluted mixture was used as the test solution in the method described above.

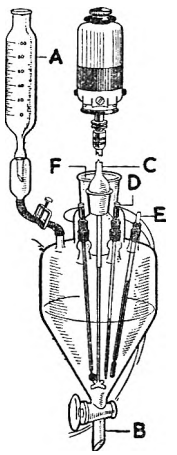

FIG. 2. The artificial stomach used for the second method of assessment devised in our laboratories.

KEY—A, measuring cylinder allowing intermittent addition of fresh artificial gastric juice; B, tap allowing intermittent removal of the mixture; C, constant stirrer; D, electrodes constantly registering pH; E, thermometer allowing control of temperature; F, wide necked opening for addition of substance under test. Heating is by a thermostatically controlled cuff which fits round the apparatus.

Solutions were tested under a number of conditions. It was found that samples of freshly homogenised milk did not vary significantly in casein concentration; that temperature exerted a marked effect upon the rate of precipitation, and that there was some variation in the potency of artificial gastric juice after storage. Although the actual precipitation time for a test solution was inconsistant, the logarithmic relation of pepsin concentration to time of precipitation remained constant being a straight line, the slope of which was always 1·12. From such a graph it was calculated that:

log pepsin concentration a = log pepsin 150 − (log precipitation time a − log precipitation time 150) × 1·12

where a = is the solution of unknown pepsin; 150 solution = solution containing 150 mg. per cent pepsin. (This solution was selected as standard since it is the artificial gastric juice used in each experiment.)

RESULTS

Brindle's Method

The results are shown in Table I. In terms of acid neutralisation, bismuth aluminate compares favourably with other substances and in the dry state is far superior to dry aluminium hydroxide the potency of which varies considerably from sample to sample. Table II shows the effect

TABLE I

BRINDLE'S TESTS. RESULTS

Substance	Neutralisation value	Change in pH and acid neutralised by adding 20 per cent excess over neutralisation value to 100 ml. A.G.J. at 38° C.							
		After 10 mins.		After 20 mins.		After 30 mins.		After 60 mins.	
		pH	HCl neutralised per cent	pH	HCl neutralised per cent	pH	HCl neutralised per cent	pH	HCl neutralised per cent
Bismuth carbonate	1·38 g.	0·15	30	0·18	33	0·18	33	0·25	41
Bismuth subnitrate	8·3 g.	0·42	56	0·45	58	0·42	56	0·40	54
*Aluminium hydroxide gel	8·707 g.	1·4	88	2·14	95	2·32	96	2·4	98
*Dried aluminium hydroxide gel (A)	0·1829 g.	0·17	30	0·24	40	0·32	49	0·45	60
*Dried aluminium hydroxide supplied as B.P.C. (B)	1·144 g.	0·98	81	1·76	91	1·93	93	1·98	94
Dried aluminium hydroxide	0·420 g.	0·46	51	0·50	55	0·61	61	0·74	65
*Magnesium trisilicate	0·435 g.	0·5	62·5	1·02	82·5	1·52	90	3·36	100
Bismuth aluminate powder	0·395 g. (0·139 g. Bi)	0·69	65	0·94	70	1·1	76	1·47	85
Bismuth aluminate cream	5·3 g. (0·367 g. Bi)	0·22	35	0·31	45	0·42	50	0·52	60

* Results taken from Brindle's original paper.

TABLE II

Substance	Neutralisation value	Change in pH and acid neutralised by adding therapeutic dose of bismuth aluminate to 100 ml. A.G.J. at 38° C.							
		After 10 mins.		After 20 mins.		After 30 mins.		After 60 mins.	
		pH	HCl neutralised per cent	pH	HCl neutralised per cent	pH	HCl neutralised per cent	pH	HCl neutralised per cent
Bismuth aluminate powder	2·01 g. (0·7 g. Bi)	2·21	82	2·34	88	2·46	89	2·62	90
Bismuth aluminate cream	10·1 g. (0·7 g. Bi)	0·63	64	0·76	66	0·86	71	1·12	82

of therapeutic doses of bismuth aluminate, virtually all the acid is neutralised within 10 minutes and the final pH (4·09, powder; 2·57, cream) is within the accepted desirable range.

These results also indicate the relative efficiency of the bismuth salts tested, in terms of acid neutralisation. This comparison is perhaps best

TABLE III

ANTACID POWER OF 2 G. OF BISMUTH SUBNITRATE

pH at:—	Time in minutes after addition of 2g. bismuth subnitrate				
	0–20	20–40	40–60	60–80	80–100
0 secs. ..	1·42	—	—	—	—
30 secs. ..	1·48	1·51	1·45	1·44	1·46
5 mins. ..	1·5	1·52	1·45	1·45	1·45
10 mins. ..	1·52	1·45	1·44	1·45	1·44
15 mins. ..	1·52	1·45	1·44	1·46	1·43
20 mins. ..	1·52	1·45	1·45	1·46	1·43

made by comparing the amount of elemental bismuth which is required to neutralise 100 ml. 0·05N HCl viz.: In the carbonate 1·10 g., subnitrate 6·05 g., aluminate powder 0·139 g., aluminate cream 0·367 g.

The marked superiority of bismuth aluminate, especially the powder allows efficient, yet economical bismuth therapy.

Results from Our Own (MCP) Method

Results are recorded in the Tables III–V.

The results for bismuth subnitrate 2·0 g. are given in full to show the method of calculation of antipeptic activity. Tables for other tests show only the final results.

At the beginning of the experiment, the precipitation time of the artificial gastric juice used is determined. This time (Table IV) (1) in the

TABLE IV

ANTIPEPTIC PROPERTIES OF 2 G. OF BISMUTH SUBNITRATE

Volume at start 150 ml.	Time in minutes				
	0–20	20–40	40–60	60–80	80–100
(1) Precipitation time at time zero (seconds)	61	86	71	75	66
(2) Pepsin concentration mg. per cent at time zero ..	150	100	123	118	135
(3) Pepsin present in 150 ml. at time zero	225	150	184	177	203
(4) Pepsin added in period (mg.)	45	45	45	45	45
(5) Total pepsin at start (mg.)	270	195	229	222	248
(6) Precipitation time at the end of period (seconds) ..	86	71	75	66	63
(7) Pepsin concentration at the end of period (mg.per cent)	100	123	118	135	140
(8) Total pepsin at the end of period (mg.)	180	221	212	243	252
(9) Pepsin inactivated in the period (mg.)	90	0	17	0	0
(10) Pepsin re-activated in the period (mg.)	0	26	0	21	4
(11) Pepsin discarded at the end of the period (mg.) ..	30	37	35	40	—

Total pepsin used mg. = 450
Total pepsin inactivated mg. = 56
Total pepsin discarded mg. = 142
Total pepsin at the end mg. = 252

56 mg. pepsin inhibited by 2 g. bismuth subnitrate = 1·46 g. Bi., i.e. 38 mg. pepsin per 1 g. Bi.

} Pepsin inactivation in relation to amount of preparation used.

0–20 minute period) corresponds to 150 mg. pepsin per 100 ml. and provides a standard. Since the initial volume in each period is 150 ml., the pepsin present therein (3) is $1\frac{1}{2}$ × the concentration (2). In each period 30 ml. of fresh artificial gastric juice is added and this contains 45 mg. of pepsin (4). The total amount of pepsin at the start (5) is

obtained by the addition (3) plus (4). The precipitation time at the end of each period (6) is converted into pepsin concentration (7). Since the volume at the end is 180 ml. the total pepsin at the end (8) equal $\frac{9}{5}$ × concentration. Pepsin inactivated or re-activated (9) and (10) is represented by the difference in total pepsin at the start and end of the period. 30 ml. of the mixture is discarded and this contains $\frac{3}{10}$ of pepsin concentration (mg. per cent) at the end (11).

The precipitation time of the pepsin concentration at the beginning of the second and subsequent periods is the same as at the end of the preceding period.

TABLE V

ANTACID AND ANTIPEPTIC PROPERTIES OF SUBSTANCES TESTED BY M.C.P. METHOD

Compound and its weight	pH at minutes						Total pepsin used = 450 mg. Pepsin inactivated: mg.	mg. pepsin inactivated per g. of element
	0	20	40	60	80	100		
Bismuth subnitrate .. .. 5 g.	1·4	1·54	1·56	1·58	1·55	1·55	74	20
Bismuth carbonate .. .. 2 g.	1·48	1·56	1·60	1·60	1·60	1·58	158	98
Bismuth carbonate .. .. 5 g.	1·51	1·53	1·55	1·55	1·55	1·53	377	94
Bismuth aluminate cream 11·0 ml. (0·7 g. Bi)	1·49	3·55	3·20	2·95	2·75	2·54	450	642+
Bismuth aluminate powder .. 2 g. (0·7 g. Bi)	1·50	3·77	3·55	3·35	3·10	2·70	450	642+
Aluminium hydroxide powder 3 g.	1·4	1·54	1·41	1·43	—	1·43	17	16·3
Aluminium hydroxide Gel BPC 5·4 g. (1 teaspoonful)	1·49	3·18	2·94	2·70	2·50	2·22	207	38
Aluminium hydroxide Gel BPC 10 g. (2 teaspoonsful)	1·49	3·88	3·75	3·55	3·42	3·22	378	37·8
Magnesium carbonate powder 0·1 g.	1·47	1·75	1·67	1·68	1·63	1·59	56	1900
Magnesium carbonate .. 1 g.	1·43	7·34	7·30	7·22	7·04	6·88	450	
Magnesium carbonate .. 2 g.	1·47	7·52	7·46	7·42	7·38	7·28	450	
Magnesium trisilicate powder 0·7 g.	1·44	1·82	1·98	1·78	1·62	1·63	47	361
Magnesium trisilicate .. 2 g.	1·41	2·78	3·00	2·78	2·55	2·28	272	715
Magnesium oxide powder .. 0·1 g.	1·5	1·90	1·78	1·73	1·69	1·65	10	166
Magnesium oxide .. .. 0·5 g.	1·4	9·50	9·30	9·20	8·88	8·70	450	
Sodium bicarbonate .. 1 g.	1·4	6·38	6·24	6·04	5·4	5·2	313	
Calcium carbonate powder 1 g.	1·49	5·53	5·42	5·37	5·18	4·88	18	
Calcium carbonate powder 3 g.	1·42	5·66	5·62	5·60	5·53	5·50	40	
Aluminium glycinate .. 2 g.	1·51	3·30	3·31	3·20	3·04	2·92	360	900
Aluminium glycinate .. 0·9 g.	1·58	2·80	2·72	2·58	2·41	2·30	211	1171

DISCUSSION

Within the conditions of the test all three bismuth salts have antipeptic power. In terms of bismuth content, bismuth aluminate is the most efficient.

It is difficult to explain why 2 g. of bismuth subnitrate should inactivate almost twice as much pepsin as 5 g. when the results are expressed in terms of mg. of pepsin inactivated per g. of bismuth. The experiments have been repeated on three occasions with similar results. With bismuth carbonate the indices for 2 doses (98 and 94) are sufficiently close to be recorded as the same. It is not possible to state the absolute antipeptic index for bismuth aluminate, since with both powder and cream, the quantity used inactivated all the available pepsin. Subsequent tests have shown that there may be slight variation, in the antipeptic power, but this never falls below 600 mg. pepsin per g. bismuth.

The antacid results confirm opinion already expressed[1,2,10], i.e. that bismuth carbonate and subnitrate are poor antacids. Conversely bismuth aluminate powder by Brindle's method neutralises 70–88 per cent of acid in 20 minutes without an abnormal increase in pH and in therapeutic doses (Table V) using our own method maintains the pH of the stomach

TABLE VI

ANTACID AND ANTIPEPTIC INDEX OF SOME COMMONLY EMPLOYED ANTACIDS

		Antacid index*	Per cent pepsin inactivated	
Group 1				
Bismuth aluminate powder	2·0 g.	100	100	Good antacid and antipeptic properties
Bismuth aluminate cream	11·0 ml.	100	100	
Aluminium glycinate	2·0 g.	100	80	
Aluminium hydroxide gel B.P.C.	10 g.	95	84	
Group 2				
Magnesium trisilicate	2·0 g.	95	60	Require potentiation of antipeptic effect
Aluminium glycinate	0·9 g.	100	47	
Aluminium hydroxide gel B.P.C.	5·4 g.	95	46	
Group 3				
Bismuth carbonate	5·0 g.	–	84	Requires potentiation of antacid effect
Group 4				
Bismuth carbonate	2·0 g.	–	35	Require potentiation of antacid and antipeptic effects
Bismuth subnitrate	2·0 g.	–	12	
Bismuth subnitrate	5·0 g.	–	16	
Magnesium carbonate	0·1 g.	–	12	
Magnesium trisilicate	0·7 g.	–	9	
Magnesium oxide	0·1 g.	15	2	
Aluminium hydroxide powder	3·0 g.	–	4	
Group 5				
Magnesium carbonate	1·0 g.	+	100	Unsatisfactory
Magnesium carbonate	2·0 g.	+	100	
Magnesium oxide	0·5 g.	+	100	
Calcium carbonate	1·0 g.	+	4	
Calcium carbonate	3·0 g.	+	9	
Sodium bicarbonate	1·0 g.	+	70	

* No. of minutes for which the acidity was within the range pH 2—4 (+ indicates pH above 4. – indicates pH below 2.)

within the desirable range of 2 to 4 throughout the 100 minute period of the test, whereas this was not so for the other bismuth salts. On the basis of this assessment bismuth aluminate is the most desirable of the three salts.

In comparing bismuth aluminate with the other substances tested, difficulties arise in that in some instances the increase of pH was of itself capable of inactivating pepsin. Thus magnesium oxide, 0·1 g. inactivates pepsin at the rate of 166 mg./g. of elemental magnesium whereas magnesium oxide 0·5 g. inactivates 1500 mg. of pepsin per g. of elemental magnesium. Under the conditions of these experiments pepsin became inactive at pH 5–6. With the small quantity of magnesium oxide the pH never rose above 2·12, and this could not account for pepsin inhibition. With the larger quantity, the pH was above 8·7 throughout.

The merits of each dose of each substance may be compared by giving them an antacid index and considering this together with the pepsin inactivated. The antacid index is defined here as the number of minutes for which the acidity was within the range pH 2–4. The pepsin inactivated is expressed as a percentage of the total pepsin used in the test.

Group 1

Substances in this group fulfil the *in vitro* requirements and therefore merit consideration as therapeutic agents. They can be relied upon to exert a desirable antacid action and at the same time inactivate pepsin. Differences of pH in the range pH 2·5–3·5 represent only a small percentage of acid and are not therefore important. Conversely differences in the pepsin inactivated as recorded in Table VI are of greater magnitude.

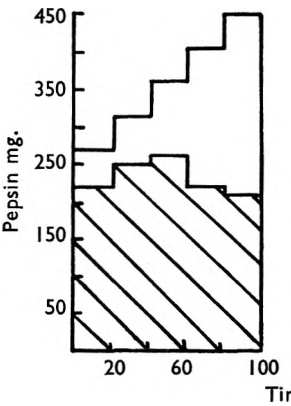

FIG. 3. Antipeptic action of 5·4 g. aluminium hydroxide gel BPC. See Table II. Note that after 60 minutes pepsin starts to be washed out and reactivated.

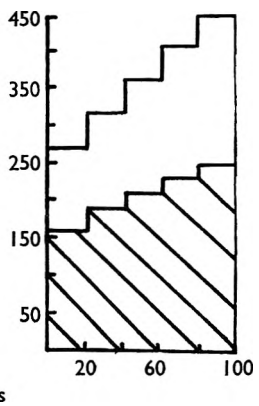

FIG. 4. Antipeptic action of a sub-minimal dose of bismuth aluminate powder. Note that although inactivation is incomplete there is no reactivation.

Unshaded area: active pepsin; shaded area: inactivated pepsin.

(1 per cent = 4·5 mg. pepsin.) Thus bismuth aluminate is a significantly better antipeptic than aluminium glycinate or aluminium hydroxide in the therapeutic doses tested. However, the antipeptic properties of aluminium glycinate would be improved by increased dosage, which would be unlikely to adversely effect the antacid index. Larger doses of glycine may be uneconomical and may give rise to undesirable side reactions such as gastro-intestinal hurry. A consideration of Table V and that of Figure 3 reveals that with aluminium hydroxide, while in the earlier 20 minute periods of the test, the pepsin inactivation is effective this power diminishes as the test proceeds, the pepsin being washed out of its combination with aluminium hydroxide. This phenomon is due to pepsin being adsorbed by aluminium hydroxide, but released again upon the addition of more HCl and the subsequent formation of soluble aluminium chloride.

Figure 4 expresses the results of a test with a subminimal dose of bismuth aluminate. Although the inactivation of pepsin is incomplete there is no evidence of pepsin being re-activated during the test.

Group 2

These substances are deficient in antipeptic effect. With aluminium glycinate this can be made good by increasing the dosage which also brings some improvement with aluminium hydroxide gel.

Group 3

Bismuth carbonate alone is an ineffective antacid and its chief use might well be to potentiate the effect of magnesium trisilicate, or some of the non-bismuth salts in Group 4.

Group 4

The preparations in this group are deficient in both antacid and antipeptic properties. With bismuth salts, the deficiency cannot be made good by increasing the dosage. Only in 3 times the B.N.F. dose is magnesium trisilcate a satisfactory antacid, and even then the antipeptic effect is poor. From Table V it can be calculated that to inactivate 450 mg. pepsin approximately 4 g. of MgO would be required, this would give far too great an antacid effect and thus this substance can only be of value when used in a mixture.

The dosage of aluminium hydroxide powder used (3 g.) is far in excess of the standard therapeutic dose, and yet both antacid and antipeptic effects are insignificant. The difference from the aluminium hydroxide gel is striking and emphasises the dangers inherent in assuming that such a salt in the dry state will have similar properties hydrated.

Group 5

All these substances gave an abnormally high gastric pH and must be considered unsuitable for therapeutic use.

Preparations administered in peptic ulcer may possess properties other than those discussed. For example, coating and sedative properties have not been considered. Furthermore, the final assessment of any therapetutic agent rests with its clinical appraisal. It is a necessary preliminary to clinical trial that the pharmacological properties of a compound should be assessed on a comparative basis. The scheme outlined here provides a method of doing this, and is considered preferable to older methods in that it includes inactivation of pepsin as well as acid neutralisation.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Amylobarbitone and Pentobarbitone Sodium in Mixtures, Identification of. E. G. Brooker. (*Analyst*, 1957, **82**, 448.) These barbiturates are not easily separable by direct paper chromatography. Separation can be achieved by preliminary treatment with concentrated sulphuric acid at 100° for one hour. Amylobarbitone is unaffected while pentobarbitone can be separated into 5-ethylbarbituric acid and an unidentified hydrolysis product. The acid reaction mixture is diluted with water, extracted with ether, the ether evaporated and the residue dissolved in chloroform. An aliquot of this is used for chromatography with the water-poor phase of the system *n*-butanol-*n*-pentanol-ammonia (1 : 1 : 1) as mobile phase. The procedure can be used for quantities as small as 0·1 mg. and on mixtures containing as little as 10 per cent of pentobarbitone sodium in amylobarbitone. D. B. C.

Analgesics, Reactions of. M. Hädicke and M. Kuntze. (*Pharm. Zentralh.*, 1957, **96**, 152.) Reactions suitable for the identification of a number of analgesics are given in the table below:

Substance	with phosphomolybdic acid	with nitric-sulphuric acid	with Marquis' reagent
Methadone	weak blue, white precipitate	dark red	slow rose
Eucopon	bluish, turbid	do.	reddish brown
Pethidine	nil	nil	orange brown
Morphine	deep blue	pale yellow	purple
Eucodal	blue colour, crystalline precipitate	yellow	yellow-violet-blue
Codeine	weak blue	yellow	violet
Ethylmorphine	blue	yellow	yellow-violet
Dicodid	weak blue	yellow	red violet
Dilaudid	deep blue	pale yellow	violet
Papaverine	crystalline precipitate on standing	orange yellow	purple red

G. M.

Barbiturates, Microscopic Identification of. H. M. Romijn. (*Pharm. Weekbl.*, 1957, **92**, 397.) The reagents used consist of a 1 and 10 per cent solution of cupric acetate (monohydrate) in ammonia (25 per cent). By adding about 1 mg. of the barbiturate to a drop of each of these reagents it is possible to identify a number of barbiturates. Actually four types of crystals may be observed—violet, blue, colourless or yellow. The preliminary classification is made according to the following scheme):—(1) One or both reactions give both blue and violet crystals: heptobarbitone, allobarbitone, *iso*butylallylbarbitone, phenobarbitone, barbitone and aprobarbitone. (2) One or both reactions give blue but no violet crystals: cyclobarbitone, cycloheptenylethylbarbitone, propylbarbitone, butobarbitone, *iso*amylethylbarbitone, pentobarbitone, and *sec.*-butylbromoallylbarbitone. (3) No reaction in the cold; after boiling and addition of ammonia, violet or colourless, but no blue, crystals: hexobarbitone and methylphenobarbitone. Illustrations are given of the characteristic crystalline forms observed in each case. Crystals are also given by a number of organic acids and other compounds. G. M.

Ergotamine and Ergotoxine, Estimation of. D. D. Jones, H. Katyama and V. E. Tyler Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 426). When ergot alkaloids are submitted to chromatography on buffered paper under controlled conditions, the R_F value increases as the quantity of alkaloid applied to the paper is increased, presumably owing to a tendency for the alkaloidal base to overload the salt-forming capacity of the buffered paper. Within limits the increase in R_F value is proportional to the quantity of alkaloid applied to a spot on the paper, and this is the basis of the proposed method of assay. Strips of filter-paper are dipped in McIlvaine's buffer solution (pH 4) and allowed to dry. Fresh alcoholic solutions equivalent to 10 to 30 mg. of the alkaloids are placed in spots on the strips, which are developed with chloroform saturated with 0·1 M citric acid, by the ascending technique. Slight variations of R_F values are encountered, as a result of which a minimum of 4 experiments for each unknown and each concentration of standard is required to achieve an accuracy of $\pm$ 2·5 to 3 μg. The method is suitable for general laboratory use when high accuracy is not necessary. G. B.

Salicylic Acid in Aspirin, Determination of. C. W. Strode, F. N. Stewart, H. O. Schott and O. J. Coleman. (*Analyt. Chem.*, 1957, **29**, 1184.) Spectrophotometric and visual techniques are described for the quantitative determination of salicylic acid in acetylsalicylic acid and in aspirin tablets (including those tinted with dyes) in amounts down to less than 0·005 per cent. Variables such as pH, temperature, rate of hydrolysis of acetylsalicylic acid are strictly controlled, a special correction being applied for this last variable which is proportional to the time elapsing during manipulation. A calibration curve is prepared by adding graded amounts of pure salicylic acid to a fixed amount of a solution of purified acetylsalicylic acid in ethanol and standard ferric alum solution and diluting to a suitable volume with water. The weight of test sample taken is such that the concentration of acetylsalicylic acid is the same as that in the solutions used for the calibration curve. Results of the spectrophotometric analysis of typical white, pink and green aspirin granulations containing 10 to 20 per cent starch show that the over-all precision is maintained. D. B. C.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Protein Component of High Molecular Weight in the Serum of Patients with Rheumatoid Arthritis. E. C. Franklin, H. R. Holman, H. J. Müller-Eberhard and H. G. Kunkel. (*J. exp. Med.*, 1957, **105**, 425.) In the sera of a number of patients with rheumatoid arthiritis an unusual high molecular weight protein could be detected by direct ultracentrifuge analysis of whole serum. The material sedimented more rapidly than the normal 19S component in the γ-globulin fraction and reached a concentration up to 340 mg. per cent. This high molecular weight material was also present in the γ-globulin fraction of serum and joint fluid. It had an $S_{20,w}$ of approximately 22S and could be dissociated into two fractions, one of which had a sedimentation coefficient of approximately 19S. The relationship between the unusual protein complex and various 19S γ-globulins and 19S antibodies is discussed. G.F.S.

Sphingosine as an Inhibitor of Blood Clotting. E. Hecht and D. Shapiro. (*Science*, 1957, **125**, 1041.) A powerful clot delaying substance, identified as sphingosine, has been isolated from brain tissue. It is a component of cerebrosides and sphingomyelins, it has been isolated from the liquid activator of pig brain and it seems to be the active principle of antithromboplastin, which appears in the blood of haemophiliacs in abnormally high quantities. Small quantities of sphingosine prolong the clotting time of chicken plasma to 40 hours or more but the inhibitory action is prevented by the liquid activator from pig brain. Sphignosine and certain derivatives have been synthesized, its structure is CH_3–$(CH_2)_{12}$–CH = CH–CHOH–CHNH_2–CH_2OH. The presence of the double bond as well as the free functional OH and NH_2 groups is essential for activity. The intensity of the reactions with sphingosine depend on its concentration, on the concentration of lipid activator and on the properties of the plasma. Two isomers of threoninol, which are the lowest homologues of dihydrosphingosine have been isolated and their oxalates found to have a powerful inhibitory influence on the clotting of chicken plasma. G. F. S.

BIOCHEMICAL ANALYSIS

Barbiturates in Biological Material, Separation and Identification of. L. G. Allgén. (*Scand. J. clin. lab. Invest.*, 1957, **9**, 71.) While spectrophotometric methods are used to differentiate between various barbiturates in some cases they are not satisfactory, particularly when low amounts are present and interfering substances are present. Paper chromatography has therefore been compared with spectrophotometric results in such cases. For paper chromatography of blood 10 ml. is extracted by shaking for 3 minutes with 50 ml. of chloroform, the chloroform is filtered and evaporated to dryness on a water bath. The residue is triturated with 0·1 ml. of ethanol for several minutes. For urine and gastric contents 50 ml. is acidified with dilute sulphuric acid and extracted with 100 ml. of chloroform and an aliquot (60 ml.) evaporated and the residue dissolved in 0·2 ml. of ethanol. Using Whatman No. 1 filter paper sheets the ethanol concentrates are applied as spots on the paper on a line about 7 cm. from one end, and one and three times 5 μl. of each solution applied by micropipette. Standards of diethylbarbituric acid are applied to each paper in amounts of 25 to 50 μg. in ethanol solution, and any barbiturate suspected is also applied as a standard. Descending paper chromatography is carried out using alkaline aqueous suspensions of various alcohols (ethanol, butanol, *iso*amyl alcohol, tertiary amyl alcohol, hexanol and benzyl alcohol). The time of running varies with the solvent used. The papers are air dried and the spots identified by ultra-violet illumination and by cutting out the spots, extracting with 4 ml. of 0·05 M pH 10 borate buffer followed by determination of the absorption curve at various wavelengths between 220 and 350 mμ in a spectrophotometer. Absorption curves were also obtained after adding 0·5 ml. of N sodium hydroxide to 3 ml. of eluate and then after adding 1 ml. of N sulphuric acid. A number of chemical spraying reagents have also been used including 0·02N potassium permanganate and 0·1N silver nitrate. The R_F values of a number of barbiturates are given and chromatographic findings from a number of patients suffering from barbiturate poisoning are surveyed. The paper chromatographic method takes too long for routine use, but it is useful in special cases. G. F. S.

Poliomyelitis Antibodies, Detection of. H. L. Hodes, H. D. Zepp, W. L. Henley and R. Berger. (*Science*, 1957, **125**, 1089.) A valuable screening test for distinguishing immune from non-immune persons is described. The upward spread of virus in strips of filter paper is decreased by serum containing specific antibody. Whatman filter paper No. 3 is cut into strips 12 by 1·75 cm. Each strip is marked off into 1 cm. spaces (numbered 1 to 12), suspended from a rubber stopper and autoclaved. Poliovirus cultivated in monkey kidney tissue is diluted to a concentration of 100 TCD50 per ml. in 0·85 per cent saline containing 10 per cent bouillon broth. Thirty ml. of the diluted virus is placed in a sterile bottle surrounded by ice. The serum to be tested (previously inactivated at 56°) is then distributed evenly over spaces 3 and 4 of the filter paper. The paper is placed in the bottle containing the virus with only the lower half of space 1 below the surface of the virus suspension. After 1 hour the strips of paper are removed, and each paper is cut off and placed in a monkey kidney tissue-culture tube. Tissue culture tubes are incubated and observed for virus cytopathogenic effects in the usual manner. Neutralising antibodies titres of the serums used in the paper tests are determined by standard tissue culture methods. In 52 successive duplicate tests with 14 human serums, virus was detected on every wet space of every paper strip on which the serum containing no antibody had been placed, while no virus was found above space 6 when the serum contained type-specific poliovirus antibody (usually not above space 4). Only 0·025 ml. of serum is required. G. F. S.

Protein in Biological Fluids, Microdetermination of. G. R. Kingsley and G. Getchell. (*J. biol. Chem.*, 1957, **225**, 545.) An investigation has been made to develop the most practical method for the microdetermination of protein in body fluids using tetrabromophenolphthalein ethyl ester (TBPEE) or its potassium salt. A simple accurate method is described for the estimation of total protein in spinal fluid and serum sensitive to 0·1 μg. For spinal fluid add 0·02 ml. of fresh spinal fluid to a standard photometer cuvette containing 4 ml. of water maintained at 25° in a water bath. Add 1 ml. of working indicator solution (a 1 in 10 dilution of a stock solution containing 134 mg. of TBPEE, both in methanol). Add 0·5 ml. of 0·004N acetic acid and mix, stand for 5 minutes at 25° and read the per cent transmittance of light at 600 mμ against a blank prepared with distilled water and set at 100 per cent transmission. For serum dilute 1 ml. of fresh serum to 100 ml. with saline, dilute 2 ml. of this mixture to 100 ml. with water, add 4 ml. of the final dilution to the cuvette and continue as for CSF. The accuracy of the new method compares very favourably with the biuret, phenol, Kjeldahl-nesslerization and turbimetric methods except with abnormal specimens where there was only good agreement in 1 out of 30 specimens. Apparently most abnormal spinal fluids contain protein which reacts as serum, and the method has been adapted satisfactorily for the determination of serum present in spinal fluid. The method was not satisfactory for the determination of urinary protein. G. F. S.

PHARMACOLOGY AND THERAPEUTICS

Antibiotic Treatment of Severe Bronchiectasis. Report by a Sub-Committee of the Antibiotics Clinical Trials (Non-Tuberculous) Committee of the M.R.C. (*Brit. med. J.*, 1957, **2**, 255). The trial was conducted at seven centres. Patients were between 15 and 55 years of age and all had had symptoms of bronchiectasis for at least three months. 112 patients were treated; 36 were treated with penicillin, 40 with oxytetracycline, and 36 with lactose. The drugs were provided as indistinguishable 0·25 g. capsules, two of which were given four times

a day on two days each week for a year. Regular measurements were made of the volume of a 24-hour sputum specimen and of the severity of cough, dyspnoea, haemoptysis and disability. Each of the groups showed a reduction in the sputum volume, the reduction being greater for the pus than for the mucus fraction, during the year. The reduction was rapid in the oxytetracycline group, and for pus to about half the pre-treatment level. The reduction in the penicillin and lactose groups was slower, and to about 70 per cent of the pre-treatment level in each. In each group there was some reduction in the severity of cough and dyspnoea and in the number of episodes of haemoptysis, the oxytetracycline group showing a slight advantage. During the period of treatment the patients receiving oxytetracycline suffered less severe interference with their lives. The number of days on which patients in this group were confined to bed was less than half the total for those receiving penicillin and a little over a quarter of those receiving lactose. The number of episodes of fever and the number of days off work was also less in the oxytetracycline group. The results as a whole indicated a definite benefit from oxytetracycline, and a probable but smaller benefit from penicillin. Even in the oxytetracycline group, however, the effect was not dramatic. It is clear that the response obtained and the expense entailed do not justify the widespread use of long-term oxytetracycline therapy in most patients with bronchiectasis, though for the relatively few advanced cases it offers a measure of relief not apparently obtainable by oral penicillin in the doses used. S. L. W.

Benzylpenicillin, Rectal Absorption of. K. Backe-Hansen. (*Scand. J. Clin. lab. Invest.*, 1957, **9**, 170.) Sodium lauryl sulphate has been shown to increase the rectal absorption of benzylpenicillin from suppositories prepared with cocoa butter or Imhausen Base H. Determination of the penicillin serum concentration in fifteen patients after insertion of suppositories containing 500,000 units of benzylpenicillin showed the absorption was rapid and maximal blood concentrations were attained within 30 minutes. These were on the average about one third of the maximum following intramuscular injection. G. F. S.

Cortisone: Influence on Teratogenic Effects of Hypervitaminosis-A. J. W. Millen and D. H. M. Woollam. (*Brit. med. J.*, 1957, **2**, 196.) An investigation was undertaken to explore the effect of cortisone administered to rats during pregnancy on the incidence of deformities of the brain and calvaria produced by hypervitaminosis-A. The pregnant rats were divided into three groups, each group consisting of 12 animals. Group I received 60,000 I.U. of vitamin A acetate daily from the 8th to the 13th day, inclusive, of pregnancy; Group 2 received 20 mg. of cortisone acetate daily from the 9th to the 12th day, inclusive; and Group 3 received 60,000 I.U. of vitamin A acetate daily from the 8th to the 13th day, inclusive, and 20 mg. of cortisone acetate from the 9th to the 12th day, inclusive. The rats were killed on the 20th day of pregnancy and the foetuses removed and inspected for abnormalities. In Group 1 the number of young with deformity of brain and calvaria was 6 out of 77 (7·8 per cent), in Group 2, there were none out of 73, and in Group 3 there were 15 out of 41 (36·6 per cent). These results suggest strongly that cortisone (while itself having no teratogenic action) potentiates the known teratogenic effect of hypervitaminosis-A. It may well be that the increased incidence of cleft-palate observed by Fraser and others in mice genetically susceptible to the condition was also due to the cortisone potentiating the expression of the inherent genetic weakness. S. L. W.

5-Hydroxytryptamine Antagonism by Lysergic Acid Diethylamide after Intracerebral Injection into Conscious Mice. T. J. Haley. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 428.) Intracerebral injection of 5-hydroxytryptamine into conscious mice was shown to give rise to scratching and stupor, whereas lysergic acid diethylamide, its 1-acetyl and 1-methyl derivatives and (+)-lysergic acid dimethylamide gave rise to hyperexcitability, piloerection, muscle incoordination and sensitivity. In addition, lysergic acid diethylamide produced a sensitivity to sound, muscle twitch in the lumbar area and a peculiar alternating stamping of the feet. Mixtures of 5-hydroxytryptamine and lysergic acid diethylamide or its derivatives were injected to detect any antagonism between these substances: lysergic acid diethylamide, and its 1-acetyl and 1-methyl derivatives were shown to block the central effects of 5-hydroxytryptamine, whereas lysergic acid dimethylamide did not. G. B.

Hypoglycaemic Drug, Pharmacology of. G. Ungar, L. Freedman and S. L. Shapiro. (*Proc. Soc. exp. Biol. N.Y.*, 1957, **95**, 190.) From a series of 200 new mono- and disubstituted alkyl and aralkyl derivatives of formamidinyliminourea *N*-β-phenylformamidinyliminourea (DBI) has been found to be a highly active oral hypoglycaemic agent in both normal and alloxan-diabetic animals. In guinea pigs, rats, rabbits, cats and monkeys hypoglycaemia reached its maximum in five hours and was back to normal in twenty-four hours. The monkey was the most sensitive. DBI failed to cause hypoglycaemia in dogs, but it reduced the blood sugar in alloxan-diabetic rats, rabbits and monkeys and maintained it at normal level. DBI differs from other orally active hypoglycaemic agents in not causing a significant change in the glycogen content of liver and muscle. Apart from its hypoglycaemic action DBI has no acute pharmacological actions and if the blood sugar is maintained by the administration of glucose, animals can tolerate very high doses. G. F. S.

Mecamylamine in the Management of Hypertension, Use of. F. H. Smirk and E. G. McQueen. (*Brit. med. J.*, 1957, **1**, 422.) The use of mecamylamine in the management of hypertension over a period of four to eight months is described. The drugs were given orally or by subcutaneous or intravenous injection to forty patients, many of whom had proved difficult to control with other ganglion-blocking agents. In contrast with the quaternary ammonium compounds mecamylamine was well absorbed from the alimentary tract so that the oral dose was little more than the parenteral dose. Tolerance to mecamylamine was slight and insufficient to cause difficulty in fixing the maintenance dose. Duration of action was longer than that of pentolinium or chlorisondamine: consequently control over blood pressure was easier. For the management of hypertension two doses a day are recommended, the evening dose being 30 per cent higher than the morning one. The average daily dose used was 33 mg. Adequate control of the blood pressure was in some instances hindered by the occurrence of side effects caused by parasympathetic ganglion block; these appeared to be rather more prominent than with pentolinium. There were, however, individual differences in patients, so that with equal falls in blood pressure, side effects were less in some patients when they were treated with mecamylamine and less in others when the drug used was pentolinium. Combinations of mecamylamine with rauwolfia alkaloids was also satisfactory. No delayed toxicity with the ganglion-blocking agent was encountered during the eight months' trial. G. P.

Muscarine Chloride, Pharmacological Actions of. P. J. Fraser. (*Brit. J. Pharmacol.*, 1957, **12**, 47.) The action of chromatographically pure crystalline muscarine chloride, prepared from *Amanita muscaria*, was compared with acetylcholine on a number of different organs from various species. Muscarine caused contraction of the smooth muscle of the gut, uterus, urinary bladder, and bronchi, both *in vivo* and *in vitro*. Isolated preparations of the ureter and carotid artery of the horse were also contracted, and the isolated auricles of the guinea pig and rabbit and frog's heart were slowed. The alkaloid caused a drop in blood pressure, although on the isolated rabbit's ear it produced either constriction or dilatation of the blood vessels. All the actions were qualitatively similar to the parasympathomimetic actions of acetylcholine, though muscarine was usually more potent, and, as with acetylcholine, the effects were readily prevented with atropine. In high concentration the drug had some contractural action on the frog's isolated rectus abdominis muscle, but had no neuromuscular blocking action on the rat diaphragm or on the cat gastrocnemius. Muscarine was destroyed neither by pepsin nor by boiling at any pH. It was inactive by mouth in a monkey in a dose many times that which would have been toxic in man. With true- or pseudo-cholinesterase, no hydrolysis or enzyme inhibition were seen. G. P.

Nalorphine, Analgesic Activity and Morphine Antagonism of Compounds related to. C. A. Winter, P. D. Orahovats and E. G. Lehman. (*Arch. int. Pharmacodyn.*, 1957, **110**, 186.) The analgesic and antimorphine properties of 70 compounds have been compared. Analgesic activity was determined in rats using the radiant heat method applied to the tail. For analgesic activity comparisons were made with morphine and for antimorphine activity with nalorphine. The results showed that length of the *N*-substituent chain had an important influence upon both analgesic activity and morphine antagonism. In the morphine series, compounds with 3-carbon chains, allyl, propyl, *iso*butyl and methallyl had some degree of antimorphine activity, with the *N*-propyl compound exhibiting activity of the same order as the *N*-allyl. Substitution of other elements or groups for hydrogen in the chain destroys the antimorphine activity without conferring analgesic properties. Lengthening the side chain beyond *N*-butyl yields potent analgesic agents. None of the morphine derivatives with aromatic side chains were antimorphine agents, but one of them, *N*-phenylethylnormorphine was an extraordinary potent analgesic. Both the *N*-allyl and *N*-propyl substituents yielded potent antimorphine compounds not only with morphine, but also with diacetylmorphine, dihydromorphine, desoxymorphine and dihydromorphinone. Both the *N*-allyl and *N*-propyl derivatives of dihydromorphinone were exceptionally potent antimorphine agents. Neither *N*-propylnorcodeine, *N*-propyldesoxynorcodeine nor *N*-propyldihydrodesoxynorcodeine was as active an antagonist as the corresponding *N*-allyl derivative. Neither the *N*-allyl nor the *N*-propyl derivative of either dihydrocodeine or of dihydrocodeinone was active. Tripropionylnormorphine and the *N*-allyl derivatives of *iso*morphinan and 6-methyl-$\triangle^{6}$-desoxynorcodeine had moderate antimorphine activity, while the 1-*N*-allyl-3-hydroxy-morphinan (levallorphan) was as active as nalorphine. *N*-Allylnorpethidine was an active analgesic like pethidine. G. F. S.

Pacatal and Chlorpromazine in Schizophrenia. J. Lomas. (*Brit. med. J.*, 1957, **2**, 78.) Fifty schizophrenic patients were treated with Pacatal and 50 with chlorpromazine. The usual dosage of either drug was 300 mg. daily. Assessment of results was made on a five-point scale by an independent observer

after 13 weeks treatment or when treatment had to be discontinued. Chlorpromazine was shown to be much more effective than Pacatal; only 8 of 50 patients receiving Pacatal were more than moderately improved as against 23 of those on chlorpromazine. Pacatal would appear to be less toxic than chlorpromazine. Dryness of the mouth occurred in almost all patients taking Pacatal; cycloplegia was also common, and constipation was occasionally troublesome, but less so than with chlorpromazine. Jaundice does not seem to be a risk in using Pacatal but, as with chlorpromazine, agranulocytosis is a dangerous complication. S. L. W.

Phenothiazine Derivatives, Central Depressant Activity of a New Series of. J. Schmitt, J. Mercier, M. Aurousseau, A. Hallot and P. Comoy. (*C.R. Acad. Sci., Paris*, 1957, **244**, 255.) Four derivatives of chlorpromazine in which the chlorine atom was replaced by an acyl radical were prepared and the pharmacological activity of one, 3-acetyl-10-(3-dimethylaminopropyl) phenothiazine (1522CB) examined. This derivative had high central depressant and antiadrenaline activity in the dog. The vasopressor response to 1 to 4 μg./kg. of adrenaline was abolished by 10 μg./kg. of 1522CB, and reversed by larger doses of the antagonist. A hypnotic action in mice was seen with subcutaneous injection of 1 to 3 mg./kg., a dose of 0·5 to 1 mg./kg. prolonging barbiturate sleeping time some two to three times. The analgesic action of morphine was also potentiated by these doses of 1522CB. Convulsions induced by direct cortical stimulation in the dog and EEG arousal responses with peripheral nociceptive stimuli were diminished, by 0·5 to 1 mg./kg. of the drug. These central actions could be explained by the depression the drug exerts on the brainstem reticular activating system. In addition 1522CB had a powerful anti-emetic action against apomorphine in the dog and reduced the body temperature in the mouse and rat. Cardiovascular reflexes such as the pressor response to anoxia or carotid occlusion were very sensitive to the action of the drug. Other actions included local anaesthetic activity comparable with cocaine, feeble atropine-like effects on the salivary glands and moderate antispasmodic and antihistamine actions. Intravenous injection in the dog caused hypotension and diminished cardiac output. Neuromuscular block in the rabbit with gallamine was increased by 1522CB. The LD50 in mice was 70 mg./kg. intravenously and 130 mg./kg. orally. Chronic toxicity tests in the rat revealed no untoward effects on hepatic or renal function or on blood formation. G. P.

Plastic and Red Rubber Giving-sets; Thrombophlebitis following Intravenous Transfusions. (*Lancet*, 1957, **272**, 595.) This is a report to the Medical Research Council on an assessment of the incidence of thrombophlebitis long-continued intravenous infusions in two series of recipients. In one series the infusions were given through red rubber sets often supplied by the National Blood Transfusion Service, in the second series the infusions were given through sets made of plastic (polyvinyl chloride with added "plasticiser" and "stabiliser"). In other respects the two series were closely comparable. Seven hospitals took part in the trial and 700 reports were received. The investigation showed that in the series in which plastic tubing was used the incidence of thrombophlebitis was approximately half of that in which red rubber tubing was used; the proportions of very severe and severe reactions were 32 out of 180 (18 per cent) for the plastic sets, and 64 out of 189 (34 per cent) for the rubber sets. It would be unwise to conclude that the cases of major thromophlebitis following infusion through plastic tubing were necessarily due to harmful substances contained in that tubing. S. L. W.

Quinine, Potentiating Effects of. P. D. Orahovats, E. G. Lehman and E. W. Chapin. (*Arch. int. Pharmacodyn.*, 1957, **110**, 245.) Studies in animals have shown that quinine potentiates analgesics, hypnotics and anaesthetics. In rats, quinine itself had no analgesic action in doses up to 1000 mg./kg., but it strongly potentiated the analgesic effect of morphine. It also potentiated methadone, pethidine, codeine and 6-methyl-δ-desoxymorphine. After quinine, nalorphine showed an analgesic action. Potentiation was only seen when the quinine was given before or with the analgesic, never when given afterwards. Quinine pretreatment in doses up to 200 mg./kg. did not increase the acute toxicity of morphine. The potentiating effect of quinine on analgesics was also seen in dogs, but it antagonised the excitant action of morphine in cats. Quinine itself did not produce signs of CNS depression, hypnosis or sedation, but it enhanced the hypnotic effect of pentobarbitone without increasing its toxicity. Quinine was also found to prolong the action of a variety of chemically non related hypnotic agents in rats, cats and rabbits. G. F. S.

Rauwolscine, Pharmacological Action of. J. D. Kohli, J. H. Balwani, C. Ray and N. N. De. (*Arch. int. Pharmacodyn.*, 1957, **111**, 108.) Rauwolscine is a stereoisomer of yohimbine isolated from *Rauwolfia canescens* Linn. which is closely related to *R. serpentina* Benth. Its adrenergic blocking activity has been compared with yohimbine and tolazoline by a number of methods. In inhibiting the adrenaline induced spasm on the guinea pig seminal vesicle, rauwolscine was about as active as yohimbine and about twice as active as tolazoline. It also markedly antagonised histamine and acetylcholine on this tissue. Rauwolsine was also as active as yohimbine in shortening the strychnine convulsion time of rats. In anaesthetised cats and dogs doses of 0·1 mg. to 2 mg/kg. caused a fall in blood pressure with recovery in 5 to 30 minutes and partial blocking of the blood pressure response to adrenaline was observed with 0·2 mg./kg. In 2 mg. doses it completely blocked the pressor effect of 10 to 20 μg. of adrenaline but not to 40 μg. so that it does not appear to be highly effective. Like dibenamine it blocks the influence of generalised sympathoadrenal stimulation. The blood pressure effects and adrenergic blockade of rauwolscine were the same as with yohimbine. Rauwolscine blocks the contracting effects of injected adrenaline and preganglionic stimulation on the cat nictitating membrane. In the cat perfused hind legs rauwolscine counteracts the vasoconstrictor effect of adrenaline. G. F. S.

Reserpine, Adrenergic Block by, in Man. B. Åblad. (*Acta pharm. tox. Kbh.*, 1957, **13**, 213.) A study is made of the mechanism of action of reserpine in man. Its effects on the reflex discharge of the sympathetic nervous system on the blood pressure, pulse frequency and muscle blood flow in healthy young adults are studied. The reflex discharge of the sympathetic nervous system was provoked by the subject immersing his feet in water at 12° for one minute. This rapidly caused a constriction of the blood vessels in the hand and forearm. To determine whether the sympathetic blockade is central or peripheral, the action of reserpine on the effects of an intravenous infusion of adrenaline or noradrenaline was studied. It was found that reserpine, 10 μg./kg. intravenously, partly inhibited the effect of small doses of adrenaline and noradrenaline on the peripheral resistance in skin and muscle, from 1 hour to 48 hours after its administration. The reflex vasoconstriction in skin and muscle was also partially inhibited by reserpine and to approximately the same degree as the effect of noradrenaline was inhibited. These results indicate that a considerable part of the decrease in peripheral resistance after reserpine depends on adrenergic block. M. M.

Senna Preparations, Clinical and Laboratory Assessment of. J. C. McC. Browne, V. Edmunds, J. W. Fairbairn and D. D. Reid. (*Brit. med. J.*, 1957, **1**, 436.) Controlled clinical trials designed to measure the relative clinical effectiveness of different senna preparations are described. In the trials comparison was made between results obtained for two B.P. preparations of syrup of senna and a dry granular preparation of senna pod (Senokot) and estimates of the potencies of the preparations by biological and chemical assay methods. Two groups of constipated patients were chosen: short-stay obstetric and long-stay chronically sick patients. In preliminary tests two criteria of potency were used: (i) the per cent of "satisfactory" results, one or more bowel movements occurring within a set period, and (ii) the total number of movements occurring within the time limit. The first criterion was found to be very subjective and was subsequently discarded in favour of the second. From the results obtained it was concluded that laxatives could be graded by the simple clinical trial described. Also, clinical results were in general agreement with those of both chemical assay of sennoside content and biological assay on mice. However, not only did apparently identical B.P. preparations of syrup of senna differ widely in their therapeutic effect, but, with average doses, the B.P. syrups tested were no better than inert controls. Deterioration of the B.P. preparations was confirmed by chemical assay. The dry granular preparation of senna pod was chemically stable and in doses usually prescribed had a potent laxative effect.

G. P.

Serotonin and Reserpine, Effects of Drugs on the Potentiation of Hexobarbitone Hypnosis produced by. G. C. Salmoiraghi and I. H. Page. (*J. Pharmacol.*, 1957, **120**, 20.) Small doses of lysergic acid diethylamide (LSD), a potent serotonin-blocking agent on isolated organs, has been shown to enhance rather than to block the potentiating effect of serotonin on hexobarbitone hypnosis in mice. A similar enhancing effect was also produced by small doses of other drugs which cause hallucinations in man (bufotenine, mescaline, and ibogaine) and also by small doses of brom-LSD (BOL), a lysergic acid derivative which does not cause mental changes in man when giving orally. The potentiating action of reserpine on hexobarbitone hypnosis is blocked by all these hallucinogen compounds in large and small doses. These results suggest that potentiation of the central effects of serotin rather than blockage may be responsible for the hallucinogenic effects of certain drugs, and that the effect of reserpine is not directly mediated by free serotonin released from body depots. G. F. S.

Tranquillising Drugs in Psychoneurosis. M. J. Raymond, C. J. Lucas, M. J. Beesley, B. A. O'Connell and J. A. F. Roberts. (*Brit. med. J.*, 1957, 2, 63). This is the report of a controlled trial of five drugs, claimed to reduce tension, on psychoneurotic out-patients selected as having a tension component in the symptomatology. Seventy-nine patients were included in the trial, but of these 4 returned faulty records, and 24 defaulted. Each patient received each drug for 2 weeks, and a placebo (lactose) for 2 weeks and recorded his assessment of the drug on a five-point scale daily. A randomised design was used. The dosage for all of the drugs was two tablets three times daily. The drugs employed (with dose per tablet) were as follows: amylobarbitone, 50 mg.; benactyzine, 1 mg.; chlorpromazine, 25 mg.; meprobamate, 400 mg.; "Sedaltine" (carbromal, 195 mg., bromyaletone 65 mg., aluminium hydroxide 100 mg., rauwolfia 0·25 mg., mephenesin 100 mg.). The average score for the placebo was close to a nil response, neither good nor bad. Amylobarbitone was highly significantly superior to the placebo. There was no significant difference between the other four drugs and the placebo. S. L. W.

APPLIED BACTERIOLOGY

Bacteria, Preservation of, by Drying on Cellulose and Alginate Fibres. D. I. Annear. (*J. appl. Bact.*, 1957, **20**, 17.) The author reports the recovery of viable organisms from dried films of suspensions of *Salmonella ndolo* on fibres of calcium alginate and cellulose (absorbent cotton). Organisms grown on nutrient agar were suspended in a preserving medium (10 per cent peptone, 10 per cent glucose) and this suspension was used to inoculate tufts of sterile absorbent cotton wool or calcium alginate. Ampoules of the tufts were connected to a vacuum pump and were sealed after drying for 24 hours. Bacteria were recovered from the fibres by shaking with 1 per cent sodium hexametaphosphate in the case of calcium alginate or with nutrient broth in the case of cellulose fibres. Viable counts were made on blood agar. A very high recovery of organisms (nearly 100 per cent) was obtained from either of the fibres. High recoveries were also obtained after the organisms had been dried on cellulose fibres and stored for a period of six months at room temperature. The method of drying was rapid and freezing, if it occurred at all, must have been very transient. The author considers that the films on the fibres, although very thin, are firm in structure, the fibres acting as shock absorbers when the ampoules are jarred.

B. A. W.

***Escherichia coli*, Measurement of Thermodynamical Quantities in the Disinfection of.** W. Kondo. (*Bull. med. dent. Univ., Tokyo*, 1957, **4**, 81.) The author gives an account of the thermodynamical interpretation of results obtained on the effects of temperature on the disinfection of *E. coli* by phenol. A temperature range of 30–42° was used over a range of phenol concentration of 0·021–0·055 M. Survivors were estimated by a colony counting method after an exposure to the bactericide of 4 hours. No regular relationship between the concentration exponent and temperature was found. It was calculated that the amounts of variation in resistance of individual bacteria to the treatment were independent of temperature. The rate constant of phenol disinfection increased exponentially with temperature in accordance with the Arrhenius equation. The value of total energy of activation was calculated as 52,520 cal., and the activation energy per mol. phenol in disinfection as 8,120 cal. Values of entropy of activation calculated by statistical theory of reaction rate were very high (near 100 cal./deg.), this being suggestive of a protein denaturation. A quantitative relation is suggested between phenol concentration, exposure time, percent survival in probability scale and temperature of exposure.

B. A. W.

Fungi, Cultivation and Identification of, with the aid of Membrane Filters. S. Funder and S. Johannessen. (*J. gen. Microbiol.*, 1957, **17**, 117.) A method of cultivating fungi on a membrane filter was found usually to give more rapid growth than could be obtained on solid media. A sterile absorbent pad was placed in a petri dish and was evenly moistened with about 2 ml. of sterile yeast water. A sterile membrane filter was placed over the pad with care not to entrap air. Filter discs were inoculated from cultures of the fungi and the dishes incubated at room temperature for 2–3 days. The filter disc, or a part of it, was dried at room temperature for 3–4 hours and placed on a few drops of immersion oil on a microscope slide. The membrane was rendered transparent within a few seconds and was then examined microscopically. The method makes the use of plate or slide cultures unnecessary.

B. A. W.

PHARMACOPOEIAS AND FORMULARIES

SUBSIDIA PHARMACEUTICA I (S.Ph.I.)*

Reviewed by J. P. Todd

The Scientific Centre of the Pharmaceutical Society of Switzerland, founded little more than two years ago, has just published a work of more than usual interest. Attention is first attracted by the style of presentation consisting of a binding cover and loose leaf system with numbering of the pages and division into sections to allow the insertion of new pages or even new sections when necessary. This system allows the work to be kept up to date without the inconvenience of addenda or new editions and is the most logical method of dealing with an ever-changing subject and might be adopted with advantage for some of our own standard works. The book is beautifully printed on high grade paper suitable to withstand the vicissitudes of a loose leaf system and the seven different sections into which it is divided are easily found by means of a thumb index printed on stout paper separators.

A second point of interest is the use of International Non-Proprietary Names as main reference titles in the Index of Names which constitute Section I. The classification of new remedies by systems which may depend on the Brand Name, the Common, Generic or Approved Name, the systematic chemical name or sometimes even on the therapeutic action makes life a little difficult for the busy practitioner in pharmacy trying to keep abreast of the modern Materia Medica. As in other spheres, whole-hearted International agreement on suitable short titles and their use in standard reference books could do much to solve this difficult problem. Our Swiss colleagues have pointed the way we should go.

Section I is, however, more than a list of new and better synthetics. In it can be found many old friends like Pyroxilin and Crystal Violet. Even in dealing with these humble substances there is valuable information in the wealth of cross reference indicating the title in other official works as well as the maker's name and the branded name of specialities where these exist. It is interesting to learn that Colloxylinum of the Ph. Helv. V. is nothing more exotic than Pyroxilin and that Crystal Violet (Internationally—Methyl Rosaniline) is the principal constituent of no less than six branded specialities.

In Section II a therapeutic system of classification is adopted. This section begins with an account of the structure, the physiology and functioning of the autonomic nervous system and concludes with a very useful list of the drugs and specialities affecting this system. The names of drugs bearing brand names or approved names are arranged in alphabetical order and their chemical structure is given along with indications for their use. Something has gone wrong with the numbering of the pages in this section and there are two pages numbered 25 and no page 23.

The remaining five sections are chiefly of domestic interest. They consist of a Section (III) obviously intended for amplification at a later date dealing with tests of identity and purity of some of the newer medicaments, and a section on galenical pharmacy, mainly an account of the preparation of sulphonamide suppositories using polyethylene glycols of varying complexity as the basis.

A commentary on the Prescriptions Magistrales which was formerly published separately, is included in Section V while Section VI describes a number of pieces of useful equipment suitable for the dispensary, including a bacterial filter, a still, a homogeniser and an ion exchange column for the preparation of purified water.

* Published by Schweizerischer Apothekerverein, Zurich 1, Sihistrasse 37

The final section gives tables and formulae for the dilution of liquids of different specific gravity and concentrations of active material.

The volume is complementary to the standard works of reference and is clearly intended to be enlarged with new material as the opportunity occurs. By widening the coverage and scope especially of Sections I and II this work could be made to occupy an important place among pharmaceutical reference books.

Professors Steiger and Büchi and their collaborators are to be congratulated on a fine beginning and encouraged to continue their efforts.

BRITISH NATIONAL FORMULARY 1957*

Reviewed by Miles Weatherall

This edition of the British National Formulary differs from its predecessors because the preparations listed in it are classified according to their use instead of their pharmaceutical form. The Notes for Prescribers of the main edition have been extended and used as preambles to the list of preparations in each group. The result appears to be as convenient for reference as the main edition and at first sight more likely to be helpful to the prescriber whose mind is not yet made up. However, this more rational arrangement of the contents, being unaccompanied by any more critical selection than in the main edition, emphasizes the tiresome consequences of prescribing "by habit and tradition", and the Joint Formulary Committee are to be congratulated on having made the unreasonableness of the present position more evident by their new arrangement. There are still 23 expectorants, 29 sedative applications to the skin and 30 purgatives listed, and it would be a remarkable physician who could quote different indications for each of them and support his indications with evidence from properly controlled clinical trials. If all these preparations are therapeutically effective (about which there may be some reasonable doubt) it would not be difficult to compare their activities experimentally and show which were best. The hard facts of such trials would be more informative than some of the conventional observations contained in the present Prescribers' Notes, and a reduction in the number of identically active (or inactive) remedies which are put before the practising doctor would be no bad thing.

*Alternative Edition based on a Pharmacological Classification. Pp. 245 (including Index). The British Medical Association and the Pharmaceutical Press, London. 7s. 6d.; interleaved copy, 10s. 6d.