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REVIEW ARTICLE

SCLERENCHYMA IN THE DIAGNOSIS AND ANALYSIS OF VEGETABLE POWDERS

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Introduction.

Sclerenchyma is the name given to any hard vegetable tissue other than vascular tissue. There are two types of sclerenchyma, *viz.*:—(a) parenchymatous sclerenchyma, the cells of which are termed sclereids or stone-cells, and (b) prosenchymatous sclerenchyma, the cells of which are termed fibres. Both sclereids and fibres have heavily thickened walls which are usually lignified; in a few plants, however, cellulosic sclerenchyma occurs, as in the endosperm of the date, *Phœnix dactylifera* Linn., and in that of the corozo or vegetable ivory nut, *Phytelephas macrocarpa* Ruiz et Pav., which are composed of sclereids, and in the pericycle of flax and hemp and the phloem of mezereon and slippery elm barks, in which the fibres are unlignified. The striking appearance and, when lignified, the strong staining reactions of these cells render them easily identifiable and, since as long as a century ago, they have been regularly used as a means of identifying vegetable materials, such as tea (sclereids) and cinchona barks (fibres), (see Fig. 1 R and G).

The identifications were based at first upon the form, abundance and manner of distribution of the sclerenchyma. Measurements were not usually given for sclereids and only rarely for fibres, although they were used for starches and blood-corpuscles and for materials like lupulin and lycopodium which are composed of discrete particles. Towards the end of the nineteenth century measurements of the length and width of cells in sclerenchyma began to be made as a routine addition to the verbal descriptions. The dimensions given were used chiefly as a record of observed facts and also for the purpose of making drawings to scale. Greenish (1903) in his "Foods and Drugs" and Greenish and Collin (1901 to 1904) in their "Anatomical Atlas" advocate and use measurements for starches, but only rarely for crystals of calcium oxalate, e.g., in rhubarb, squill, and orris, and very occasionally for the dimensions of cells; otherwise only vague statements such as "very large cells", "short hairs", "small rosettes", etc., are made about dimensions. Tschirch and Oesterle (1890) introduced many measurements into their "Anatomischer Atlas" and, about the same date, measurements began to be used for the characterisation of certain drugs, such as the different cinchonas and for the exclusion of particular adulterants, such as cassia bark when substituted for cinnamon. Ludwig Koch in his atlas "Die mikroskopische Analyse der Drogenpulver" 1900-1908 made a still more systematic use of dimensions of cells and carefully recorded the linear measurements of all the structures present in the powders and drugs he

examined. These dimensions, however, considered as criteria of purity, are not very satisfactory, since they fail to exclude quite large percentages of adulterants and, moreover, may be indecisive when attempting to establish merely the identity of a powdered drug.

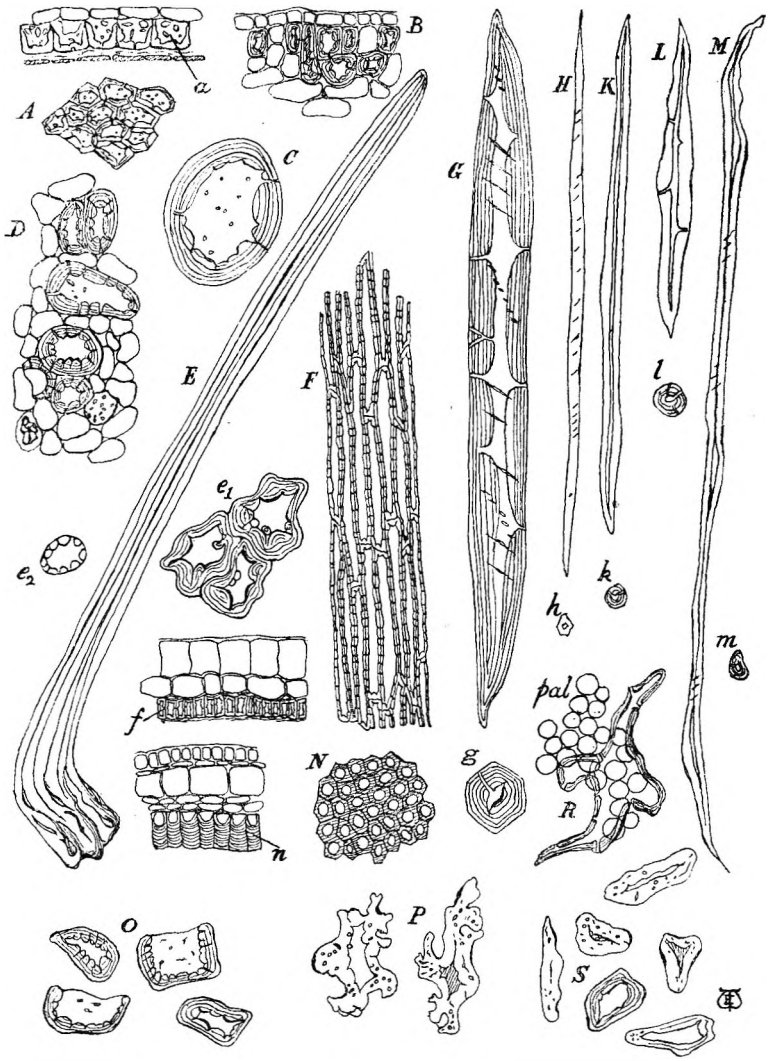

FIG. 1.—Typical Sclereids and Fibres, all × 150. Sclereids of A, beaker-cell layer of *Piper nigrum* Linn., *a*, the same in section; B, hypodermal layer of *Piper nigrum* Linn., C, *Juniperus phœnicea* Linn., D. *Eugenia caryophyllus* (Spreng.) Sprague; E, *Strychnos nux vomica* Linn., e_1, section of trichome bases; e_2, section of limb of trichome; F. *Linum usitatissimum* Linn., f, the same in section; N. *Elettaria cardamomum* Maton var. *minuscula* Burkill, n, the same in section; O. *Cinnamomum zeylanicum* Nees; P. *Viburnum prunifolium* Linn.; R. *Camellia sinensis* (Linn.) O.Ktze. pal, palisade cells; S, *Rhamnus purshianus* D.C.; G. Fibres of *Cinchona succirubra* Pav.; H, *Rhamnus purshianus* D.C.; K. *Cinnamomum zeylanicum* Nees; I, *Sassafras variifolium* (Salisb.) O. Kuntze; M. *Quillaia saponaria* Molina; g, h, k, l, m transverse sections of the corresponding fibres.

SCLEREIDS PER SQUARE MILLIMETRE.

When the cells to be measured form a continuous layer one cell in thickness, it is possible to make an improvement upon simple linear dimensions; this is effected by counting the number of cells per sq.mm. of the layer. The values so obtained provide an automatic averaging of the breadth and length of a very large number of cells, thus yielding data which can replace or supplement linear measurements, the ranges of which frequently overlap so much as to give inconclusive results, when such measurements are used to differentiate between similar tissues derived from closely related plants. The improvement effected has been demonstrated for the sclerenchymatous layer of the testa of various types

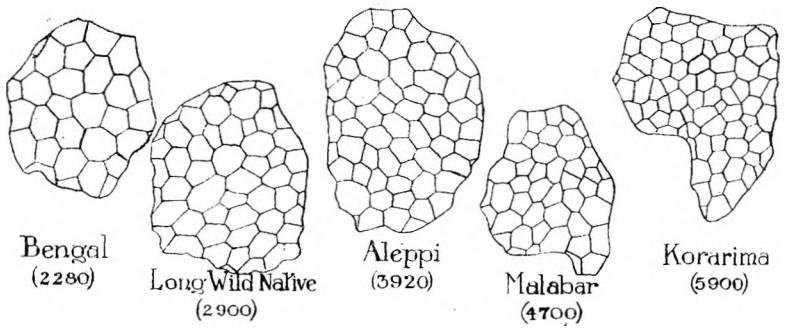

FIG. 2.—Outline of cells in typical fragments of sclerenchymatous layer of cardamom seeds (× 175).

of cardamom seeds (Fairbairn[1]). The drawings in Figure 2 show how difficult it is to make distinctions by the measurements of the diameters of individual cells, but the corresponding numerical values given beneath each piece of sclerenchyma are evidence of the much increased differentiation obtained by their use. These quantities allow eight varieties of cardamoms to be arranged in four distinct groups and, by supplementing the counts with other histological characters, the individual varieties can all be distinguished both in the unground condition and in the form of powder (Fairbairn[2]). This method deserves consideration wherever it is applicable and, in addition to the results for cardamom seeds, values already exist for the sclerenchymatous beaker-cell layer of pepper, viz. 1464 ± 200 beaker-cells per sq.mm. (Wallis and Santra[3]) and for the epidermal sclerenchyma of nux vomica seeds, viz. 570 epidermal sclereids per sq.mm. (Wallis and Fairbairn[4]).

SCLEREIDS PER MILLIGRAMME

Further advances in the use of sclerenchymatous tissues have been made by introducing the concept of mass into the values obtained. When this is done, it becomes possible, not only to identify the materials concerned, but also to assess the purity and to determine the proportions present in a given powder. Where the sclereids are isolated as in French savin, *Juniperus phœnicea* Linn., see Figure 1C (Flück and Haller[5]), or are loosely associated as in clove stalks, see Figure 1D (Wallis and

Santra[6]), and in the hypodermal sclerenchyma of pepper, see Figure 1B (Wallis and Santra[7]) and in the pericycle of ipecacuanha stem (Lupton[8]), the number of sclereids per mg. is easily counted. This is done by using the lycopodium method (Wallis[9]) and staining the material with phloroglucin and hydrochloric acid. The results for the materials quoted are as follows:—

Materials Examined.	Sclereids per mg.
French savin	210
Clove stalks	1,067
Pepper (hypodermal sclereids)	4,585
Black Pepper Husks (hypodermal sclereids)	13,230
Mysore Cardamom seeds (beaker-cells)	9,154
Black Pepper (beaker-cells)	1,500
White Pepper (beaker-cells)	1,619
White Pepper shells (beaker-cells)	10,783
Ipecacuanha stem	33

Measurement of Length per Milligramme

Although the number of epidermal sclereids per mg. of nux vomica seeds has been determined by careful manipulation and found to be 288 per mg. (Wallis and Fairbairn[4]), this value cannot be used as a means of determining nux vomica in powder, because the trichomes obscure the outlines of the cell bases. Each epidermal sclereid is prolonged into a trichome, the limb of which is traversed longitudinally by several (average number 11) narrow lignified strips varying in width from 3 to 10μ, see Figure 1E, e_1 and e_2. In the powder of the seed the strips separate and become broken into small fragments, which have a very characteristic appearance and are easily recognised. It is preferable therefore and comparatively simple to determine the length per mg. of the fragments of lignified rib derived from the trichomes. For this purpose, the powdered nux vomica is mixed with lycopodium and stained with safranin and the lengths of the fragments of rib are measured by using a camera lucida In this way, it is found that there are, on the average, 184 cm. of rib per mg. of air-dry nux vomica (Wallis and Fairbairn[4]).

All these values involving structural units per mg. can be used either to assess the purity of the material concerned or to determine the amount of any one of them in admixture with other substances.

Measurements of Area

Fibres in vegetable materials are more difficult to count than sclereids; this is largely because of their length and the difficulty of deciding how many fibres are represented by the broken portions found in the powders. To obtain satisfactory results with powders of No. 85 fineness, work must, at present, be limited to those materials in which the fibres occur either isolated, as in sassafras bark, or are arranged in single rows, as in cinchona, cassia and cinnamon barks; fibres in bundles cannot be successfully counted in a No. 85 powder. The difficulty of counting the fragments present is best surmounted by finding the total area of the

fragments of fibres present; this is done by tracing the outlines of the fragments with a camera lucida and finding their area by cutting out the tracings and weighing them. In conjunction with the lycopodium method, the area of fibre per g. is determined. This procedure was adopted for the fibres of powdered cinnamon and powdered cassia barks. The number of fibres in the phloem of cinnamon is considerably greater than in the phloem of cassia; moreover, the cork and cortex are removed from cinnamon, but not from cassia and this still further increases the difference between them. The fibres of cinnamon are somewhat more slender, but slightly longer than those of cassia, so that the area of the outlines of individual fibres in the two barks is not very different. The area of fibre-outline per mg. which summates number, length and breadth is therefore markedly greater in cinnamon than in cassia. The values obtained (Saber[10]) are:—

Cinnamon 80 to **85** to 91 sq.cm. per g.
Cassia 11 to **12** to 13 sq.cm. per g.

For these two barks therefore the values are widely separated and can be used, not only to characterise the barks themselves, but also to determine accurately the proportion of either in a mixture of the two or in compound powders.

Area measurements are also used for sclereids which form a layer one cell thick, as in linseed (see Fig. 1 F) (Saber[11]) and in cardamom seeds, see Fig. 1 N, (Fairbairn[1]), the result being expressed as area of the layer per mg. The values found for these seeds are:—

Linseed 28 to **32** to 35 sq. cm. per g.
Cardamom (Mysore) 27 to **28·5** to 29·7 sq. cm. per g.

These values can be used to determine the proportion of linseed in mixed cakes, etc., and of cardamom seeds in mixed spices and compound powders.

Many aërial stems are strengthened to withstand lateral strains by a tubular development of sclerenchyma, often in the pericycle, sometimes in the inner layers of the cortex. The stem bases attached to the rhizomes of *Valeriana officinalis* Linn., show a well-developed cylinder of rectangular sclereids in the inner layer of the cortex and there are similar cells in the bases of the petioles of the same plant. It has been proposed to use these diagnostic elements to determine the proportion of stem and leaf-bases present in powdered valerian rhizome (Flück and Haller[5]). The amount of these sclereids is measured in terms of their area obtained by multiplying together the length and breadth of the particles of sclereid layer seen in the powder. In this way 6·83 sq.cm. of sclereid layer was found to be present in one gram of powdered stem and leaf base. Flück and Haller suggest that the Swiss Pharmacopœia might introduce a standard for powdered valerian rhizome of not more than 0·35 sq.cm. of these sclereids per g., corresponding to just over 5 per cent. of stems in the drug.

Three-Dimensional Masses of Cells

In many substances, sclereids are present in masses, which may be limited in extent and approximately ovoid in shape, as in pimento, or

they may form a dense tissue, as in olive stones. These groups and tissues occur in the powder as three-dimensional particles and it is impossible to count accurately the individual cells in these particles. This difficulty can be overcome either by breaking down the particles into individual cells or by finding some method of calculating the number of cells from the number visible on the upper surfaces of the masses. For breaking down the particles, the oxidising agent used is nitric acid; an important drawback to this method is that the removal of lignin by the oxidising agent modifies or destroys the staining reactions of the cells, thus making it more difficult to identify them in the operation of counting. In attempting to devise a method for making calculations; it is evident that the shape of a mass built up of cells must depend to a large extent upon the shape of the constituent cells. If the cells are chiefly longer than wide, an ovoid mass might be expected, but if they are isodiametric, a subspherical mass would result. For both types of cell aggregate a count of cells along two axes at right angles would give a good estimate of the diameter of the sphere, when the mass is subspherical, or of an imaginary equivalent sphere, when the mass is more or less ovoid (see Fig. 3). Then using the formula for volume of a sphere, viz. $\frac{4}{3}\pi r^3$, the number of cells is calculated. If a sufficient number of particles is used—about 12 to 20—the result of the calculation method agrees with that obtained by disintegration and, since calculation is more rapid and involves no change in staining reaction of the cells, it is to be preferred. Proceeding in this way it has been found (Wallis and Santra[12]) that pimento contains 3546 ($\pm$ 200) sclereids per mg.

Powdered olive stones consist of a certain number of individual cells

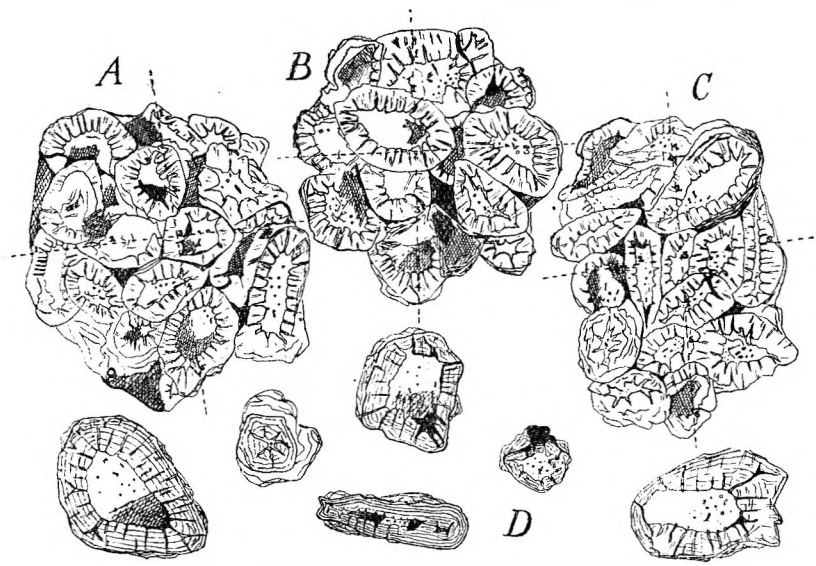

FIG. 3.—A, B, C, three typical masses of sclereids isolated from powdered *Pimento officinalis* Lindl.; D, individual sclereids of various shapes and sizes from powdered pimento. All × 200. The dotted lines are the diameters across which the numbers of cells were counted in calculating the radii of the equivalent spheres.

and broken pieces as well as particles of various shapes consisting of masses of sclereids. The counting of the separated individual sclereids presents no special difficulty, but the particles consisting of masses of cells are much more irregular than the ovoid groups found in pimento. When, however, the same convention of an equivalent sphere is applied to them, it was found that, if at least 12 particles are used, the number of sclereids obtained by calculation agrees with the number found by disintegration. A standard value for olive stones was thus determined, viz:— 15,140 (±900) sclereids per mg. (Wallis and Santra[13]). This value has been successfully applied for the determination of powdered olive stones added to pepper.

Quantitative Distribution of Tissues

By utilising the number of sclereids per mg. of black and white pepper and of the various products (see above) obtained commercially in the grinding of pepper, it has been possible to obtain a quantitative measure of the distribution of the tissues in the fruit of *Piper nigrum* Linn. The values thus established can be used to assess the proportions of the different parts of the fruit, which should be present in commercial products obtained from peppercorns (Wallis and Santra[3]). The quantitative distribution of the tissues in black pepper berries and their products is as follows:—

Black pepper shell in pepper fruit	34·6 per cent.
White pepper shell in pepper fruit	13·9 per cent.
Perisperm in pepper fruit	51·5 per cent.
White pepper shell in white pepper	14·3 per cent.
Perisperm in white pepper (by difference)	85·7 per cent.

Reliability of the Numerical Values

Justification for accepting, as reliable and satisfactory, the general method of working by the use of lycopodium has been provided in connection with many of the experiments. This has been done by making a duplicate and independent determination of the result by a method which did not involve the use of lycopodium. For several commodities the required value can be found by the use of calculations based upon geometrical data derived from measurements of the area or volume or some other character of the unground substance. Whenever this has been done, working with sclereids it has been used for linseed, nux vomica and pepper, the values obtained have always been in remarkably close agreement with those found by the lycopodium method applied to the powder of the same material. Although this type of independent check cannot be made for every powder, the fact that it has confirmed values for a number of materials to which it is applicable gives justification for claiming a similar accuracy for all the materials examined. In this way complete confidence in the results has been established so that, when they happen to differ markedly from results obtained from powdered materials by other methods, the cause of disagreement must

be sought either in some defect inherent in the other method or in some difference in the actual substance examined.

When determining the number of cells per sq.mm. of cardamon seeds the results from the first series of experiments showed a rather large variation, which was greater than was desirable and trials showed that altering the details of manipulation yielded no improvement. It therefore appeared that the variations were probably due to variations in the number of pieces of sclerenchymatous layer used for each value obtained; these varied from 8 to 16 pieces selected at random from a powder of No. 85 fineness. Although a fairly precise estimate of the minimum number of pieces to be used could be made by considering the experimental figures obtained, it seemed desirable to obtain mathematical confirmation of the validity of the deductions made from the experiments. It was, therefore, determined to examine statistically the effect of using different numbers of pieces of the sclerenchyma. For this purpose the mean of the individual results from each of 98 pieces was found and the standard deviation was determined. Curves were then constructed to represent the limits of error above and below the mean that could be obtained by using gradually increasing numbers of pieces of the sclerenchyma. Two curves were constructed, one showing limits for errors in 67 per cent. of the counts and the other in 99 per cent. of the counts. In this way it was shown (Fairbairn[14]) that, when the number of pieces is about 36, the limits of error become fairly constant and for 99 per cent. of the determinations the limit of error is ± 8 per cent. and for 67 per cent. it is ± 3 per cent. This statistical examination creates confidence in the experimental figures based on counts of 36 pieces; it also gives a reliable measure of the amount of variation to be expected and therefore assists in attributing a correct degree of specificity to the values obtained.

Conclusion

A review of these studies of sclereids and fibres, made during the last 15 years, reveals the much extended information which can be gained by applying to them the concepts of number, length, area, volume and mass in addition to the simple observational concepts of form and location. Similar advances have been made in the study of other groups of tissues, but they cannot be discussed in this article.

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RESEARCH PAPERS

A TITRATION METHOD FOR THE DETERMINATION OF PROCAINE IN PROCAINE PENICILLIN AND ITS OILY SUSPENSIONS

By W. H. C. Shaw

Received March 31, 1949

The essential characteristics of procaine benzyl penicillin ("procaine penicillin") have been described by Salivar *et al*[1]. It is a compound of one molecule each of procaine base and of benzyl penicillin (Penicillin G) with one molecule of water of crystallisation. Thus the theoretical composition is procaine 40·12 per cent., water 3·06 per cent. and penicillin potency 1008 I.U./mg., calculated on the basis of sodium benzyl penicillin as 1667 I.U./mg.

Procaine penicillin has been formulated for injection as a water-dispersible powder and also as a sterile suspension in arachis oil. Of these the latter is at present the more commonly used and normally contains 300,000 units of penicillin combined with 120 mg. of procaine base in each ml. This preparation may also contain 2 per cent. of aluminium monostearate as suspending agent[2]. The high dosage and the toxicity of free procaine required the development of a rapid and accurate method for determining the procaine content of procaine penicillin both in the dry state and also when in oily suspension. Published methods for the determination of procaine include bromination, titration of the base with standard acid following either extraction with chloroform or separation by distillation[3] and a colorimetric method for small amounts[4]. A spectrophotometric method for the determination of procaine in procaine penicillin G has recently been described[5]. It is well known, however, that most primary and secondary aromatic amines can be made to react quantitatively with nitrous acid. This method is used in the Pharmacopœial assay process for sulphanilamide and other sulphonamides but has apparently not been applied to procaine hydrochloride.

The object of the work described here was to investigate whether this reaction could be applied to the determination of procaine, particularly when in the forms already mentioned. The high cost of penicillin required that the amount used for each assay should be as small as possible and for this reason attention was directed to testing on a semi-micro scale.

Experimental

(I) *Titration of procaine hydrochloride.* Procaine hydrochloride B.P. was recrystallised twice from water and dried first at 60°C. and finally *in vacuo* over phosphorus pentoxide. Moisture content (Fischer reagent) 0·04 per cent., m.pt. 154·8° to 155·5°C. Found: C, 57·4; H, 7·72; N, 10·1*; ionisable chlorine, 13·00 per cent.

* I am indebted to Dr. F. R. Cropper for the results of all micro analyses quoted in this paper.

$C_{13}H_{20}N_2O_2$,HCl requires C, 57·23; H, 7·76; N, 10·27; ionisable chlorine, 12·99 per cent.

About 0·8 g. of the purified salt was dissolved in 15 ml. of 3N hydrochloric acid and the solution diluted to 150 ml. with distilled water. The solution was adjusted to 15°C. and titrated slowly (0·1 ml. at a time towards the end of the titration) with N/10 sodium nitrite solution. The end-point was taken when a drop of the titrated liquid gave an immediate blue colour on starch-iodide paper 2 minutes after the last addition of nitrite.

A blank determination omitting the procaine was carried out and the titre deducted. The results of a series of these titrations are given in Table I.

The determination was repeated on a semi-micro scale by dissolving about 0·1 g. of procain hydrochloride in 15 ml. of 3N hydrochloric acid and diluting to 50 ml. with water. After adjustment of temperature the solution was titrated as before with N/10 sodium nitrite added from a 5 ml. microburette in 0·02 ml. quantities towards the end of the titration. These results are also given in Table I.

TABLE I

TITRATION OF PURIFIED PROCAINE HYDROCHLORIDE WITH STANDARD SODIUM NITRITE SOLUTION

Procaine hydrochloride taken	N/10 sodium nitrite f.1·005	Procaine hydrochloride
g.	ml.	per cent.
0·8009	29·22	99·93
0·8547	31·18	99·91
0·7525	27·44	99·88
0·8055	29·40	99·95
0·9033	32·93	99·86
0·1104	4·04	100·2
0·1238	4·51	99·8
0·1168	4·28	100·3
0·1218	4·45	100·0
0·1203	4·385	99·8

(II) *Titration of procaine penicillin.* (*a*) *Extraction of procaine from procaine hydrochloride.* When the above semi-micro procedure was applied to procaine penicillin fictitiously high results were obtained, apparently due to reaction of penicillin with nitrous acid. This was confirmed by titrating a sample of crystalline sodium penicillin in dilute hydrochloric acid solution with N/10 sodium nitrite. Slow absorption of nitrous acid occurred and no definite end-point was obtainable. Separation of procaine from penicillin was, therefore, necessary and an extraction technique was devised for this purpose. The procedure was first applied to procaine hydrochloride itself in order to check the efficiency of the extraction. The procaine hydrochloride used for this assayed 99·5 per cent. by the macro method given under (I).

About 100 mg. of procaine hydrochloride, accurately weighed, was dissolved in 50 ml. of water and the solution transferred to a separating funnel. 5 ml. of M ammonium hydroxide was added and the solution extracted successively with 20, 5, 5, and 5 ml. quantities of chloroform, previously washed by thorough shaking with an equal volume of

water.* Completion of extraction was checked by carrying out a fifth extraction with 5 ml. of chloroform, this being extracted with dilute hydrochloric acid and the acid solution tested for traces of procaine as described later. Each chloroform extract was run in turn into a 4 oz. wide-mouth glass stoppered bottle containing 35 ml. of water and 15 ml. of 3N hydrochloric acid. After adjusting the temperature to 15°C. the chloroform and acid layers were titrated with vigorous stirring with N/10 sodium nitrite solution in the manner described above until a reaction on starch-iodide paper was obtained. The stopper of the bottle was then inserted and the contents vigorously shaken for 30 seconds. After separation had occurred the titration of the aqueous layer was continued with stirring until an end-point reproducible after 2 minutes was obtained.

It was observed that any delay between addition of ammonia and extraction with chloroform led to low results. Decomposition of procaine in alkaline solution with the formation of *p*-aminobenzoic acid is well known[6] and this was thought to be the cause of the low results. To test this and to show that the presence of penicillin did not interfere with the extraction the following three separate procedures were adopted:— (i) Extraction after addition of ammonia was delayed for a series of time intervals. (ii) 50 mg. of *p*-aminobenzoic acid was added before making alkaline. (iii) An approximately equimolecular proportion of crystalline sodium benzyl penicillin was added before making alkaline. The results obtained are given in Table II.

TABLE II

RESULTS OBTAINED USING THE EXTRACTION PROCEDURE ON PROCAINE HYDROCHLORIDE

Procaine hydrochloride	Crystalline sodium penicillin G added	Delay in extraction	N/10 sodium nitrite (f. 0·994)	Procaine hydrochloride (Mol. Wt. 272·6)
mg.	mg.		ml.	per cent.
117·4	—	None	4·31	99·5
120·4	—	,,	4·44	99·9
122·5	—	,,	4·43 (f 1·009)	99·4
127·5	151	,,	4·61 (f 1·009)	99·4
123·7	158	,,	4·545	99·6
122·1	150*	,,	4·49	99·6
119·7	—	½ hr.	4·36	98·7
122·9	—	,,	4·37 (f 1·009)	97·8
123·2	—	1 hr.	4·33 (f 1·009)	96·6
116·0	—	,,	4·14	96·7
118·6	—	3 hrs.	3·99	91·1
115·1	—	,,	3·86	90·9

* 50 mg. of *p*-aminobenzoic acid also included in this assay.

(b) *Extraction from procaine penicillin.* Direct application of the method used in (a) was inapplicable to procaine penicillin on account of its low solubility in water. About 280 mg. of procaine penicillin was, therefore, dissolved by warming in 15 ml. of chloroform contained in

* NOTE: In the presence of chloroform B.P. slow absorption of nitrous acid occurred in the blank titration but this could be prevented by prior washing of the chloroform with distilled water.

a beaker and the solution poured into a separating funnel containing 50 ml. of water. A further 5 ml. of chloroform and 5 ml. of M ammonia were used in turn to rinse the beaker, each being added to the separating funnel. Extraction and titration procedure were as described above.

The sample* of procaine penicillin G used for this and replicate determinations assayed 1038 I.U./mg. (*Staphylococcus aureus*), 1016 I.U./mg. (*Bacillus subtilis*) water content (Fischer reagent) 3·4 per cent. Found: C. 59·15; H, 6·75 per cent.; N, 9·8; S, 5·31 per cent. $C_{29}H_{38}N_4O_6S,H_2O$ requires C, 59·17; H. 6·85; N, 9·52; S, 5·45 per cent.

As a check on possible interference by low potency penicillin one assay where this was added is included in Table III.

TABLE III

REPLICATE DETERMINATIONS OF PROCAINE IN PROCAINE PENICILLIN

Weight taken	N/10 sodium nitrite f. 1·006	Procaine
mg.		per cent.
290·0	4·90	40·1
279·5	4·71	40·0
292·1	4·91	39·9
281·5	4·72	39·8
274·6	4·60	39·8
283·1	4·755	39·9
291·6	4·92	40·0
271·0	4·54	39·8
280·2	4·73	40·0
273·1	4·57	39·8
282·2	4·73	39·8
270·1	4·54	39·9
290·1*	4·86	39·8

* Sodium penicillin 0·1 g. (potency 547 I.U./mg.) added.

(III) *Titration of Suspensions in Oil.*

Accurate weights of the procaine penicillin used in (II) (b) were converted into suspensions in oil, using:—(i) 300,000 units in 1 ml. of arachis oil, (ii) as above, but with 2 per cent. of aluminium monostearate.

The procaine in these preparations was determined using the same technique as that previously adopted for procaine penicillin itself. The results are given in Table IV.

TABLE IV

DETERMINATION OF PROCAINE IN PROCAINE PENICILLIN WITH ADDED ARACHIS OIL AND ALUMINIUM MONOSTEARATE

Procaine penicillin taken	N/10 sodium nitrite f. 1·006	Procaine
mg.	ml.	per cent.
With arachis oil ... ... 290·5 ...	4·89	40·0
293·8 ...	4·93	39·9
With arachis oil containing 2 per cent. w/w aluminium monostearate ... ... 284·1 ...	4·78	39·9
281·7 ...	4·71	39·7

The same procedure was then applied to several routine manufactured batches of procaine penicillin suspension in arachis oil (with and without aluminium monostearate).

* I am indebted to Mr. D. H. Geard for this sample and to Mr. C. R. Bond for the results of all penicillin assays quoted.

These results are given in Table V.

TABLE V
REPLICATE DETERMINATIONS ON PROCAINE PENICILLIN OILY SUSPENSION (300,000 I.U./ML.)

Sample	Density at 20° C.	Penicillin potency I.U./ml. (Iodimetric assay)	Replicate procaine determinations (calculated to Mol. Wt. 236·1)
			per cent. w/v
A	0·994	295,000	12·0 12·0 12·0 12·0 12·0
B	0·996	303,000	12·2 12·1 12·2 12·1
C	1·001	309,100	12·6 12·6 12·6 12·4
D*	1·002	304,600	12·4 12·3 12·3 12·3

* Containing 2 per cent. w/w of aluminium monostearate.

CONCLUSIONS

Results given in Table I show that procaine may be determined satisfactorily under macro and semi-micro conditions by direct titration in acid solution with standard sodium nitrite.

By employing an extraction procedure, interference by penicillin may be eliminated with no loss of accuracy. The results in Table II show that extraction should follow immediately after addition of ammonia, otherwise, low results, due to decomposition of procaine, are obtained. For the same reason the chloroform extracts should be run directly into dilute acid.

The presence of chloroform in the final solution for titration in no way affects the results, but the emulsion formed by stirring or shaking during titration should be allowed to separate before removing a drop of the aqueous layer for spotting on to starch-iodide paper. In the presence of penicillin the first chloroform extract is normally turbid, but this has not been observed to affect the results. Vigorous shaking may produce emulsions that separate slowly and should, therefore, be avoided.

Although with a suitable microburette the volume of titrant used may be read to 0·002 ml., experience has shown that the end-point cannot be estimated to better than 0·02 ml., corresponding to an error of about 0·5 per cent. on a volume of 4 to 5 ml. This is borne out by the results quoted in Table III. Whilst this error would be correspondingly reduced by taking a larger sample for assay, it was considered tolerable for routine application.

It will be noted that starch-iodide papers have been used throughout

in place of the starch iodide paste recommended in the British Pharmacopœia for use in the assay of sulphanilamide. Papers have been found much more convenient, and they are reasonably stable if prepared and stored as described below.

Recommended Method

Special Reagents.

(a) *Chloroform.* Wash chloroform B.P. by shaking thoroughly with an equal volume of distilled water. Allow complete separation to take place and run off the chloroform for use.

(b) *Starch-iodide papers.* (i) Dissolve 4 g. of cadmium iodide in 50 ml. warm water.

(ii) Make 5 g. of soluble starch into a thin smooth paste with a little distilled water. Pour the suspension into 450 ml. of boiling distilled water. Boil for 1 minute, add solution (i) and boil again for 1 minute. Cool the solution to 70° to 80°C. and impregnate strips of suitable filter paper (e.g., embossed filter paper No. 633 made by Evans, Adland and Co., Postlip Mills) by immersion in the solution and then removing as much surplus liquid as possible by means of a glass rod. Dry the impregnated filter paper in a warm atmosphere free from fumes. Cut off the edges of the strips and cut the remainder into strips for use. Store in a well-stoppered amber-coloured bottle.

Procedure.

Weigh accurately 0·27 to 0·29 g. of procaine penicillin (or about 0·9 g. of procaine penicillin suspension in oil, 300,000 I.U./ml.) and dissolve in 15 ml. of chloroform by warming. Pour the solution into a separating funnel containing 50 ml. of water. Rinse the beaker with 5 ml. of warm chloroform, then with 5 ml. of M ammonia, adding each in turn to the separator. Shake the contents of the separator gently for 2 minutes. Allow to separate, ignoring a turbidity of the chloroform layer and run the lower layer into a 4-oz. wide-mouthed bottle (provided with a well-fitting stopper) containing 35 ml. of water and 15 ml. of 3N hydrochloric acid.

Extract with 3 further portions each of 5 ml. of chloroform, running each in turn into the bottle. Adjust the temperature of the contents of the bottle to 15°C. and titrate with N/10 sodium nitrite with vigorous stirring until a drop of the aqueous portion of the titrated solution yields an immediate blue colour when spotted on starch-iodide paper. Insert the stopper of the bottle and shake vigorously for 30 seconds. Allow the layers to separate and complete the titration of the aqueous layer with gentle stirring. The end-point must be reproducible after allowing the titrated liquid to stand for two minutes after the last addition of nitrite, added 0·02 ml. at a time towards the end of the titration. Carry out a blank determination omitting the sample and deduct the titration figure. 1 ml. of N/10 sodium nitrite is equivalent to 0·02361 g. of procaine base.

Note 1: The extraction procedure described normally extracts the procaine quantitatively, the following test for traces of procaine may, however, be applied for confirmation.

Extract 5 ml. of chloroform solution with 4 ml. of water containing 0·2 ml. of N/10 hydrochloric acid. Allow to separate, discard the chloroform layer, warm the aqueous layer gently to expel chloroform, cool and add 1 ml. of N/10 iodine. No turbidity should be produced.

NOTE 2: Unless preparations of approximately known strength are being tested it is advisable to carry out a preliminary titration in order to establish the approximate end-point.

SUMMARY

1. A semi-micro method for the determination of procaine in procaine-penicillin and in its suspensions in arachis oil is given.

2. The method is based upon extraction of procaine base with chloroform followed by acidification of the extract and titration with sodium nitrite solution.

3. No interference has been encountered in the presence of low potency penicillin or aluminium monostearate.

The author wishes to thank Mr. A. G. Fishburn and Mr. R. T. Parry-Jones for their interest and helpful criticism during this work.

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A NEW METHOD FOR MEASURING DIFFUSION OF ANTISEPTICS FROM OINTMENT BASES

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Although it is not yet possible to determine by laboratory tests the clinical efficacy of medicated ointments, methods have been suggested for the measurement *in vitro* of certain physical properties which may influence their therapeutic action. For instance, it has frequently been found useful to study the rate of liberation of active agent from base under standard conditions. It is realised that data so obtained cannot be related directly to release of drug *in vivo* but it may, nevertheless, serve as a guide to the suitability of bases for specific purposes. The information may also provide a check on possible incompatibility between drug and excipients. Attention has so far been directed chiefly to the study of the diffusion of water-soluble drugs, usually antiseptics, from various bases and two general methods of measurement have been evolved:—(a) Measurement of zones of inhibition observed on inoculated agar following diffusion from a small cup[1]; (b) Estimation by chemical or other suitable methods, of the amount of drug which diffuses into water through a cellophane or similar membrane[2,3].

Method (a) usually involves a minimum period of 24 hours contact between ointment and agar before any readings are made. It is thus not possible to compare different bases in respect of diffusion-rates over shorter periods of, say, 2 hours. There is also the drawback that it is not always easy to fill the cups completely and uniformly, as a result of which there may not be good agreement between duplicate experiments. In addition, the interpretation of results obtained by method (a) is very difficult, since not all antiseptics penetrate the agar gel in the same way. The antiseptic may—(1) diffuse in a simple manner through the agar gel, in which case the concentration of antiseptic falls steadily as the distance from the cup increases; (2) be weakly adsorbed on the agar, giving rise to a gradual fall in concentration of antiseptic from the cup outwards; the penetration of antiseptic is not, however, so extensive as in the first case; (3) be strongly adsorbed on the agar so that, from the cup outwards, there is a narrow zone of substantially constant antiseptic concentration followed by a rapid decrease to zero. Before conclusions can be drawn from any method involving transfusion into agar the precise nature of the process must be known. It is possible to compare different concentrations of the same antiseptic (e.g. penicillin) once the diffusion mechanism is known; it is very difficult to compare different antiseptics which may have dissimilar diffusion mechanisms.

Method (b) has the advantage of being adaptable to all water-soluble drugs, whether bacteriostatic or not, but requires a rather elaborate technique.

The object of the work described here was to devise a simple technique capable of detecting different rates of diffusion over shorter time intervals. It has resulted in the development of a test in which the active agent of an ointment diffuses through cellophane on to inoculated agar, and the minimum time of contact sufficient to cause inhibition of bacterial growth is recorded.

Attempts were made to devise a method of applying an ointment to inoculated agar in such a manner that it would be removable easily and completely after any desired time-interval. After a number of preliminary tests the procedure described below as Method I was selected as the most satisfactory and was thereafter evaluated by:—(a) Confirmation of reproducibility, (b) Comparison against other methods, (c) Utilisation to distinguish between bases of different type.

Method 1. 10 ml. of molten agar is poured into a petri dish and allowed to set. The plate is inverted in an incubator and dried for 2 hours. On to the layer of agar is poured 5 ml. of molten agar containing 1 ml. of a 1 in 10 broth dilution of a 24-hour broth culture of *Staphylococcus aureus*. Similar results are obtained if, instead of using two layers of agar, the culture is pipetted directly on to the surface of alcohol-dried agar, but it is difficult to ensure an even spread of the culture and it may take several hours for the liquid to become absorbed into the agar. The first method of inoculation was therefore considered preferable since it results in a dry, evenly-contaminated surface. By means of sterile forceps, four 1 inch squares of sterile cellophane are placed on the surface of the agar and incubated for 45 minutes. The incubation causes the cellophane to spread evenly on the agar. To absorb moisture a disc of filter paper is trapped in the lid of the dish. The preparation to be tested is applied to the surface of the cellophane by means of an all glass hypodermic syringe without needle, so as to leave a border about $\frac{1}{8}$" wide on the cellophane. The plate is then returned to the incubator and pieces of cellophane with the adherent preparation under test, removed at suitable time-intervals. After incubation overnight the plates are examined for growth-inhibition which is indicated by a clear area of agar at the site of the cellophane square. The minimum time required to cause inhibition of growth is recorded. This period may vary from a

TABLE I

DIFFUSION OF ANTISEPTIC FROM PHENYLMERCURIC NITRATE JELLY 0·001 PER CENT. AS DETERMINED BY CELLOPHANE METHOD

Date Tested	Period of contact between treated cellophane and agar							
	1 min.		5 mins.		10 mins.		15 mins.	
15.1.48	∓	∓	∓	+	+	+	+	+
16.1.48	−	−	+	+	∓	+	+	+
20.1.48	∓	−	+	+	+	+	+	+
23.1.48	−	−	∓	∓	+	+	+	+
27.1.48	−	−	+	+	+	+	+	+

+ =complete clearance ; ∓ =partial clearance ; − =no clearance.

few minutes to several hours and sighting experiments are therefore necessary when a preparation is tested for the first time.

Reproducibility. The test was applied to a range of active agents in a variety of bases (see Appendix) and no difficulty was experienced in obtaining replicate results. This is illustrated by Table I, which records successive tests made on a dilute phenylmercuric nitrate jelly.

Comparison with other methods. Although there may be objections to using variations of the agar cup method for evaluation of the diffusion of an antiseptic from ointment bases, there are no other methods available which are based on a biological technique. Accordingly, for comparison, it was decided to use three variations of the agar diffusion method, as detailed below.

Method 2. A cylindrical hole 15 mm. in diameter is cut in an agar plate inoculated as in Method 1, and a few drops of molten agar added, from a Pasteur pipette, to cover the glass surface so exposed. This hole is then filled with the preparation to be tested. The plate is incubated for 24 hours and the diameter of clearance measured.

Method 3. This method is similar to Method 2, but the preparation is placed in a ditch 10 mm. × 40 mm. instead of in the cylindrical hole.

Method 4. Four sterile glass cylinders of internal diameter 10 mm. and depth 13 mm. are filled with the preparation to be tested and placed on the surface of a poured inoculated agar plate.

The results are recorded in Table II.

TABLE II

ANTISEPTIC ACTIVITY AS TESTED BY FOUR DIFFERENT METHODS

Base	Active agent		Method 1	Method 2	Method 3	Method 4
			Minimum time for inhibition	Average diameter or clearance of 4 tests		
		per cent.	minutes	mm.	mm.	mm.
Jelly	crystal violet	1·0	5	20	20	20
	sodium ethyl mercurithiosalicylate	0·02				
Jelly	sulphanilamide	5·0	5	20	18	10
Jelly	4:4′-diamidinodiphenoxypropane di-(hydroxyethanesulphonate)	0·15	10	4	4	4
Oil-in-water emulsion	potassium hydroxyquinoline sulphate	0·2	15	20	18	10
	chlorocresol	0·2				
Petroleum/lanolin	benzoyl peroxide	10·0	60	10	14	4
	chlorohydroxy quinoline	0·5				
Simple ointment	phenylmercuric nitrate	0·001	60	7	6	4
			hours			
Glycerin/wool fat White soft paraffin	Resorcinol	12·5	2	2	4	1
Oil-in-water cream	diamidinodiphenoxypropane dihydrochloride	0·1	4	2	4	0

A number of preparations when tested in the same way gave negative results by all four methods, i.e., no diffusion of active agent could be detected. These included Scarlet Red Ointment B.P.C., Ichthamol Ointment B.P.C., Coal Tar Ointment B.P.C., and Salicylic Acid Ointment B.P.C. It will be noticed that a certain parallel exists between the results obtained by Method 1 and those from the remaining three inasmuch as increased times of contact usually correspond to decreased zones of inhibition, but Method 1 provides a much sharper distinction.

Distinction between different bases: Phenylmercuric nitrate and neutral proflavine sulphate were formulated into a series of five different types of base and at different concentrations. The results of applying Method 1 to these preparations are recorded in Table III.

TABLE III

MINIMUM TIME FOR INHIBITION OF BACTERIAL GROWTH BY VARIOUS OINTMENTS WHEN TESTED BY THE CELLOPHANE METHOD

Active ingredient	Fatty Base	Water-in-oil Base	Oil-in-water Base 1	Oil-in-water Base 2	Jelly
per cent					
Phenylmercuric nitrate ... ... 0·001	60 mins.	10 mins.	15 mins.	15 mins.	2 mins.
" " ... ... 0·002	30 mins.	10 mins.	4 mins.	4 mins.	3 mins.
Neutral proflavine sulphate ... ... 0·05	24 hrs.	24 hrs.	4 hrs.	45 mins.	30 mins.
" " " ... ... 0·1	24 hrs.	24 hrs.	4 hrs.	25 mins.	15 mins.
" " " ... ... 0·2	24 hrs.	24 hrs.	4 hrs.	15 mins.	5 mins.

The results obtained with this test seem to indicate that, other factors being equal, a jelly type of base is superior to oil-in-water emulsions, which in turn are better than water-in-oil emulsions or fatty bases. In fact, for water-soluble antiseptics, it would seem that the oil phase is largely redundant, its purpose being solely to increase the consistency of the preparation so that it will remain at the site of application. This function can be fulfilled equally well with the jelly base which has the added advantage of being cheaper and easier to prepare.

DISCUSSION

There will probably always be a wide gap between the clinical evaluation of an ointment and laboratory tests made *in vitro*. Nevertheless, pharmaceutical technique has advanced beyond the stage when an ointment was selected merely on account of its elegance. The latter practice must have inevitably resulted in the formulating of many ineffective preparations whose faults might have remained undetected for a long time.

In the formulating of antiseptic preparations there is often available a series of ointment bases all equally suitable in stability and appearance. The final choice should therefore take into account the rapidity with which the antiseptic is required to be released. It is suggested that the test described above provides a convenient method of doing this since it can be performed with standard apparatus available in most hospital dispensaries and manufacturing laboratories. The test can also be applied as a routine measure to control manufacture.

Summary

1. A test is described for measuring the release of antiseptics from ointments.

2. The significance of the test is discussed and some applications suggested.

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APPENDIX

Materials

Ointment Bases:		
Fatty Base:	Simple Ointment B.P.	
Water-in-oil Base:	Hydrous Ointment B.P.	
Oil-in-water Base 1:	Active Agent	a sufficient quantity
	Emulsifying Wax B.P.	7 g.
	Hard Paraffin B.P.	5 g.
	Liquid Paraffin B.P.	40 g.
	Distilled Water, sufficient to produce	100 g.
Oil-in-water Base 2:	Active Agent	a sufficient quantity
	Castor Oil B.P.	20 g.
	Cetyl Alcohol	6 g.
	Diethyleneglycol distearate	9 g.
	Polyglyceryl ricinoleate	5 g.
	*Emulsifying Agent (non-ionic) ...	3 g.
	Distilled Water, sufficient to produce	100 g.

* A condensation product of cetyl alcohol with ethylene oxide.

Jelly:	Active Agent	a sufficient quantity
	Cellofas W.F.Z.	6 g.
	Distilled Water, sufficient to produce	100 g.
Cellophane:	Described as non-waterproof of 0·0009 inch thickness	
Staphylococcus aureus:	N.C.T.C. 4163	per cent.
Culture Medium:	Sodium Chloride	0·125
	Peptone	1·5
	Yeastrel	0·5
	Potassium dihydrogen phosphate (anhydrous)	0·1
	Dipotassium hydrogen phosphate (anhydrous)	0·1
	Dextrose	0·5
	Agar	2·0
	Distilled water (*p*H 7·2 to 7·6) to produce	100

THE BROMINATION OF *p*-AMINOSALICYLIC ACID, SODIUM *p*-AMINOSALICYLATE AND *m*-AMINOPHENOL

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THE quantitative bromination of phenols by acid bromide/bromate solution, first described by Koppeschaar[1] in 1876 is based upon the general assumption that aqueous nascent bromine will substitute quantitatively only in the *ortho* and *para* positions of phenols. The presence of certain groups other than hydrogen in these positions has been found to give anomalous results. Ruderman[2] showed that certain alkylated phenols brominate quantitatively regardless of the bromine excess used while others overbrominate to an extent which varies with the magnitude of the bromine excess. Day and Taggart[3] found that Koppeschaar's method was unsatisfactory for materials like *o*- and *p*-cresol but satisfactory for *m*-cresol, phenol and a number of salicylates. Sprung[4] found that phenols substituted in the *meta* position brominate quantitatively. The present paper was undertaken to study the behaviour of *p*-aminosalicylic acid and *m*-aminophenol on bromination with the object of developing an assay method for these compounds.

EXPERIMENTAL

The *p*-aminosalicylic acid used was purified by two crystallisations from methyl alcohol. A melting-point is not quoted since the material is known to decompose on heating at temperatures over 110°C., the melting-point being variable according to the rate of heating[5]. The sodium *p*-aminosalicylate was purified by recrystallisation from hot 90 per cent. aqueous alcohol. The product contained two molecules of water of crystallisation. The *m*-aminophenol was recrystallised from boiling water; m.pt. 122° to 123°C.

Procedure.—An accurately weighed quantity of the material was dissolved in distilled water using sufficient sodium hydroxide in the case of *p*-aminosalicylic acid and *m*-aminophenol to effect solution and made up to 1 l. A 25 ml. aliquot of the solution was transferred to a 250 ml. iodine flask, to which were added varying volumes of N/10 potassium bromate/bromide solution, together with a volume of distilled water to ensure that the total volume of bromate/bromide solution and diluent water was 50 ml. 5 ml. of concentrated hydrochloric acid was then added, the flask was immediately stoppered and allowed to stand for 5 minutes, being shaken intermittently during this time. 5 ml. of a 20 per cent. w/v solution of potassium iodide was then added, the flask quickly stoppered and allowed to stand for a further 5 minutes. The stopper and sides of the flask were then washed down with water and the excess of iodine estimated by titration with N/10 sodium thiosulphate, using starch solution as indicator. A blank was run using an additional 25 ml. of distilled water in place of the test solution.

The number of reactive positions R in the molecule of *p*-aminosalicylic acid, sodium *p*-aminosalicylate or *m*-aminophenol was calculated from the formula $R = \frac{EM}{50C}$ where E is the number of milli-equivalents of bromine absorbed, M is the molecular weight of the compound and C is the concentration of the test sample in g./l.

Results and Discussion

The results of a series of titrations on the three materials is set out in Tables I, II and III. It appears that the materials examined brominate quantitatively under the conditions used and the reaction could be made the basis of an assay process.

TABLE I

THE BROMINATION OF *p*-AMINOSALICYLIC ACID
(ASSAY BY ALKALI TITRATION 99·74 PER CENT. W/W)
CONCENTRATION OF SOLUTION 1·200 G./L.

Bromine added milli-equivalents	$Na_2S_2O_3$ required milli-equivalents	Bromine absorbed milli-equivalents	No. of reactive positions found	Percentage w/w of $C_6H_3COOH.OH.NH_2$
1·500	·330	1·170	2·99	99·5
2·000	·822	1·178	3·01	100·2
2·000	·828	1·172	2·99	99·7
2·500	1·320	1·180	3·01	100·3
2·500	1·323	1·177	3·00	100·1
2·500	1·323	1·177	3·00	100·1
2·500	1·325	1·175	3·00	99·9
2·500	1·324	1·176	3·00	100·0
2·500	1·328	1·172	2·99	99·7
3·000	1·828	1·172	2·99	99·7
3·500	2·330	1·170	2·99	99·5
3·500	2·326	1·174	2·99	99·8
4·000	2·822	1·178	3·01	100·2
5·000	3·820	1·180	3·01	100·3

TABLE II

THE BROMINATION OF SODIUM *p*-AMINOSALICYLATE DIHYDRATE
(CONTAINS 82·9 PER CENT. W/W OF $C_6H_3COONaOH.NH_2$)
CONCENTRATION OF SOLUTION 1·600 G./L.

Bromine added milli-equivalents	$Na_2S_2O_3$ required milli-equivalents	Bromine absorbed milli-equivalents	*No. of reactive positions found	Percentage w/w of $C_6H_3COONaOH.NH_2$
1·500	·365	1·135	2·48	82·8
2·000	·866	1·134	2·48	82·7
2·500	1·360	1·140	2·49	83·2
2·500	1·365	1·135	2·48	82·8
3·000	1·865	1·135	2·48	82·8
3·500	2·360	1·140	2·49	83·2
4·000	2·860	1·140	2·49	83·2
5·000	3·867	1·133	2·48	82·6
5·000	3·866	1·134	2·48	82·7

* Theoretical figure equivalent to three bromine atoms when calculated for the anhydrous salt.

The results on *m*-aminophenol substantiate the results of Sprung[4] while the results on *p*-aminosalicylic acid and its sodium salt would appear to agree with Day and Taggart's[3] conclusions regarding a number of salicylates. The analyses suggest that three atoms of bromine are absorbed per molecule of the compounds studied. Although their point of reaction is a matter for conjecture it is interesting to note from Allen's Commercial Organic Analysis (4th Ed., 1910, Vol. III) that salicylic acid

brominates with loss of the carboxyl group and the formation of 2:4:6 tribromphenol.

From the present work it would appear that pure specimens of *p*-aminosalicylic acid and its sodium salt brominate quantitatively regardless of the excess of bromine used. It is interesting to notice, however,

TABLE III

THE BROMINATION OF *m*-AMINOPHENOL
CONCENTRATION OF SOLUTION 0·8000 G./L.

Bromine added milli-equivalents	$Na_2S_2O_3$ required milli-equivalents	Bromine absorbed milli-equivalents	No. of reactive positions found	Percentage w/w of $C_6H_4OH.NH_2$
1·500	·403	1·097	2·99	99·7
1·500	·407	1·093	2·98	99·3
1·500	·405	1·095	2·98	99·5
1·500	·400	1·100	3·00	99·9
2·500	1·394	1·106	3·15	100·4
3·000	1·896	1·104	3·01	100·3
3·000	1·897	1·103	3·01	100·2
3·500	2·400	1·100	3·00	99·9
3·500	2·395	1·105	3·01	100·4
4·000	2·910	1·090	2·97	99·0
4·000	2·903	1·097	2·99	99·7
4·500	3·398	1·102	3·00	100·1
5·000	3·900	1·100	3·00	99·9
5·000	3·890	1·110	3·02	100·8
5·000	3·895	1·105	3·01	100·4

that as mentioned in an earlier communication[5], less pure material shows a slight variation in bromine absorption which does depend upon the available bromine. Accordingly, a direct bromination method using an external indicator has been suggested for assay purposes, although the present method is more convenient where the degree of purity of the material warrants its application.

SUMMARY

1. The bromination of *p*-aminosalicylic acid, sodium *p*-aminosalicylate and *m*-aminophenol has been studied using a variable excess of bromide/bromate solution.

2. *p*-Aminosalicylic acid, sodium *p*-aminosalicylate and *m*-amino phenol brominate quantitatively regardless of the excess of bromide/bromate solution used.

3. The basis for an assay process for *p*-aminosalicylic acid, sodium *p*-aminosalicylate and *m*-aminophenol has been described.

Thanks are expressed to the Directors of Herts Pharmaceuticals Limited for permission to publish these results.

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A PHOTOELECTRIC COLORIMETRIC METHOD FOR THE ESTIMATION OF KHELLIN

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Khellin, $C_{14}H_{12}O_5$ (2-methyl-5 : 8-dimethoxy-6 : 7-furano-chromone[1,2]) m.pt. 154° to 155°C., the main active principle of the fruits of *Ammi Visnaga* Linn., is identified by the rose red colour which it gives with potassium or sodium hydroxide[3]. This test has been used as a spot reaction by Abdel-Rahman[4] for the determination of khellin in solutions. It makes use of the fact that one drop of khellin solution gives with solid sodium hydroxide a rose red colour, only, if the quantity of khellin is not below a certain limit (1 : 505,000), which is the identification limit, Accordingly, 0·066 μg. of khellin can be detected in one drop, assuming that 1 ml. of the solution equals 30 drops.

Applying this method quantitatively, a given test solution is progressively diluted with constant testing of drops taken from the various dilutions until the colour reaction fails. The concentration of khellin in the original solution can then be calculated from the dilution required to reach this limit.

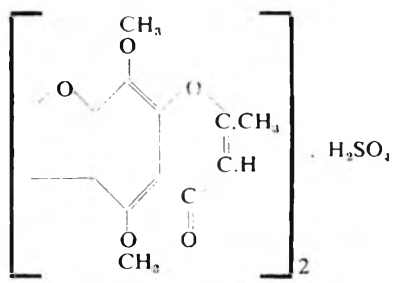
The repeated dilutions and testing of drops required by this procedure, are time consuming, and the results are only approximate, even if a special standardised dropper is used; this is because the " identification limit " is not the same for different observers; there is almost always a certain range of variation which depends on the individual characteristics of the observer. The range of dilution in which a test sometimes succeeds and sometimes fails is called by Feigl the " region of uncertain reaction[5]," and a quantitative estimation based on the examination for the failure of a test always extends into that region and therefore cannot be exact. Furthermore, even slight experimental errors become quite significant because the subsequent computation involves a multiplication. On applying this method, the results obtained by the authors varied to a great extent. In some determinations the results obtained were 40 to 60 per cent. lower than the amount of khellin taken.

The method of Anrep and co-workers[6] for assaying khellin, using a saturated solution of potassium hydroxide, is a modification of the above method. Its advantage lies in the fact that it makes use of controls, the colour given by the unknown being matched with that of a standard solution; but a saturated solution of potassium hydroxide is not a stable reagent as it easily absorbs carbon dioxide from the atmosphere leading to precipitation of potassium carbonate, the result being a turbid solution which interferes with correct comparison of colours. By applying this method on a photoelectric colorimeter, the authors found that the transmission values of the coloured solution are different for the same amounts of khellin, when examined under the same experimental conditions. This shows that this method is not reliable for the quantitative

estimation of khellin. In this communication, a photoelectric colorimetric method is described. This method has the advantage of being accurate, simple and suitable for the assay of pharmaceutical preparations.

The Photoelectric Colorimetric Method

Khellin gives with sulphuric acid a citron yellow colour which is stable in relatively dilute solutions. This colour is due to the formation of an oxonium salt (khellin sulphate) which has been isolated by the authors in a pure crystalline form as golden yellow needles having the structural formula.



The yellow colour of khellin sulphate is a property of the non-ionised salt. In presence of excess of water, khellin sulphate splits into khellin and sulphuric acid and the colour disappears as shown by the following equation: —

$$K + H_2SO_4 \rightleftarrows K\,S$$

where K and K S represent khellin and khellin sulphate respectively.

This is a reversible reaction which follows the law of mass action. This equation in terms of concentration becomes:

$$\frac{(K\,S)}{(K).\,(H_2SO_4)} = \text{Constant.}$$

brackets being used to represent concentrations.

If the concentration of sulphuric acid or of khellin is increased; the concentration of khellin sulphate increases proportionally; and as khellin sulphate is responsible for the citron yellow colour produced; the optical density of the solution will be proportional to the concentration of khellin and of sulphuric acid present in the solution.

On keeping the concentration of sulphuric acid constant, the concentration of khellin sulphate and consequently the optical density of the solution will be proportional to the concentration of khellin.

$$\frac{(K\,S)}{(K)} = \text{constant or } \frac{D}{(K)} = \text{constant.}$$

where D represents optical density.

This is true within certain limits of concentration, as shown in the experimental part.

Experimental

Preparation of Khellin Sulphate. 10 g. of khellin is dissolved in 50 ml. of glacial acetic acid in the cold; then 25 ml. of concentrated sulphuric

acid is gradually added and the mixture is kept in the ice-chest for 24 hours. 150 ml. of absolute alcohol is then added slowly to the mixture, stirred and again kept in the ice-chest for 1 week. The golden yellow crystals of khellin sulphate which separate, are filtered by suction, washed twice with ethyl acetate, and dried at room temperature. Yield, 9·5 g. (95 per cent.); m.pt. 175° to 185°C.

The crystals when treated with water split into pure khellin, m.pt. 154° to 155°C., and sulphuric acid. Acid released, 15·635 per cent. w/w of H_2SO_4. Khellin sulphate $C_{14}H_{12}O_5)_2,H_2SO_4$ requires 15·85 per cent. w/w.

Relation Between the Concentration of Khellin, the Concentration of Sulphuric Acid and the Optical Density of the Solution

Solutions required:—

(1) 10N sulphuric acid (A.R.).

(2) Standard stock solution of khellin (0·5 per cent. w/v). 0·5 g. of pure crystalline khellin, m.pt. 154° to 155°C. dissolved in 100 ml. of alcohol (60 per cent. v/v).

(3) Standard dilute solution of khellin (0·025 per cent. w/v); freshly prepared by diluting 5 ml. of the standard stock solution to 100 ml. with distilled water.

The relation between the concentrations of khellin and sulphuric acid and the optical density was determined at room temperature (25°C.).

The concentration of sulphuric acid in the solution is controlled by diluting known volumes of 10N sulphuric acid with distilled water to a constant volume, and the optical density of the solution is calculated from the equation:

$$D = 2 \log_{10} T$$

where D represents the optical density and T the percentage transmission of the solution. T is read directly on the scale of the colorimeter. Blue filter 420 is found to be the most suitable, as it gives minimum transmission values and a better spread of readings.

The following general procedure has been adopted. Into a volumetric flask of 50 ml. capacity, containing x ml. of a standard solution of khellin, n ml. of 10N sulphuric acid is added. The volume is then completed to 50 ml. with distilled water, well mixed, and left to stand for about 5 minutes; about 10 ml. of the solution is transferred to a colorimeter tube; and its percentage transmission is read in a Lumetron Photoelectric colorimeter Model 400-A using blue filter 420 against water as the blank set at 100 per cent. transmission, x = 1 ml. of the standard stock solution of khellin (0·5 per cent. w/v), representing a concentration of 10 mg. per cent. w/v of khellin, or 1, 2, 4, 8 and 10 ml. of the standard dilute solution of khellin (0·025 per cent. w/v) representing concentrations of 0·5, 1, 2, 4 and 5 mg. per cent. w/v of khellin respectively; n = 10, 15, 20, 25, 30 and 40 ml. of 10 N sulphuric acid, representing concentrations of 98·08, 14·712, 19·616, 24·52, 29·424 and 39·232 g. per cent. w/v of sulphuric acid respectively.

From the results obtained it may be concluded: —

(a) For the same concentration of khellin; the optical density of the solution increases with an increase in the concentration of sulphuric acid.

(b) For the same concentration of sulphuric acid the optical density of the solution increases with an increase in the concentration of khellin.

(c) For concentrations up to 4 mg. per cent. w/v of khellin the optical density is proportional to the concentration of khellin provided that the concentration of the acid is more than 29·424 per cent. w/v of sulphuric acid.

(d) Transmission readings obtained with a concentration of 39·232 per cent. w/v of sulphuric acid are spread enough to allow a determination of khellin to be done within concentrations ranging from 0·5 to 10 mg. per cent. w/v of khellin.

(e) The graph shows that within the above concentrations (0·5 to 10 mg. per cent. w/v of khellin) there is a slight deviation from Beer's Law. Therefore, a calibration table will replace more conveniently the graph and give more accurate results, when the calibration table and the estimations are made at the same room temperature and under the same conditions.

Calibration Table

As a concentration of 39·232 per cent. w/v of sulphuric acid may be practically obtained by mixing 10 ml. of 10N sulphuric acid with 2·5 ml. of aqueous khellin solution the following procedure has been adopted for the preparation of the calibration table.

From the standard stock solution of khellin (0·5 per cent. w/v) are prepared standard dilutions so that 2·5 ml. of each dilution contain an amount of khellin ranging from 0·05 to 1 mg. and increasing in the order of 0·05 mg.; 2·5 ml. of each dilution are accurately measured in a dry colorimeter tube; 10 ml. of 10N sulphuric acid are added, well mixed; left to stand for about five minutes, then the percentage transmission of the solution is read, in a Lumetron Photoelectric colorimeter using blue filter 420 against water as the blank set at 100 per cent. transmission.

The results obtained at room temperature (25°C.) are shown in Table I.

TABLE I

Calibration table

Concentration of Khellin in mg. per cent.	Amount of Khellin in mg.	Percentage Transmission	Concentration of Khellin in mg. per cent.	Amount of Khellin in mg.	Percentage Transmission
0·4	0·05	93·0	4·4	0·55	49·0
0·8	0·10	87·0	4·8	0·60	47·0
1·2	0·15	80·5	5·2	0·65	44·0
1·6	0·20	75·5	5·6	0·70	41·0
2·0	0·25	71·0	6·0	0·75	39·0
2·4	0·30	65·0	6·4	0·80	37·5
2·8	0·35	61·0	6·8	0·85	35·0
3·2	0·40	57·0	7·2	0·90	33·0
3·6	0·45	54·0	7·6	0·95	31·5
4·0	0·50	51·0	8·0	1·00	30·0

ESTIMATION OF KHELLIN

Method of Assay

(1) For the estimation of khellin in a test solution the following method is recommended. Make a dilution of the test solution so that 2·5 ml. contain an amount of khellin ranging between 0·05 and 1 mg. Accurately measure 2·5 ml. of this dilution into a dry colorimeter tube, add 10 ml. of 10N sulphuric acid; mix well; leave to stand for about 5 minutes; measure the percentage transmission of the solution and read the amount of khellin corresponding to the percentage transmission from the calibration table. The amount of khellin in the original test solution can then be obtained by calculation.

(2) For the estimation of khellin in oily preparations the following method is recommended. Dilute a known volume of the oily preparation with light petroleum so that 10 ml. of this dilution contains an amount of khellin ranging between 1 and 8 mg. Accurately measure 10 ml. of this dilution in a dry separating funnel; add 100 ml. of 39·232 per cent. w/v of sulphuric acid (obtained by diluting 80 ml. of 10N sulphuric acid to 100 ml. with distilled water); shake the mixture for about 15 minutes, then leave to stand for 15 minutes to separate. Filter about 15 ml. of the aqueous layer into a dry colorimeter tube; measure the percentage transmission of the solution and read the concentration of khellin corresponding to the percentage transmission from the calibration table. The figure obtained is the amount in mg. of khellin contained in 10 ml. of the diluted oil solution.

Discussion

It has been found experimentally that, on mixing at room temperature 2·5 ml. of an aqueous solution of khellin with 10 ml. of 10N sulphuric acid the optical density of the solution becomes stable after leaving the mixture to stand for about 5 minutes at room temperature; and it remains stable for more than 24 hours.

On mixing the solution of sulphuric acid with the solution of khellin a rise of about 2°C., in the temperature and a contraction in the volume of about 0·5 ml. per cent. of the solution takes place. The rise in temperature and the contraction in the volume of the solution within these limits do not affect, to any appreciable extent, the optical density of the solution, i.e., a difference of about 2°C. in the temperature and a variation in the volume up to 0·5 ml. per cent. of the solution does not affect the optical density of the solution to any appreciable extent. On applying this method to accurately weighed amounts of pure khellin the results obtained did not differ by more than ±2 per cent.

Visnagin, another constituent of the fruits of *Ammi Visnaga* Linn., may be present, as an impurity, in pharmaceutical preparations of khellin to an extent of about 5 to 10 per cent. of the weight of khellin present. As visnagin gives with sulphuric acid a yellow colour which, when compared with that given by khellin, is found to be about 50 per cent. less in intensity. In this case, the results obtained will not differ by more than ± 5 per cent.

Moreover, alcohol was found to interfere to a certain extent with the proper development of the colour. Therefore, alcoholic solutions of

khellin should be diluted with water before applying this method, so that the alcohol content of the coloured solution does not exceed 1 per cent. v/v.

Summary

(1) A photoelectric colorimetric method for the assay of khellin is described.

(2) This method is recommended for the assay of pure khellin in pharmaceutical preparations.

(3) The assay is carried out within the limits of 0·1 to 1 mg. of khellin.

Work is proceeding for the application of this method on galenical and other pharmaceutical preparations. The results will be compared with those obtained by the other colorimetric methods.

The authors wish to express their thanks to the Memphis Chemical Co. for the help extended to them in carrying out this work and for the supply of pure khellin and instruments involved.

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KHELLIN AND ITS ASSAY IN INJECTIONS AND TABLETS

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THE most important constituent of *Ammi Visnaga* fruits, as regards pharmacological activity, is khellin, $C_{14}H_{12}O_5$, m.pt. 153° to 155°C. Khellin is a furano-chromone derivative with a pronounced antispasmodic action on smooth muscle viz.: ureter, bronchial muscle, intestine and bile duct[1,2]. It has, moreover, a specific dilator action on the coronary arteries which makes it useful in the treatment of angina pectoris[2].

Besides khellin, the fruits contain two other constituents which have been isolated in chemically pure form and their molecular and structural formulæ established viz.: a glucoside, khellol-glucoside[3], $C_{13}H_9O_3C_6H_{11}O_5,2H_2O$, m.pt. 175°C. and visnagin[4], $C_{13}H_{10}O_4$, m.pt. 142° to 145°C., both of which are also furano-chromone derivatives. The glucoside is devoid of the antispasmodic action of khellin while visnagin has a much lower activity than khellin[5]. The fruits contain about 1 per cent. of pure khellin, 0·1 per cent of pure visnagin and 0·3 per cent. of pure khellol-glucoside.

Khellin is usually obtained from the powdered fruits by extracting with ether or light petroleum, concentrating the extract and leaving it to crystallise; or alternatively the powdered fruits are extracted with alcohol, the alcohol is distilled and the residue extracted with chloroform. After distillation of the chloroform, crude khellin is obtained. By both methods, khellin is extracted in association with visnagin; the glucoside being insoluble in ether, in light petroleum and in chloroform. Khellin must be purified by several crystallisations from alcohol to free it from impurities and from visnagin. Nowadays, a number of pharmaceutical laboratories in Egypt prepare khellin on a semi-large scale. It is generally dispensed in the form of tablets or injectable solutions.

Up to the present, no standard description of khellin has been given. In this communication, the description, solubilities, identification, purity test of khellin and the method of its assay in the above preparations are described.

Description.—Khellin (2-methyl-5 : 8-dimethoxy-6 : 7-furano-chromone, Mol.Wt. 260), occurs in colourless needle-shaped crystals, odourless, taste bitter. It should contain not less than 99 per cent. of $C_{14}H_{12}O_5$.

Solubilities.—Khellin is very soluble in chloroform, less soluble in cold ether and in light petroleum, more soluble in the hot liquids. Soluble at 25°C. in 130 parts of alcohol (95 per cent.) in 6750 parts of water[6], in 500 parts of a saturated aqueous solution of theophylline, and in 33 parts of a saturated solution of sodium benzoate. It is soluble in glacial acetic acid and in dilute mineral acids from which it is regained unchanged.

Identification Tests.—(a) When one drop of 0·01 per cent w/v solution

in alcohol or in water is added to a piece of solid sodium or potassium hydroxide, a rose red colour is developed within 2 minutes[7,8].

(b) When a few crystals are treated with 1 drop of concentrated sulphuric acid on a white porcelain plate, a deep orange colour is developed which on dilution with water becomes yellow[8].

(c) When a solution of 10 mg. in 2 ml. of alcohol (50 per cent.) is poured on a freshly prepared mixture of 0·5 ml. of N/2 iodine and 0·5 ml. of 10N potassium hydroxide solution, a yellow colour is formed followed by a yellow precipitate, which redissolves gradually on shaking, imparting to the solution a wine red colour.

Test for Purity.—m.pt. 153° to 155°C.

Injection of Khellin

In consequence of the greater solubility in water in presence of theophylline or sodium benzoate, solutions for injection in an aqueous medium containing one or both of these compounds are already to be found on the Egyptian market.

Identification and Test for Purity.—Place in a separating funnel a volume of the injection equivalent to about 0·2 g. of khellin. Extract the khellin from this solution with three successive quantities, each of 10 ml. of pure benzene. Evaporate the combined benzene extracts to dryness on a water-bath and dry the residue at 100°C. The residue should comply with the tests for khellin.

Assay.—In solutions for injection, khellin may be assayed colorimetrically by the sulphuric acid method[6]. Dilute a volume of the solution equivalent to about 0·1 g. of khellin with distilled water to 100 ml. Dilute 10 ml. of this dilution to 50 ml. Measure 2·5 of this dilution in a dry colorimeter tube, add 10 ml. of 10 N sulphuric acid, leave to stand for about 5 minutes and read the percentage transmission in a photoelectric colorimeter against water as the blank, set at 100 per cent. transmission using blue filter No. 420. Read the amount of khellin corresponding to the percentage transmission from a calibration table, prepared under the same conditions, using standard dilutions of pure khellin. The result obtained, multiplied by 200, gives the amount of khellin present in the original volume taken.

Tablets of Khellin

Identification and Test for Purity.—Triturate a quantity of the powdered tablets, equivalent to 0·2 g. of khellin, with two successive quantities, each of 10 ml., of chloroform. Filter, evaporate the chloroform to dryness, on a water-bath, and dry the residue at 100°C. The residue should comply with the tests for khellin.

Assay.—Weigh and powder 20 tablets; treat an accurately weighed quantity of the powder, equivalent to about 0·1 g. of khellin, on a dry filter with successive small quantities of hot alcohol (95 per cent.) until the khellin is completely extracted. Concentrate the alcoholic extract to about 15 ml. and transfer it to a volumetric flask of 100 ml. capacity, washing the flask with two successive quantities, each of 5 ml., of alcohol

(95 per cent.) and make up to volume with distilled water. Dilute 10 ml. of this solution to 50 ml. with distilled water. Carry out the assay, using 2·5 ml. of this dilution, as described for injections. The result obtained, multiplied by 200, gives the amount of khellin in the original weight taken.

Discussion

The determination of the melting point of the extracted khellin from injections and tablets is required as a test for purity. If the khellin used is contaminated with visnagin or with other impurities from the fruits, the product will begin to melt below 140°C. In such cases the result of the assay may be reported as "total chromones of *Ammi Visnaga* fruits calculated as khellin."

In the extraction of khellin from injections, benzene is used instead of chloroform, to avoid the extraction of any theophylline present, theophylline being insoluble in benzene. Theophylline and sodium benzoate do not interfere with the method of assay.

Summary

(1) The characters and identification tests of khellin are described.

(2) The standards of purity of khellin used in pharmaceutical preparations is given.

(3) The method of assay of khellin in injections and tablets is described.

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THE RESPONSE OF THE HEART TO VISAMMIN AND TO KHELLININ

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Introduction

It has already been reported that various active principles are present in the fruit of *Ammi Visnaga* Linn, with different pharmacological actions and therapeutic idications[1,2,3]. At the time of isolation of these principles in 1930 in this Department, the literature found on the chemistry of this drug was by Ibrahim Mostapha[4] and T. Malosse[5]. The latter described three crystalline principles which he named visnagine α, visnagine β, and visnagine γ. Ibrahim Mostapha described a crystalline principle which he named khellin, and which was stated by Malosse (*loc. cit.*) not to be identical with visnagine α, but was supposed to be formed during the process of extraction. In view of this and other anomalies (variation of m.pt., etc.) it was deemed preferable to give new names to the isolated compounds, names which are derived from the Latin and Arabic names of the drug—Ammi Visnaga and Khella respectively.

Two compounds of the various principles isolated, namely visammin and the glycoside khellinin, are of great interest pharmacologically.

Anrep *et al.*[6,7,8], who recently worked on the active principles of Ammi Visnaga, refer to khellinin as khellol glycosides regardless of the fact that khellinin is not an alcohol, and that names of glycosides end in "in." The same authors refer to visammin as khellin.

The value of Ammi Visnaga as an antispasmodic and as a coronary dilator was indicated by one of us as early as 1930[9]. In what concerns the action of visammin and of khellinin on the circulation, experimental animals being the toad, rabbit and dog, the following points were indicated. (a) Visammin[1] in a concentration of 1 : 100,000 diminishes the amplitude of beat and slows the heart with no arrest. Stronger solutions (1 : 50,000 to 1 : 20,000) produce more evident slowing and a great diminution of the amplitude of beat. The diminished amplitude is relatively much more marked than the slowing, and is, to a great extent, due to diminution of systole rather than of diastole. Atropine added to the perfusion fluid produces no evident changes in the amplitude. The action is considered to be a direct depressant effect on the muscle fibres. Visammin is a systemic and coronary vasodilator. The intravenous injection of visammin produces an immediate fall of blood-pressure which returns to normal. Later it falls slightly below the normal and remains so for a long time. The first fall of blood-pressure occurs before and after atropine, but is more marked when no atropine is injected before injecting the drug. Similarly the fall of blood-pressure is more marked when the vagi are intact than when severed. Pre-

sumably, therefore, the initial fall of blood-pressure is mainly cardiac in origin and is due to the direct action of visammin on cardiac muscle and to cardiac inhibition through stimulation of the vagal centre. The intestinal volume shows an early slight decrease which may be explained as a secondary effect to the immediate action of the drug on the heart. Soon, the splanchnic vessels dilate as evidenced by the increase in intestinal volume. The vasomotor centre appears to be stimulated as indicated by the return of blood-pressure to normal after the first fall with a secondary diminution in intestinal volume, while, lastly, the direct action of visammin on the muscle wall of the blood vessels overshadows the stimulant effect on the vasomotor centre and results in a final slight fall of blood-pressure with increase in intestinal volume. (b) Khellinin[1,3] in a concentration of 1 : 100,000 increases the contractility of the cardiac muscle by producing a more complete systole and a more complete diastole with a corresponding increase in cardiac output. It increases the coronary flow, and this increase is more pronounced if the coronary vessels were first rendered in a state of partial spasm by barium chloride (1 : 40,000). The intravenous injection of the glycoside with the vagi intact slightly raises the blood-pressure with an increase in intestinal volume. The rise in blood-pressure is cardiac in origin.

Recently Anrep *et al.*[6,7,8] and Kenawy and Barsoum[10,11] state that visammin is not a cardiac depressant, and that the administration in a heart-lung preparation of a dog of 100 mg. of the drug produces no change in the heart volume. The same authors record that the glycoside khellinin is not a cardiac stimulant and does not increase the coronary flow, and is devoid completely of pharmacological activity. Nevertheless, Bagouri[12], just recently, has admitted in his experiments that visammin causes a diminution in the amplitude of the heart beat which is of a temporary nature, and that khellinin produces a slight increase in the strength of heart beat with large doses of the glycoside.

This being the case, we thought it worth while to record the following experimental results and observations, in view of the fact that we have examined both principles in their crude forms and in their various stages of purity obtained during the processes of their final isolation—results and observations which may throw light on these variations of results.

Experimental

(A) *Intact animal:* The dog was used in all experiments. Anæsthesia was maintained by the intravenous injection of 0·22 g. of barbitone sodium per kg. supplemented by ether for the preliminary operative procedure. Blood-pressure was measured from the femoral artery. Injections were made in the femoral vein of the opposite side. In those experiments in which the ventricular beats were recorded, uniform artificial respiration was maintained by the use of Brodie's ideal respiration pump. The thorax was opened in the usual way. The pericardium was snipped through and ligatured to the anterior thoracic wall on each side.

A fine hook was applied to the tip of the left ventricle and connected to a recording lever. In some experiments the vagi were severed. We restricted ourselves to the injection of limited doses of visammin and of khellinin so as not to interfere with blood volume—the solubility of these principles in Ringer's solution being, indeed, very limited—and we avoided the interference of solvents (alcohol or sodium benzoate for visammin and alcohol or pyridine for khellinin) and the use of controls.

The injection of 3 to 4 mg. of pure visammin (m.pt. 153° to 154°C.) per kg. reduced the contractility of the ventricular muscle, producing principally a less complete systole. The diminution of the amplitude of beat was always evident, but was more marked when the vagi were intact (Fig. 1), and slowing of the rate of beat may later occur. The first

FIG. 1 (reduced).—Intact dog under barbitone sodium and uniform artificial respiration. The ventricular heart beats are recorded with the vagi intact. Intravenous injection of 4 mg. of visammin per kg. reduced the contractivity of the ventricular muscle producing principally a less complete systole.

effect on blood-pressure was an initial fall followed by recovery with fluctuation—as previously reported the direct depressant action of visammin on the heart and the vasodilatation by its direct action on the muscle wall of the blood vessels contribute to the lowering of blood-pressure, whereas the stimulant action of the drug on the vasomotor centre may overshadow this effect.

On the other hand, the injection of 1 to 1·5 mg. of pure khellinin (m.pt. 175° to 176°C.) per kg, increased the contractility of the ventricular muscle producing a more complete systole and a more complete diastole. The increase in amplitude of the ventricular beats was always evident, but was more marked when the vagi were severed (Fig. 2), the rate of beat hardly changed though, relatively, slowing of the heart beat may occur with the vagi intact. The glycoside in similar doses raised the blood pressure in the intact animal especially when the vagi were severed (Fig. 3). This effect is, however, in favour of increased cardiac output—the glycoside does not directly stimulate the vasomotor centre and has no

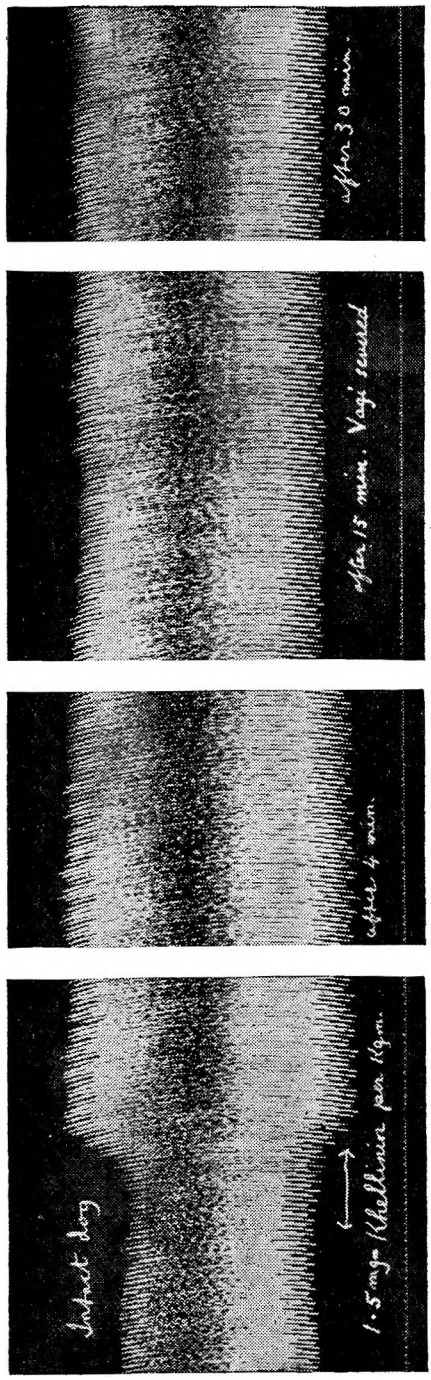

FIG. 2 (reduced).—Intact dog under barbitone sodium and uniform artificial respiration. The ventricular heart beats are recorded with the vagi severed. Intravenous injection of 1·5 mg. of khellinin per kg. increased the contractility of the ventricular muscle producing a more complete systole and a more complete diastole. The rate of beat hardly changed. Record after 4, 15 and 30 minutes of injection is demonstrated.

direct vasoconstricting action on blood vessels. The stimulant action of khellinin on the heart is well exhibited in such small doses and is persistent and rather "selective"—the glycoside, as already described by one of us, has no appreciable action on other organs in even larger doses.

(B) *Toad's heart perfusion:* Several experiments were carried out with various concentrations of visammin (1 : 10,000 to 1 : 100,000) and of khellinin (1 : 20,000 to 1:200,000). All the results were depression with visammin and stimulation with khellinin, with no cumulation. We record (Fig. 4) a triple perfusion, first with Ringer's solution, then with visammin (1 : 35,000 in Ringer's solution) and lastly with the same solution of visammin in which khellinin is dissolved in the same concentration. Depression under visammin is noticeable and the stimulant effect of khellinin is illustrated. In fact this antagonism was referred to by one of us as early as 1932 when it was recorded. "The cardiac depression caused by solutions of visammin is much more marked than equivalent concentrations of tinc-

ture of Ammi Visnaga —the presence of khellinin in the tincture partly accounts for this."

Moreover, Figure 4 demonstrates a greater amplitude of heart beat with visammin and khellinin together than under normal Ringer's solu-

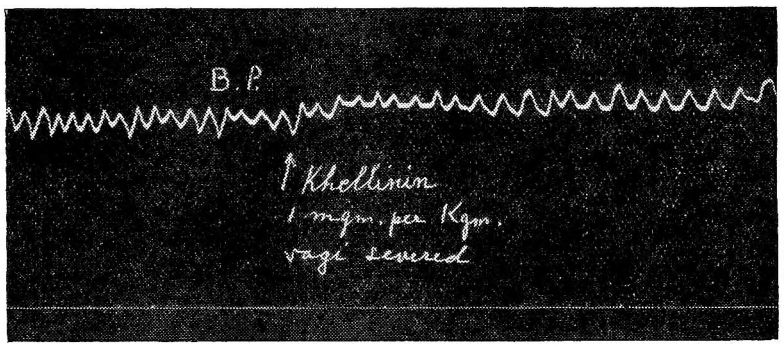

FIG. 3 (reduced).—Blood pressure of a dog under barbitone sodium. Vagi severed. Intravenous injection of 1 mg. of khellinin per kg. raised the blood-pressure.

tion. Presumably, however, one may infer that in the same concentration khellin is more a cardiac stimulant than visammin is a cardiac depressant. The perfusion of a strong solution (1 : 10,000) of visammin (Fig. 5) greatly diminished the degree of systole with arrest of the heart.

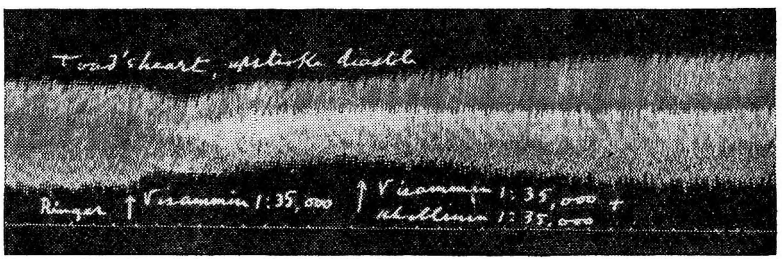

FIG. 4 (reduced).—Triple perfusion of a toad's heart, first with Ringer's solution then with visammin 1:35,000 and lastly with the same solution of visammin in which khellinin is dissolved in the same concentration. Depression of heart beat principally due to a less complete systole with visammin and recovery with stimulation under visammin and khellinin together are demonstrated. The amplitude of beat is greater under visammin and khellinin together than under Ringer's solution. Upstroke diastole.

The continued perfusion of the same solution of visammin to which khellinin was added to a concentration of 1 : 20,000 produced a fairly good recovery of the arrested heart with promotion of systole.

Similar triple heart perfusion experiments were carried out using chloral hydrate, quinine hydrochloride or alcohol in suitable concentrations against the same concentration of each one of these drugs in which khellinin was dissolved (1 : 25,000 to 1 : 50,000), depression of the heart beat was first established with subsequent stimulation in the presence of the glycoside.

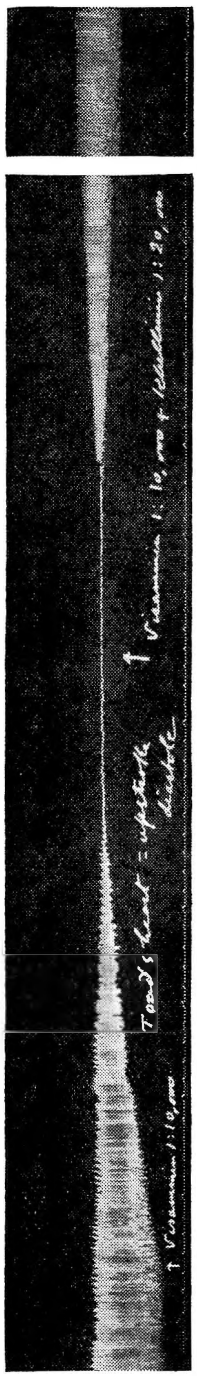

FIG. 5 (reduced).—Triple perfusion of a toad's heart, first with Ringer's solution then with visammin 1:10,000 and lastly with the same solution of visammin in which khellinin 1:20,000 is dissolved. Under visammin alone, systole is greatly reduced with final arrest of the heart, while under visammin and khellinin together, recovery with promotion of systole is seen. Upstroke diastole.

(C) *Isolated rabbit's heart perfusion:* The heart was perfused by a modification of Gunn's method. Pressure of perfusion was kept at 100 to 120 mm. Hg. Outflow was determined every 2 minutes and 5 readings were registered for each change of liquid. In all perfusions partial experimental spasm was induced by the use of barium chloride and for this a concentration of 1:40,000 was maintained throughout—barium chloride Locke's solution was first prefused followed by the same solution of barium chloride containing either visammin (1:40,000) or khellinin (1:40,000) and lastly barium chloride Locke's solution. Only 6 experiments were carried out with the one concentration of visammin and similarly 6 experiments with khellinin giving respectively an average increase in coronary flow of about 200 and 300 per cent. Owing to the limited number of experiments carried out and, moreover, on one concentration only, no conclusion could be drawn as to their relative values in this respect.

Experiments on the intact animal and on the isolated toad's heart similar to those mentioned under (A) and (B) respectively but using various crude samples—obtained from the plant in various stages of impurity—of visammin and of khellinin gave variation and ambiguity of results. In many instances the cardiac depression of visammin or the cardiac stimulation of the glycoside was marked to a great extent. This, however, is very natural, Ammi Visnaga contains quite a number of active bodies. Products which differed from the pure principles by a few degrees in m.pt. gave quite appreciable differences in the response of the heart which is a fairly sensitive organ to these bodies.

Indeed, in addition to determination of m.pt., it would be wise to confirm the purity of material biologically by heart perfusion experiments.

SUMMARY

1. Pure khellinin, m.pt. 175° to 176°C., possesses a persistent, rather selective stimulant action on the heart producing a more

complete systole and a more complete diastole with a corresponding increase in cardiac output. It raises blood pressure. The glycoside is active and doses of 1 to 1·5 mg./kg. of dog by intravenous injection. It increases the coronary flow, and is non-cumulative.

2. Pure visammin, m.pt. 153° to 154°C., depresses the heart, producing principally a less complete systole with diminished cardiac output. The drug is active in doses of 2 to 4 mg./kg. of dog by intravenous injection. It increases the coronary flow.

3. The impurities present in crude samples of khellinin or of visammin influence to an appreciable degree the normal response of the heart to the pure products.

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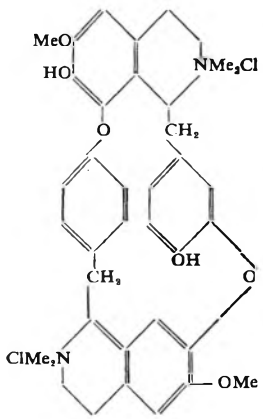
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS



Curare Alkaloids, Constitution of *dextro*-Tubocurarine Chloride. H. King. (*J. chem. Soc.*, 1948, 267.) *dextro*Tubocurarine chloride, on O-ethylation, gave amorphous O-ethyltubocurarine chloride which when submitted to a two-stage Hofmann degradation gave the nitrogen-free O-ethylbebeerilene identical in properties with the substance obtained from bebeerine. The same distribution of methoxy- and phenolic groups is therefore present in *dextro*tubocurarine chloride and bebeerine; since their particular orientation is known in bebeerine, *dextro*tubocurarine chloride must have the structure shown.

R. E. S.

ANALYTICAL

Antihistaminic Drugs of the Thenyl Series, Identification of. T. J. Haley and G. L. Keenan. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 85.) The optical crystallographic properties and physical constants of antihistaminic drugs of the thenyl series are recorded. Those studied were thenylene or histadyl (N : N-dimethyl-N-(2-pyridyl)-N-(2-thenyl)-ethylenediamine) and its halogenated derivatives, chlorothen (N : N-dimethyl-N-(2-pyridyl-N-(5-chloro-2-thenyl)-ethylene diamine) and bromothen (N : N-dimethyl-N-(2-pyridyl)-N-(5-bromo-2-thenyl)-ethylenediamine). A means for the identification and differentiation using 6 common alkaloidal colorimetric reagents is described. Tests were made by placing a drop of reagent on a microscope slide and adding about 1 mg. of the drug to it. Changes taking place were observed for about 30 minutes. The alkaloidal colorimetric reagents gave better results than three precipitation reagents also investigated but as the tests were almost identical for each of the thenyl compounds the optical crystallographic properties described offered the best means for their identification.

G. R. K.

Barbiturates; Xanthydrol as an Identification Reagent. R. S. McCutcheon and E. M. Plein. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 24.) Xanthydrol may be prepared by treating an alcoholic solution of xanthone with sodium amalgam or by reducing xanthone with zinc dust, Xanthone may be prepared by refluxing phenyl salicylate and distilling the product at high temperature. Xanthyl derivatives of 18 barbiturates were obtained by reaction of the barbiturates with xanthydrol in glacial acetic acid. Pure white crystals with characteristic m.pts. were obtained for most, clearly identifying these barbiturates. Two of the group of 20 studied were N-substituted barbiturates and did not react. The derivatives were dried to constant weight and analysed for nitrogen by the Kjeldahl-Gunning method. Deviation of the percentages found from those calculated did not exceed

9·20 except for barbitone (calculated 5·15, found 4·86). M.pts. were determined by both the block and U.S.P. methods. The former checked consistently and were useful; rate of heating was 0·5°C. per minute. G.R.K.

Paper Chromatography, Streaming Potential in. L. Rutter. (*Nature*, 1949, **163,** 487.) The net resultant flow of solvent through the capillary channels of paper used in chromatography is in a direction away from the point of feed, resulting in the setting up of a streaming potential. Distilled water flowing through a washed and dried strip of No. 3 Whatman paper showed a potential gradient of approximately 10 mV. per cm. With a 1 per cent. thorium nitrate solution in place of water, the potential was reduced almost to zero; 1 per cent. sodium chloride solution showed a potential gradient of approximately 4 mV. per cm. The rate of flow of liquid and the nature of the electrodes used for measurement affect the values. Such potentials effect chromatographic development and it was found that development of 0·01 ml. of 0·1 per cent. aqueous solution of a mixed colour (Edicol Green 37113) with distilled water failed to separate the components, the band moving with the solvent front, whereas development with 1 per cent. sodium chloride solution achieved complete separation into blue and yellow bands. With either the strip or central feed technique, the flow of one liquid over another in partition chromatography may result in streaming potentials of varying sign, of possible significance in considering mechanisms of partition separations. R. E. S.

Paper partition Chromatography, Deposition and Simultaneous Concentration of Dilute Solutions in. K. F. Urbach. (*Science* 1949, **109,** 259.) The concentrations of histamine encountered in ordinary paper partition chromatographic procedures, where 0·01 to 0·1 ml. volume of fluid is used, were too low relative to the sensitivity of the colour reaction used as indicator in the development of the chromatogram; a procedure was devised therefore so that the entire 3 to 5 ml. of butyl alcohol extract could be deposited without allowing excessive spreading of the solvent on the strips. Horizontal paper strips were fixed over a hot plate and maintained at 60° to 70°C. The extract was then dropped on to the strip from a capillary ended tube at a controlled rate of 1 ml. per hour, at which rate the solvent evaporates. The total spread of the spots is not more than 2 to 3 cm. Details of the apparatus and capillaries, together with diagrams are given. Other organic solvents, such as ether, acetone, alcohol, dioxane, etc., can be applied in a similar manner, although aqueous solutions spread excessively on the paper; solutions containing weak hydrochloric or sulphuric acid charred the paper even at moderate temperatures. R. E. S.

Stilbœstrol, Polarographic Studies of. L. E. Bingenheimer Jr. and J. E. Christian. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 117.) Electrolysis of solutions of stilbœstrol in concentrations of 10^{-4} to 3×10^{-4} M failed to give polarographic oxidation or reduction waves when the supporting electrolyte was N/10 potassium chloride in alcohol (30 per cent.) or in N/10 potassium hydroxide. Stilbœstrol suppressed the oxygen maximum in N/1000 potassium chloride solutions in alcohol (10 per cent.) or N/100 potassium hydroxide. The suppression was complete when the concentration of stilbœstrol was as little as 10^{-5} molar but negligible when the concentration was reduced to 10^{-6} molar. Repeated electrolysis decreased the size of the maxima and lowered the *p*H; buffering did not counteract this

effect but interfered, since the buffer exerted a suppressive action. Tablets of stilbœstrol were extracted by the U.S.P. method except that an extra step was necessary to remove gelatin which interfered with the results. The residue after removal of the ether was taken up in sodium hydroxide solution, potassium chloride was added and the solution diluted to correspond to $0{\cdot}4 \times 10^{-5}$ M stilbœstrol in N/100 potassium chloride and N/1000 potassium hydroxide. Standards corresponding to the U.S.P. limits of 90 and 110 per cent. of the labelled strength were similarly prepared. When the solutions were saturated with oxygen and electrolysed, the size of the maximum in the unknown solution was between those in the standard solutions. The main advantage of this method over the U.S.P. method is the saving of about 1 hour. G. R. K.

Tragacanth Flake, Evaluation of. Report No. 2 of the Tragacanth Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts. (*Analyst*, 1949, **74**, 2.) The following recommendations are made: *Viscosity*—The flake is ground rapidly until all passes a No. 30 mesh sieve and a quantity of the powdered gum equivalent to the required weight of dry gum is wetted with 5 ml. of 95 per cent. alcohol. Cold distilled water is then added quickly, the mixture is shaken, allowed to stand for 1 hour and is then heated in a boiling water bath, the determination being completed as described in Report No. 1 (*Analyst*, 1948, **73**, 368; *J. Pharm. Pharmacol.*, 1949, **1**, 44.). *Suspending Power*—Owing to the variations in suspending power in gums of the same viscosity it is recommended that the purchaser should carry out a form of test using the concentration of tragacanth normally employed in his process and using all the materials that he desires to suspend. *Ash*—Direct ashing failed to give concordant results and, after preliminary treatment the sulphated ash (at about 850°C.) was chosen. *Volatile acidity*—The method used was described in " Methods of Analysis of the Association of Official Agricultural Chemists," 6th Edition, 1945, p. 709. The detailed procedure necessary to obtain concordant results is given in each case. R. E. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Digitalis Glycosides, Chemistry and Pharmacology of. E. W. McChesney, F. C. Nachod, M. E. Auerbach and F. O. Laquer. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 364.) Analytically pure samples of gitoxin, digitoxin and their aglucones have been prepared. Experiments on rats showed gitoxin to be only slightly less toxic than digitoxin. It is known that gitoxigenin gives a red colour with ferric chloride in the presence of strong sulphuric acid, the intensity of the colour increasing rapidly up to 5 minutes and then fading. Without the ferric chloride the red colour develops slowly, reaching a maximum in about 24 hours and remaining unchanged for several days. Digitoxigenin treated similarly gives a pale lemon-yellow colour which gradually deepens. The glycosides give similar colour reactions but the aglucones are much better adapted to colorimetric work, since with the glycosides there is admixed a brown colour resulting from the reaction of sulphuric acid on the digitoxose. The absorption spectra of the aglucones in sulphuric acid were studied and their different behaviour was found to provide the basis for an analytical method which gives the proportion of digitoxin and gitoxin plus gitalin in a mixture.

S. L. W.

ESSENTIAL OILS

Ascaridol, Assay of; Iodination of Terpenes. A. Halpern. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 465.) The assay of ascaridol in oil of chenopodium depends on the liberation of iodine from potassium iodide. The author shows that the major part of the iodine is released within the first minute; this is followed by a slower steady release of iodine. In order to elucidate whether the olefinic linkage was responsible for this property an attempt was made to iodinate several related unsaturated hydrocarbons under conditions similar to those of the assay process. No iodination of the olefinic linkage occurred, so that the atypical behaviour of ascaridol toward the iodide reagent cannot be explained on this basis.

J. W. F.

GUMS AND RESINS

***Sterculia setigera*, Composition of the Gum of.** E. L. Hirst, L. Hough and J. K. N. Jones. (*Nature*, 1949, **163**, 177.) The analysis of reducing sugars by means of paper partition chromatography yields inconclusive results for mixtures of sugars with very similar R*g* values. The use of a column of powdered cellulose was found to afford a method for the separation of sufficiently large amounts of the individual sugars to allow identification by the normal determination of physical constants; results are given from a study of the gum of *Sterculia setigera*. Partial hydrolysis of the gum gave a mixture of sugars and a degraded material containing the uronic acids. The uronic acid portion had properties corresponding to a trisaccharide containing two D-galacturonic acid residues and a sugar residue, probably mainly L-rhamnose. The chief components of the non-acidic portion were D-galactose, L-rhamnose and a ketose, which, from its properties, could be fructose, tagatose or sorbose. A column of powdered cellulose was used to separate the sugar mixture with a solution of *n*-butyl alcohol saturated with water as the mobile phase. Two fractions crystallised spontaneously yielding D-galactose and L-rhamnose hydrate; D-tagatose was obtained from an intermediate fraction after oxidation of the accompanying aldose to the aldonic acid, which was removed as the barium salt. The D-tagatose thus isolated was identical with the sugar prepared synthetically. In all, five fractions were obtained containing respectively: a ketose, probably a methyl pentose; L-rhamnose; an aldose and D-tagatose; an aldose, tagatose and D-galactose; and D-galactose; D-tagatose has not hitherto been reported as a constituent of any natural product.

R. E. S.

ORGANIC CHEMISTRY

***p*-Aminobenzoic Acid and its Sodium Salt.** C. J. Kern, T. Antoshkiw and M. R. Maiese. (*Anal. chem.*, 1948, **20**, 919.) Sodium *p*-aminobenzoate was purified by charcoal treatment and three recrystallisations from aqueous solution. Purified *p*-aminobenzoic acid, prepared from the sodium salt by precipitation with hydrochloric acid, washing and drying at 100°C., melted at 187° to 187·5°C. A curve is given of the *p*H changes during titration of the acid with standard alkali and the equivalence point of 7·85, $pk^{\wedge}=4.65$ is derived. For acidimetric determination an indicator with a colour change between 7·0 and 8·7 is therefore suitable. A characteristic absorption spectrum was obtained in *iso*propyl

alcohol, wave length maximum = 288mμ. $E_{1\ cm.}^{1\ per\ cent.} = 1370$. In water both *p*-aminobenzoic acid and its sodium salt show about the same characteristic wave length, maximum = 266mμ, and $E_{1\ cm.}^{1\ per\ cent.} = 1070$, Beer's law being obeyed in both *iso*propyl and aqueous solutions. A table is given of comparison of results obtained by the spectrophotometer, titration, and diazo methods, all three being suggested for complete characterisation of pure *p*-aminobenzoic acid. R. E. S.

2-Amino-3-hydroxybenzoic Acid, Synthesis of. J. F. Nye and H. K. Mitchell. (*J. Amer. chem. Soc.*, 1948, **70**, 1847.) This substance was synthesised by two routes. In the first, 2-nitro-3-methoxybenzoic acid was reduced by catalytic hydrogenation to 2-amino-3-methoxybenzoic acid, followed by demethylation with hydriodic acid. The second method involved oxidation of 8-methoxyquinoline to give 2-(N-methyl-N-formyl)-amino-3-methoxybenzoic acid; appropriate treatment with hydriodic acid gave 2-amino-3-hydroxybenzoic acid m.pt. 254 to 255° C. (corr.). The compound of Keller (*Arch. Pharm.*, 1908, **246**, 1) reported as 2-amino-3-hydroxybenzoic acid was in reality the 3-methoxy derivative. Graphs of the ultraviolet absorption spectra of the 3-hydroxy and 3-methoxy derivatives are given. R. E. S

Mercurial Derivatives of Sulphanilamide. G. Rodighiero. (*Ann. Chim. appl. Roma.*, 1949, **39**, 27, 34.) In organic mercurial compounds the bond C-Hg usually shows greater resistance to reagents than the bond N-Hg and this influences the toxicity, antisyphilitic action and the uses in therapy and hygiene of these compounds. Mercurial derivatives of sulphanilamide are particularly interesting since they give the possibility of the mercury being linked to a carbon atom of the aromatic nucleus or to amino nitrogen or amido nitrogen. One molecule of sulphanilimide with 1 molecule of sodium hydroxide and 1 molecule of mercuric acetate gives (A) $H_2N.C_6H_4SO_2.NH.HgOH$. This is a white powder, decomposing on heating, insoluble in water, organic solvents and alkalis, but dissolving in hydrochloric acid to give the chloride (B) $HCl.H_2N.C_6H_4.SO_2.NH.HgCl$. This can be crystallised, has m.pt. 155° to 157°C. and is soluble in alcohol. These compounds have a notable antibacterial activity. The substance B dissolved in water and 0·5 to 1 molecule of sodium nitrite added at 0°C. gives (C) $HOHg.HNO_2SC_6H_4.NH.=NC.{}_6H_4SO_2NH.HgOH$. This is a yellow powder insoluble in water, organic solvents and alkalis, but dissolves in hydrochloric acid, the solution liberating nitrogen on warming. The substance A, dissolved in 4 or more molecules of hydrochloric acid gives on the addition of 1 molecule of sodium nitrate at 0°C. (D) $H_2N.SO_2C_6H_4.N=NCl.HgCl_2$. This is a white powder which can be crystallised from water; soluble in acetone and alcohol, insoluble in ether, benzene, and chloroform. It deflagrates on heating. One molecule of the substance D dissolved in acetone and shaken with 2 molecules of powdered copper gives (E) $H_2N.O_2S.C_6H_4.HgCl$. Crystallised from acetone, this occurs in white needles, melting with decomposition at 315° C. It is insoluble in water and dilute acids, but soluble in sodium hydroxide, being reprecipitated unaltered on acidification. It is insoluble in ether and light petroleum, slightly soluble in hot alcohol, readily soluble in pyridine. If the substance E is dissolved in pyridine and poured into water a white amorphous unstable compound is precipitated, which when kept in a vacuum desiccator over sulphuric acid finally loses its

pyridine leaving (F) $C_6H_4.SO_2NH$ (with Hg bridging) This is a white, amorphous powder insoluble in water, acids, bases and organic solvents, except pyridine, slightly soluble in acetic acid. It does not melt on heating and is unchanged at 320° C. H. D.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Aminobenzoic Acids, Production of, by Sulphonamide-resistant Bacteria. R. Lemburg, J. P. Callaghan, D. E. Tandy and N. E. Goldsworthy. (*Austral. J. exp. Biol.*, 1948, **26,** 9.) It is widely held that drug-resistance can be explained by an increased rate of production, or an increased total production, of *p*-aminobenzoic acid by a resistant strain of bacteria as contrasted with the susceptible parent strain. The main difficulty in demonstrating this has been the lack of specific methods for the isolation of *p*-aminobenzoic acid from the bacterial cultures. Attempts have therefore been made to develop a method of extraction and separation of aromatic animo-acids which would be sufficiently sensitive to be used with the low concentrations of these substances found in bacterial cultures. The method adopted by the authors consisted in the transformation of the aromatic amino-acids into azo-dyes by diazotisation and coupling with dimethyl-α-naphthylamine, in the separation of the acidic from the non-acidic dyes, and in separation of the former by chromatography on alumina. A strain of *Bacterium coli*, growing in synthetic Kisch's medium, produced diazotisable amino-acids in the supernatant; these were identified for the most part as anthranilic acid and *p*-aminobenzoic acid. After this strain had become adapted to sulphathiazole it did not produce more *p*-aminobenzoic acid that the parent susceptible strain, and the amount of *p*-aminobenzoic acid was insufficient to account for the resistance to sulphathiazole. The same held for the sum of *o*- and *p*-aminobenzoic acid produced. The production of anthranilic acid was variable and decreased with the number of sub-cultures. The authors therefore concluded that *p*-aminobenzoic acid production does not explain acquired drug-resistance so far as *Bacterium coli* is concerned. It may be true that the method of drug adaptation is different in different organisms. S. L. W.

Antibiotic Activity, as Shown by a Highly Amylolytic Strain of *Bacillus subtilis* B. S. Lulla. (*Nature*, 1949, **163,** 489.) *Bacillus subtilis* when grown on wheat bran medium, showed a pronounced antibiotic activity, the maximum being found in the aqueous extract from a 24-hour old culture; during this period the amylase formation was found to be low, but steadily increased with further incubation. Results are given of a study of the relationship between the antibiotic production and amylase formation by *B. subtilis* (N.C.T.C.: 2027 N) when grown on wheat bran. The antibiotic activity, at a maximum on the first day of growth, gradually disappears with further incubation, while amylase production, although negligible on the first day, steadily increases as the incubation period proceeds, and reaches its peak value on the fourth day. There is therefore a relationship between amylase formation and the production of antibiotic substance. R. E. S.

Insulin, Regeneration from Insulin Fibrils by the Action of Alkali. D. F. Waugh. (*J. Amer. chem. Soc.*, 1948, **70,** 1850.) Reversion by alkali treatment of insulin fibrils produces a crystalline product (termed r-insulin) similar to native insulin. A detailed method is given for the complete conversion of insulin into freely suspended insulin fibrils by heating crystalline zinc insulin at 100°C. with 0·05N hydrochloric acid in sealed glass ampoules. Limiting conditions for regeneration procedure were determined by studying the effect of alkali on native insulin. Using 0·5 ml. of 2 per cent. insulin (10 mg.) and 5·0 ml. of sodium hydroxide, experiments indicated that 0·03N alkali, 0°C. and a 45-minute treatment time gave the best results. Reversion of the fibrils was greatly accelerated by increasing the number of available fibril ends (by a freezing and thawing cycle which breaks up the longer fibrils into short segments), suggesting that disaggregation occurred mainly at these positions. The presence of sodium chloride in N/1 concentration in the alkali caused a 90 per cent. inhibition of reversion; the repulsive forces between similarly charged groups may thus play a part in the mechanism of disaggregation. The crystalline product from reverted fibrils was not found to be significantly different from native insulin in crystallisation properties, in biological activity (20 I.U. per mg.), in ultracentrifuge pattern (sedimentation constant 3·3 to 3·6), and in fibril formation (at 20° and 100°C.). The irreversible loss of one or more of the characteristic properties following demonstrable changes in internal structure was not found with r-insulin; tests for changes in labile groups, such as amino and disulphide, have been negative. The retention by r-insulin of characteristic insulin properties known to be sensitive to structural changes, the absence of changes in labile groups, and the fact that fibril elongation may take place at low temperatures in the *p*H region of maximum stability, are interpreted as showing that only small structural changes take place during fibril formation and that the process is one in which globular or corpuscular units are linked endwise. R. E. S.

Neomycin, a New Antibiotic. S. A. Waksman and H. A. Lechevalier. (*Science,* 1949, **109,** 305.) The organism producing neomycin was isolated from the soil, and is related to *Streptomyces fradiæ*. When the newly isolated culture was grown in various media containing a source of nitrogen, a carbohydrate, and salt, it was found to produce neomycin under both stationary and submerged conditions of culture. The antibiotic can easily be removed from the culture medium and concentrated by the methods of adsorption and elution applicable to streptomycin. Neomycin is a basic compound, most active in an alkaline medium. It is soluble in water and insoluble in organic solvents. It is thermostable. It is active against numerous Gram-positive and Gram-negative bacteria, especially mycobacteria, but not against fungi. The antibiotic spectrum of crude neomycin is quite distinct from that of streptomycin or streptothricin. Neomycin preparations were found to posses the following desirable properties: (1) similar activity against both streptomycin-sensitive and streptomycin-resistant bacteria; (2) considerable activity against various forms of -resistant mycobacteria; (3) limited or no toxicity to animals; (4) activity against various bacteria *in vivo*, including Gram-positive and Gram-negative organisms and against both streptomycin-sensitive and streptomycin-resistant organisms; (5) lack of resistance against neomycin among the organisms sensitive to it, or only limited development of such resistance. Neomycin has not

yet been obtained in crystalline form, but preliminary results point to its being distinctly different chemically from streptothricin and from streptomycin.

S. L. W.

Penicillin, Diffusion of. L. Mosonyi, R. Held and Ch. Kocsàn, (*Acta med. scand.*, 1942, **132**, 487.) The diffusion properties of penicillin are different from those of other crystalline substances. It is evenly absorbed by collidal substances, such as agar, without losing its efficiency and its diffusion is greatly influenced by this adsorption. A thrombin-fibrinogen membrane was shown to exert an adsorptive action on penicillin similar to that of agar, a fibrin layer of 3 mm. preventing the action of 0·5 to 1 unit of penicillin when interposed between the penicillin and an infected agar plate, though a fibrin layer of this thickness is not able to inhibit the action of penicillin when used in larger quantities (5 to 10 I.U.). As the concentration of penicillin in the blood, even when given intravenously, reaches only 0·3 to 0·4 units, and this for a very short time only, this explains why bacteria at the base of vegetations such as occur on the endocardium in endocarditis, and which often exceed a thickness of 4 or 5 mm., are found to retain their full virulence in spite of administration of penicillin. This adsorptive action of fibrin can be lessened, and the penicillin rendered more diffusible, by the addition to the penicillin solution of 20 per cent. of sodium dehydrocholate. S. L. W.

Penicillin. Enhancement of Therapeutic Activity. G. A. Hobby, T. F. Lenert, W. Reed and D. Renne. (*J. Bact.*, 1949, **57**, 247.) As a result of further work to discover the reason for the enhanced activity of impure penicillin as compared with crystalline penicillin G, evidence has been found suggesting that certain degradation products of penicillins G and dihydro-F respectively enhance the activity of highly purified samples of the two penicillins. The products possibly responsible are *p*-hydroxyphenylacetic acid, caprylic acid, penillic and penicilloic acids. H. T. B.

Progesterone, Stability of. R. B. Woolf and W. M. Allen. (*Proc. Soc. exp. Biol., N.Y.*, 1948, **67**, 79.) Samples of α and β-progesterone isolated from pigs' ovaries in 1936 were found on assay to be as active, after storage for 10 years, as the first preparations obtained by Wintersteiner and Allen in 1934. The preparations had been stored in small, unsealed glass-stoppered vials at room temperature. Both α and β-forms were assayed according to a slight modification of the original Corner-Allen method and the results were compared with the data obtained in 1934 by the original method. The comparison showed that the quantities necessary to produce full proliferation, and the quantities which produced little or no proliferation, were virtually the same, establishing beyond doubt that there had been no great change in activity in the interim. The dosage response curves from the data of 1934 were found not to differ significantly from those obtained from assay in 1946. S. L. W.

Steroids, Deuterium-labelled. Infra-red Spectrometry in Metabolic Studies. K. Dobriner, T. H. Kritchevsky, D. K. Fukushima, S. Lieberman, T. F. Gallagher, J. D. Hardy, R. N. Jones and G. Cilento. (*Science*, 1949, **109**, 260.) Infra-red absorption spectra of steroids, with one or more of the hydrogen atoms replaced by deuterium, are of value in the detection, analysis and identification of these compounds. The spectrum of pregnanol-3-(α)-one-20 is compared with the spectrum of the same compound where a hydrogen atom at C-11 and at C-12 has been

replaced by deuterium. Two absorption bands appear at 2,165 and 2,125 cm^{-1} in the deuterium-containing compound and in the neighbourhood of 1,200 $cm.^{-1}$ pronounced differences are apparent in the two spectra. The C-D absorption bands in the neighbourhood of 2,150 $cm.^{-1}$ are useful for the identification of a deuterium-containing molecule as this region, in the concentrations used, is transparent in the absence of deuterium. The method was very sensitive and the presence of deuterium could be established in as little as 25 μg. of pregnan-11 : 12-d_2-ol-3-(α)-one-20 containing 5 atoms per cent. excess of the isotope. In a metabolic experiment allopregnan-5,6-d_2-ol-3-(β)-one-20-acetate was injected into a normal woman and the urine and fæces were collected over 20 days; a fractionation procedure for treatment of urine and fæces followed by spectrum examination showed that the isotope was present in both the crude α- and β-hydroxy-ketonic fractions as well as in the α- and β-hydroxy non-ketonic fractions from both urine and fæces. Graphs are given of the infra-red spectra of normal and deuterium steroids in carbon disulphide solution for pregnanolone and pregnan-11 : 12-d_2-ol-3-(α)-one-20.

R. E. S.

Tricothecin, Isolation and Chemical Properties of. G. G. Freeman and R. I. Morrison. (*Biochem. J.*, 1949, **44**, 1.) The isolation of trichothecin from the culture filtrate of *Tricothecium roseum* is described. The nitrate was extracted with chloroform and the residue after evaporation of the chloroform was dissolved in ether and fractionated on a column of activated alumina using ether to develop the column. After fractional precipitation if inactive material from light petroleum and chloroform, the residue obtained was dissolved in carbon tetrachloride and again fractionated on an alumina column until pure tricothecin, m.pt. 118°C., was obtained. The substance dissolved in chloroform, ethyl alcohol, acetone, and benzene, and was slightly soluble in water (400 mg./l. at 25°C.); it had optical activity $[\alpha]^{18°C.}$ + 44° (*c*.1, in chloroform); analytical data were consistent with the molecular formula $C_{15}H_{18}O_4$ or $C_{15}H_{20}O_4$. The molcule contained one ketone group, one ethylenic group and three methyl groups attached to carbon; free carboxyl, hydroxyl, alkoxyl and aldehyde groups were absent. On hydrolysis with alcoholic potassium hydroxide, tricothecin combined with one equivalent of alkali. The ultra-violet absorption spectrum of tricothecin contained two main bands which, together with the shift of the bands occurring with a change in polarity of the solvent, indicated that the molecule contained conjugated ethylenic and carbonyl groups. Tricothecin was found to be relatively stable in acid solution and at *p*H 10, but at *p*H 12 hydrolysis took place with liberation of a carbonyl group and with virtually complete loss of antifungal activity in 6 hr. at 20°C. Acidification of the inactivated alkaline solution led to the formation of an inactive neutral ketone.

R. E. S.

BIOCHEMICAL ANALYSIS

Alcohol in Blood and other Biological Fluids, Colorimetric Determination of Microquantities of. R. J. Henry, Carol F. Kirkwood, S. Berkman, R. D. Housewright and J. Henry. (*J. Lab. clin. Med.*, 1948, **33**, 241.) The method depends upon the oxidation of the alcohol to acetaldehyde and determination of the latter colorimetrically with *p*-hydroxydiphenyl. Up to 20 ml. of sample is diluted with 20 ml. of water and 0·1 ml. of 10 per cent. sodium hydroxide solution and distilled. The

distillate, which should contain all the alcohol, is added to a solution of potassium dichromate and sulphuric acid and again distilled. The distillate is collected in a graduated tube in an ice-bath, the quantity collected depending on the amount of alcohol in the original sample. The loss of alcohol by complete oxidation to acetic acid is controlled by strict adherence to the routine described. The acetaldehyde is estimated by adding to 1 ml. of the distillate cooled in ice, 1 drop of 5 per cent. copper sulphate solution, 6 ml. of arsenic and nitrogen-free sulphuric acid, and, with constant shaking, 0·1 ml. of a 1·5 per cent. solution of *p*-hydroxydiphenyl in 0·5 per cent. sodium hydroxide solution. The mixture is warmed at 30° C. for 30 minutes, placed in a boiling water-bath for 90 seconds to dissolve excess of reagent, and cooled in ice to room temperature. The deep violet colour, which is stable for several hours, is read in a photoelectric colorimeter against a blank prepared by using 1 ml. of water instead of the distillate. Substances which interfere with the results include oxalacetic acid, α-glycerophosphates, glyceraldehyde and other alcohols. Among the non-interfering substances listed are methyl alcohol, glucose, acetone, pyruvic acid, urea and various amino-acids. The method is suitable for the determination of blood in amounts obtainable from finger puncture. The blood should be diluted 1 in 20, and the proteins and erythrocytes precipitated with tungstic acid. The method gives results within ±6 per cent. on a single blood determination.

G. R. K.

Alcohol, Ether and Volatile Reducing Substances in Blood and Gases, Determination of. A. Hemingway, L. A. Bernat and J. Maschmeyer. (*J. Lab. clin. Med.*, 1948, **33**, 126.) Various methods used for the determination of alcohol and ether in blood and air are reviewed and their disadvantages are examined. In the procedure adopted the reducing substance is absorbed in a known excess of standard potassium dichromate solution in the presence of sulphuric acid and the excess of chromic acid is determined by titration with ferrous sulphate solution using the redox indicator barium diphenylamine sulphonate. In the presence of phosphoric acid the indicator gave a sharp end-point from violet-blue to colourless, which is the main advantage of the method. Details are given for the determination of reducing substances in small quantities of blood using the Widmark flask method, and also for the determination in gases. The effect of variation in distillation time is studied and results are given of tests carried out on the reducing power of sulphuric acid, on the oxidation of acetic acid, and on the recovery of ether from prepared solutions of ether in blood.

R. E. S.

***p*-Aminosalicylic Acid in Blood and Urine, Estimation of.** H. G. Dickenson and W. Kelly. (*Lancet*, 1949, **256**, 349.) To a solution of 1 per cent. sulphanilic acid in 10 per cent. hydrochloric acid, cooled in ice, add 10 per cent. sodium nitrate solution until the reaction is just positive to starch iodide; then add the sulphanilic-acid solution until the starch iodide test is negative. This solution is kept cold and made up freshly for each test. Blood serum (2 ml.) containing *p*-aminosalicylic acid equivalent to 10 to 20 mg./100 ml. diluted with water (2 ml. is deproteinated with 10 per cent. trichloracetic acid (2 ml.), and filtered. The filtrate (2 ml.) is made strongly alkaline by adding 30 per cent. sodium hydroxide solution (0·25 ml.) and the diazo solution (0·25 ml.) added. The stable cherry colour produced is compared with that obtained from standard aqueous solutions of *p*-aminosalicylic acid equivalent to 10 to 20

mg./100 ml. Sulphonamides and *p*-aminobenzoic acid give no coloration, and normal blood only a negligible coloration. Salicylic acid gives a coloration only about 5 per cent. of that obtained for *p*-aminosalicylic acid. The recovery of added *p*-aminosalicylic acid from blood and blood serum is from 90 to 100 per cent. For the estimation in urine, 5 to 10 drops of a 10 per cent. calcium chloride solution is added to an aliquot (10 ml.) of the urine sample, the *p*H brought to 8 to 9 with 3N ammonia, the solution filtered, and the filtrate and washings adjusted to *p*H 2 with 10 per cent. hydrochloric acid. The intensity of colour on adding 2 drops of 10 per cent. ferric chloride solution is observed on a sample, and the urine diluted to give a colour approximately equivalent to that obtained from a standard solution of *p*-aminosalicylic acid. A colorimetric estimation can be made with an accuracy of ± 5 per cent. Salicylic acid will, of course, interfere with this estimation.

S. L. W.

Anti-Pernicious Anæmia Factor, Estimation of. W. F. J. Cuthbertson. (*Biochem. J.*, 1949, **44**, v.) The microbiological assay of Shorb for the growth factor present in highly refined liver extracts was found to be unsatisfactory. The organism used, *Lactobacillus lactis* Dorner ATCC 8000, required, in addition to the medium of Shorb, tomato juice and "Tween 80" as well as the anti-pernicious anæmia factor. Thymidine allowed the growth of the organism on vitamin B_{12}-deficient media and it was not possible to obtain a response to the anti-pernicious anæmia factor using the technique of Shorb. The cup-plate assay was adaptable to the determination of the anti-pernicious anæmia fraction and to the detection of other members of the B_{12} group of microbiological growth factors present in purified liver extracts. The medium used was that found suitable in the ordinary microbiological assay with the addition of 2 per cent. of agar. For an assay the sterile medium is melted, held at 45°C., and inoculated with a culture of *Lb. lactis* Dorner ATCC 8000; 12·5 ml. samples are then poured into Petri dishes. Holes are cut in the covered agar plates with a 10 mm. cork borer. Three drops of test or standard solution are placed in each of the appropriate holes and the plates are incubated overnight. After 16 to 24 hr. the colonies developing around holes form sharply defined zones of exhibition; zone diameters are proportional to the logarithms of the anti-pernicious anæmia fraction concentrations over the range 0·02 to 0·5μg./ml. Both factors contribute to microbiological activity and unless the ratio of clinical to microbiological activity is the same for both of these substances this test alone will not exactly predict the clinical potency of liver extracts. The method is rapid and simple, but it is relatively insensitive and somewhat susceptible to interference by other members of the B_{12} group, preservatives and antibiotics.

R. E. S.

Penicillin. Paper Strip Chromatography. R. G. Kluener. (*J. Bact.*, 1949, **57**, 101.) A modified paper strip technique which can be completed in 24 hours is described for determining separately penicillins X, G, F and K. The method is based on the differences in the distribution coefficients of the varieties of penicillin between ether and a phosphate buffer. The ether used for development must be anhydrous and of reagent grade. In analysing 35 known mixtures in buffer solution and 24 known mixtures in broth, the difference between percentages added and those found did not exceed 13 per cent. and averaged ± 5 per cent.

H. T. B.

Penicillin G, Determination of. G. B. Levy, D. Shaw, E. S. Parkinson and D. Fergus. (*Anal. Chem.*, 1948, **20**, 1159.) The light absorption in the ultra-violet region due to the benzyl group in penicillin G, is used to determine this component in mixtures of penicillins. The total light absorption of a penicillin mixture is due to a "background" non-selective absorption upon which is superimposed the benzene band spectrum which can be evaluated by several methods; graphs are given illustrating this procedure. For routine analysis of penicillin G preparations, a simplified technique was used based on the fact that commercial penicillin G preparations usually do not contain penicillin X or inactivated penicillin G, but, besides benzylpenicillin, only small amounts of penicillins F, K, and dihydro F, and some inert pigment. Under these conditions an average value for the angular displacement of the spectrum due to impurities, i.e. the slope of the "background" absorption—together with the measurement of the height of a characteristic band (maximum and minimum) affords a rapid method for determining the benzyl content of penicillin G preparations. Comparisons of assays by this method with the gravimetric procedure, based on the precipitation of penicillin G by N-ethyl piperidine, showed that the average deviation for the gravimetric method is 1·43 per cent., and for the spectrophotometric method 1·40 per cent., while the maximum deviation is 4·5 and 5·2 per cent. respectively. The method evolved is particularly suited to routine use but extraneous benzyl groups will interfere. A photoelectric spectrophotometer is necessary to produce the required accuracy of extinction readings. R. E. S.

Sodium in Biological Fluids, Microcolorimetric Determination of. A. A. Albanese and M. Lein. (*J. Lab. clin. Med.*, 1948, **33**, 246.) The reagent is a solution of uranyl zinc acetate prepared by adding a boiling solution of 10 g. of uranyl acetate in 50 ml. of water and 2 ml. of glacial acetic acid to a boiling solution of 30 g. of zinc acetate in 50 ml. of water containing 1 ml. of glacial acetic acid, allowing to stand overnight, filtering, diluting with an equal volume of alcohol (95 per cent.), cooling at 4°C. for 48 hours and again filtering. A mixture of 0.2 ml. of urine or spinal fluid and 1 ml. of reagent is cooled at 4°C. for one hour, and centrifuged. The supernatant liquid is discarded, the residue drained and washed with 2 ml. of alcohol (95 per cent.), again centrifuged and drained, and dissolved in 5 ml. of water. Any turbidity due to an excess of phosphate is removed by centrifuging and the intensity of the yellow colour of the solution measured in a photoelectric colorimeter. A parallel determination is done for 0.2 ml. of a standard solution of sodium chloride in water containing 2 mg. of sodium per ml. The content of sodium per ml. is calculated from: reading of unknown/reading of standard × 0.4 mg. of Na × 5. Fer sera, plasma and whole blood, 0·2 ml. is treated with 0.6 ml. of a 20 per cent. solution of trichloracetic acid, centrifuged, and 0.4 ml. of the supernatant liquid treated as described. The experimental error is about ±5 per cent. G. R. K.

Steroids in Urine, Determination of. S. L. Tompsett. (*Analyst*, 1949, **74**, 6.) A review is made of the methods available for the isolation and identification of the steroids found in human urine. The origin of the principal steroid hormones, male and female, is given, together with a detailed table of the principal natural steroids found in man. Steroids in urine are separated from other materials by three procedures: (1) extraction of the steroid conjugates with butyl alcohol followed by acid hydrolysis, (2) a short acid

hydrolysis followed by extraction of the free steroids with an organic solvent, (3) simultaneous hydrolysis by acid and extraction with a comparatively high-boiling solvent. Methods for the determination of total 17-ketosteroids, of non-alcoholic 17-ketosteroids, and of α- and non-alcoholic 17-ketosteroid content after precipitation of the β-alcohols with digitonin, are given. The 3:20-ketosteroid fraction is discussed and a method is given for the determination and separation of ketonic and non-ketonic steroids using Girard reagent T. Results are quoted for urine samples from a wide variety of clinical cases. For a complete picture of steroid hormone metabolism the following determinations are necessary: œstrogens; 17-ketosteroids; total ketones indicative of the presence of the 20-ketosteroids; the non-ketones; pregnanediol-3(α):20(α); the corticosteroids. R. E. S.

CHEMOTHERAPY

Diamidines as Antibacterial Compounds. R. Wien, J. Harrison and W. A. Freeman. (*Brit. J. Pharmacol.*, 1948, **3**, 211.) In the diphenoxyalkanes there was a graded increase in bacteriostatic activity, which was maintained in the presence of blood, against staphylococci, rising to a maximum from the propane to the hexane and nonane derivatives. This increase was accompanied by an increase of intravenous toxicity but by only a relatively small increase in local toxicity to phagocytes. Gram-positive bacteria were more susceptible than Gram-negative bacteria. The introduction of halogen into one or both benzene nuclei in the diphenoxyalkanes increased bacteriostatic activity, with little alteration in local toxicity to phagocytes. The mono-halogen derivatives were more active than the di-halogen against staphylococci, whereas the di-halogen derivatives were more active against Gram-negative bacteria. Dibromopropamidine and iodohexamidine were amongst the most active of the compounds examined for their possible use in surface infections. They both showed bacteriostatic and bacterical activity, these effects being decreased in an acid and increased in an alkaline medium. Drug-resistant strains of bacteria could easily be induced by repeated sub-cultivation *in vitro*. Cross-resistance experiments showed that: (1) staphylococci resistant to penicillin or to 5-aminoacridine were susceptible to diamidines, (2) staphylococci and streptococci resistant to one diamidine were resistant also to other diamidines, (3) staphylococci resistant to diamidines were not resistant to penicillin or 5-amino-acridine. Little therapeutic activity can be demonstrated when the compounds are given by injection. S. L. W.

Miracil D. D. M. Blai, and F. G. Loveridge. (*Lancet*, 1949, **256**, 344.) Miracil D was given by mouth twice a day for 3 to 6 days to African school-children. Of 82 children who received a total dosage of at least 60 mg./kg. of body weight 74 ceased to pass living eggs or active miracidia, and none of the cured cases had relapses up to 12 weeks after treatment. There seems to be no advantage in giving more than 15 mg./kg. daily. This dosage caused no symptoms in about half the children. The others complained of abdominal pain, loss of appetite, nausea, and/or headache and dizziness, but none were ill enough to seek medical aid. A few children infected with *S. mansoni* were also treated with the same dosage but the results were unsatisfactory. S. L. W.

PHARMACY

GALENICAL PHARMACY

Cetyltrimethylammonium Bromide, Efficacy of in Ointment Bases. L. P. Prusak and A. M. Mattocks. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 67.) Six ointments comprising 1 per cent. of cetyltrimethylammonium bromide in hydrophilic ointment U.S.P., hydrophilic petrolatum U.S.P., simple ointment U.S.P., tragacanth glycerite N.F., pectin paste N.F., and a carbowax ointment base prepared by mixing carbowax 1500 with 10 per cent. of water, were added in amounts of 0·5 g. to 5 ml. quantities of nutrient broth inoculated with *Staphylococcus aureus*, and the mixtures were incubated at 37°C. for 3- and 24-hour periods. One loopful (0·05 ml.) of each mixture was sub-cultured in a medium containing lecithin, which inhibits the action of the bactericide, incubated for 48 hours and the tubes were read for turbidity. Control tests were carried out to determine the extent to which the ointment bases interfered with the action of the bactericide. Pectin paste and carbowax ointment base proved to be suitable ointment bases. The ointments prepared with hydrophilic ointment and hydrophilic petrolatum had no bactericidal activity, while those prepared with simple ointment and tragacanth glycerite released such small amounts of bactericide as to be practically ineffective. G. R. K.

Saccharated Iron Oxide for Intravenous Administration. J. H. Nissim and J. M. Robson. (*Lancet*, 1949, **256**, 686.) Samples of saccharated iron oxide differ widely in toxicity owing to differing methods of preparation. The following method permits of better control and provides material of lower toxicity. Dissolve 25 g. of anhydrous sodium carbonate in 1 l. of distilled water and add 50 g. of ferric chloride ($FeCl_3$,$6H_2O$). Carbon dioxide is evolved and the ferric hydroxide formed dissolves in the excess of ferric chloride to give a dark purple solution. Add sodium carbonate solution gradually to reprecipitate all the iron as ferric hydroxide, and adjust to *p*H 7. Wash away the sodium chloride formed by repeated additions of distilled water in quantities of 1000 to 2000 ml., allowing the ferric hydroxide to settle and decanting, continuing until the supernatant fluid acquires a brownish tinge. Add 166 g. of sucrose, followed by 30 ml. of 15 per cent. sodium hydroxide solution, pour the mixture into a flat dish and heat in the oven at 130°C. The saccharated hydroxide gradually dissolves to an almost black solution. If to a sample of this solution (enough to colour 10 ml. of distilled water red-brown) dilute hydrochloric acid is added drop by drop it precipitates when *p*H 8 is reached. With continued heating, the *p*H at which this precipitation takes place falls gradually from 8 to 3. When the required precipitation point is reached (the original specimen used clinically with success had *p*H 5·7) the temperature of the oven is reduced to 90°C., the product is evaporated to dryness, and dissolved in 500 ml. of distilled water to give a solution containing 2 per cent. of elemental iron. After filtering through a Whatman No. 50 filter paper, and autoclaving, it is ready for use. Variations in the method of preparation are discussed and an account given of toxicity tests with these preparations leading to the selection of the best samples for intravenous administration. The macroscopical and microscopical findings in mice receiving lethal doses are described and the manner in which saccharated iron oxide produces

its toxic effects is discussed. Until the method of preparation is sufficiently reliable to ensure the constant production of satisfactory samples the authors consider that biological standardisation is necessary. S. L. W.

PHARMACOGNOSY

Indian Belladonna, Studies on. R. Chatterjee and J. K. Lahiri. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 11.) Indian belladonna, *Atropa acuminata* Royle, was compared morphologically with *A. belladonna* Linn. and found to differ mainly in the structure of the leaves. As a result of a critical study of specimens of *Atropa* from the Calcutta Herbarium, the authors suggested that the Indian species should be reduced to a variety of the type species and henceforward be called *A. belladonna* Linn. var. *acuminata* (Royle) R. Chatterjee and J. K. Lahiri. *A. lutescens* Jacquemont was said to be synonymous with *A. belladonna* Linn. var. *acuminata* (Royle), hence the statement by Chopra that *A. lutescens* (which is of low alkaloidal content) is used as an adulterant to Indian belladonna seemed without foundation. The hyoscyamine content of the roots from Indian sources is fairly high; commercially the roots are used for the preparation of atropine, and can be used for hyoscine since this constitutes about 15 per cent. of the total alkaloids. G. R. K.

***Juniperus occidentalis*, Hooker, Sierra Juniper Wood.** E. F. Kurth and H. B. Lackey. (*J. Amer. chem. Soc.*, 1948, **70**, 2206.) Trees of this species grown in Oregon were collected, the bark removed, and representative specimens of sapwood, heart-wood, whole wood, stumpwood and root-wood obtained. After room-drying to a moisture content of less than 10 per cent. the various samples were extracted with ethyl ether; the extractive ranged from 2·96 per cent. (stumpwood) to 6·50 per cent. (heart-wood). Approximately 1 per cent. of additional material, soluble in acetone, was chiefly a catechol phlobaphene. The ether extract was soluble in light petroleum to an extent of 2·53 per cent. on the weight of the sap-wood and 3·01 per cent. on the weight of the whole wood. The light petroleum extract consisted of resin acids, oleic acid and high molecular weight lactonic acids, a mixture of α- and β-sitosterol, and a hydroxyresene $C_{18}H_{31}O$. The volatile oil from the trunk of the tree ranged from 0·9 to 1·25 per cent. and appeared to consist of cedrol; the oil obtained from the rootwood contained cedrene and cedrol. R. E. S.

***Remijia pedunculata*, Observations on the Bark of.** H. W. Youngken. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 27.) Studies of the physical characteristics and histology of *Remijia pedunculata* bark, a recognised source of quinidine sulphate in the U.S.P. XIII, and of quinine during the war, are reported. The materials and methods used in these studies are outlined. The unground bark is described and shown to differ from cinchona in having brittle cork which readily separates from the bark and is therefore absent or partly absent from most commercial specimens. A detailed description of the histology of the stem bark is given and differences between it and cinchona bark noted, a striking difference being the absence of microcrystals. New additional anatomical data are also reported and the powdered bark is described. Illustrations of pieces of the unground drug, the cross section of the stem bark and the diagnostic tissue elements of the powdered

bark are included. *Remijia pedunculata* bark is shown to satisfy the identification test for cinchona in the N.F. VIII; hence it is suggested that this test be deleted in the N.F. IX or made more specific for cinchona. G. R. K.

***Opuntia vulgaris*, a new Source of Pectin.** H. Diacon and V. Massa. (*Ann. pharm. Franc.*, 1949, **6**, 457.) *Opuntia vulgaris* Mill., which is common in Tunisia, gives a good yield of pectin. From 2 kg. of the fresh twigs, 14 g. of calcium magnesium pectate were prepared. The anti-hæmorrhagic action of this material was determined, and it was concluded that the action was superior to that of the pectin used by previous authors. This superiority, it is considered, is due solely to the high content of calcium and magnesium.
G. M.

PHARMACOLOGY AND THERAPEUTICS

Amethocaine Hydrochloride. Severe Toxic Effects when used for Bronchoscopy. C. A. Jackson. (*Brit. med. J.*, 1949, **1**, 99.) The occurrence of toxic reactions in 2 patients treated with amethocaine to secure local analgesia before bronchoscopy is reported. The procedure adopted was to give 2 lozenges of benzocaine 200 mg. to suck 40 minutes pre-operatively followed after 10 minutes by 11 to 16 mg. of morphine. In the anæsthetic room, the fauces, the posterior pharyngeal wall and both pyriform fossæ were painted with less than 2 ml. of a 2 per cent. solution of amethocaine hydrochloride containing adrenaline 1 in 5000, and finally 2 ml. of the same solution was injected between the cords. Soon after injection, both patients became unconscious and had convulsions. In one patient, endotracheal oxygen and carbon dioxide and venepuncture effected recovery, but in the second, who had received 4 ml. by injection, deep cyanosis supervened and the pulse stopped, necessitating cardiac massage. Although the total period of cardiac arrest was about 4 minutes and unconsciousness persisted for 4 days, complete recovery took place. To avoid the occurrence of severe reactions the following precautions are suggested: (a) a barbiturate should be given by mouth pre-operatively; (b) an amethocaine pastille should be sucked 30 minutes before examination; (c) the total dose of amethocaine should not exceed 80 mg., and the solution should contain adrenaline, 1 in 5000; (d) application by spray should not be used; (e) amethocaine should not be applied to inflamed, traumatised or highly vascular surfaces (especially in the urethra), and (f) it should not be used for allergic, severely debilitated or cachectic patients. Treatment of toxic reactions should include artificial respiration with oxygen-carbon-dioxide mixture, using an endotracheal tube if necessary, intravenous administration of a rapidly-acting barbiturate to control the convulsions, and administration of respiratory and cardiac stimulants.
G. R. K.

Antabuse, Preliminary Report on Clinical Trials. R. G. Bell and H. W. Smith. (*Canad. med. Ass. J.*, 1949, **60**, 286.) Antabuse is the Danish trade name for tetraethylthiuramdisulphide. Patients receiving this drug have an abnormal reaction to alcohol in the body, though the drug itself has few effects when administered in daily doses of 0·5 g. over a period of several months. The symptoms produced by alcohol after administration of antabuse are probably due to interference with the oxidation of alcohol so that abnormally high levels of acetaldehyde are produced in the body.

This report is mainly concerned with the symptoms produced when alcohol is taken after antabuse. 5 to 10 minutes after ingestion of a moderate amount of alcohol (20 g. of ethyl alcohol) there is a sensation of heat in the face, accompanied by flushing of the face and upper part of the body, with throbbing of the head and neck and accelerated pulse. After larger doses of alcohol (40 to 50 g. of ethyl alcohol) nausea may begin 30 to 60 minutes after the cardiovascular symptoms and may result in copious vomiting. A considerable fall in blood pressure may occur. The duration of the symptoms may last from half an hour to several hours but after a few hours sleep the patient feels completely well again. Antabuse will prove a valuable adjunct in the treatment of the alcoholic patient. S. L. W.

Curare; A Method of Assay Using Rats. M. G. Allmark and W. M. Bachinski. (*J. Amer. pharm., Ass., Sci. Ed.*, 1949, **38**, 43.) A unit consisting of 15 separate compartments floored partly with wire netting and partly with galvanised iron and fitted with a cover to keep the rats in their respective compartments was attached to a frame at a 60° angle. Commercial samples of intocostrin and *d*-tubocurarine chloride were used for the tests, diluted with water to a concentration required to produce responses when injected subcutaneously into each rat. After injection, each rat was placed in a separate compartment and observed for 20 minutes; if it fell off the wire netting within this time it was considered a reactor. It was found that very few rats fell off after 20 minutes. To test the validity of the method several three-dose assays were carried out using 15 rats on each dose. *d*-Tubocurarine chloride was used as a standard for each assay. Results were tabulated and the methods of Bliss were followed in making the calculations. The slopes of the regression lines were found not to differ significantly for the 10 assays reported and in no assay did the slope of the regression line for intocostrin differ from that of *d*-tubocurarine chloride, nor could it be determined that the weighted means of the slopes differed, indicating that the responses in rats are the same for both products. Further tests of accuracy were recorded using solutions of known potency. The mean actual error for these assays was 5·7 per cent. Changing the slope of the frame to 75° did not increase the accuracy of the method. It compared favourably with the rabbit head-drop cross-over test and the mouse method. G. R. K.

Decamethonium Iodide (C10) in Anæsthesia. G. Organe. (*Lancet*, 1949, **256**, 773.) The use of decamethonium iodide in 150 operations of many different types, have established that this drug is a safe and satisfactory substitute for *d*-tubocurarine and that it may be used in unselected cases. It is roughly five times as potent as *d*-tubocurarine chloride but has a shorter effect, and it produces a neuromuscular block which is not affected by anticholinesterases. A single intravenous injection of 3 mg. in light surgical anæsthesia produces in most patients good muscular relaxation without unduly depressing respiration. Its action is relatively evanescent and further injections are made at intervals of 10 to 40 minutes as required. The dose depends on the preceding interval—after 40 minutes a further 3 mg. will probably be necessary. Pentamethonium iodide in a dose 10 times that of decamethonium iodide is an effective antidote. Thoracic and abdominal breathing fail, and recover, together. It seems to act similarly to *d*-tubocurarine in reducing laryngeal muscular irritability. There appears to be no

direct effect on the cardiovascular system, even with relatively large doses. Post-operative vomiting occurs in less than 25 per cent. of patients, post-operative collapse seems considerably less frequent than after *d*-tubocurarine chloride, and there have been no cardiovascular complications. Urinary retention lasting for 24 hours occurred in 9 per cent. of patients. All the common anæsthetic agents have been used with no obvious difference in effect, and a mixture of decamethonium iodide 4 mg. with thiopentone 1 g. has been used successfully. S. L. W.

Dextran as a Plasma Substitute. G. Thorsén. (*Lancet*, 1949, **256**, 132.) A proprietary form of dextran is described as a 6 per cent. solution of a polydispersoid glycose-polymer dextran, in which most of the molecules have been hydrolytically given a molecular weight conforming to that of an albumin, with 0·9 per cent. of sodium chloride added. Its viscosity lies between that of blood and plasma, and its specific gravity somewhat exceeds that of human plasma. It is non-toxic and does not injure the tissues either locally or systematically. After an intravenous injection of 1 or 2 l. of dextran the plasma-dextran level rises to 1 to 2·5 g./100 ml., and after an initial fall due to elimination of a low molecular fraction through the kidneys it falls at an even rate. During the initial renal excretion, when about a quarter of the dextran given is excreted, the urine-dextran level rises to 7 g./100 ml. without sign of renal injury. After that no dextran can be detected in the urine, the remainder of the dextran of a higher molecular weight being presumably metabolised. In Sweden today the hospital transfusion services rely to a large extent on dextran for emergency cases. It has been given to 5000 patients, and as much as 4 l. has been given in a single infusion. Very good results have been obtained both in the treatment and prevention of shock, and it is stated to be as good as plasma in shock from burns. Its use has not been found to affect fertility, foetal development or growth. S. L. W.

Dimethyl Ether of *d*-Tubocurarine Iodide, Pharmacology of. H. O. J. Collier, S. K. Paris and L. I. Woolf. (*Nature*, 1948, **161**, 817.) The dimethyl ether of *d*-tubocurarine iodide was compared with *d*-tubocurarine in the following ways: (1) in the intact mouse, rat and rabbit; (2) in a preparation of the rat under nembutal anæsthesia, similar to that of Raventos, in which the contractions of the rectus femoris muscle, in response to repeated condenser charges applied to its motor nerve, are recorded on smoked paper; (3) in the rat phrenic nerve-diaphragm preparation *in vitro*. In the intact rabbit the intensity of action of the dimethyl ether compound was shown to be many times greater than that of *d*-tubocurarine, while the slopes of the two duration curves were closely similar, indicating that the two substances are removed from their site of action at similar rates. The dimethyl ether compound, therefore, has a greater specific action at the myoneural junction in this species; this is again shown in the rat, though to a somewhat less extent than in the rabbit, by the *in vitro* experiments on the phrenic nerve-diaphragm preparation. In the intact mouse, the myoneural junction is slightly less sensitive to the dimethyl ether compound than to *d*-tubocurarine, but the rate of elimination of the former is slightly less than that of the latter. In the rat, *in vivo*, the duration of action of the dimethyl ether compound is many times greater than that of *d*-tubocurarine, whereas it has only about three times the intensity of action, which indicates that it is removed much more slowly from its site of action in this species. In the

Raventos preparation of the rat, the weight of the dimethyl ether compound required to reduce the tension of the muscular response to a given extent was one-third to one-half that of *d*-tubocurarine, when each drug was administered by jugular cannula, and the reduction lasts considerably longer. Ligation of the renal arteries and veins in the preparation prior to administration of either substance increases duration of action of each but does not prevent recovery of the muscular contraction. The dibenzyl and di-isopropyl ethers of *d*-tubocurarine iodide were prepared. The dibenzyl compound exhibited in the mouse about one-third the activity of *d*-tubocurarine and about one-half the intensity of action in the rat and the rabbit; it did not exhibit any more prolonged action. The dimethyl ether of *l*-bebeerine di-methochloride was also prepared. The ED50 and LD50 of this compound were found to be about twice those of *d*-tubocurarine. It was three times as potent as *d*-tubocurarine in reducing tension of muscular contraction in a Raventos preparation, and the effect lasted four times as long. S. L. W.

Local Anæsthetics, a Comparison of. H. S. Hamilton, B. A. Westfall and J. K. W. Ferguson. (*J. Pharmacol.*, 1948, **94**, 299.) A new series of indices, to be called Relative Ratings (R.R.) is proposed for the comparison of potency and toxicity of local anæsthetics in relation to cocaine or procaine. For each the drug of reference should be named, e.g., the Relative Rating with reference to cocaine should be designated R.R. (cocaine). In addition, the bases of comparison should be specified in tables or in the text. Example for any drug X:

$$\frac{\text{LD50 of cocaine}}{\text{LD50 of X}} = \text{Relative Toxicity (cocaine) or Toxicity relative to cocaine.}$$

$$\frac{\text{EC50 of cocaine}}{\text{EC50 of X}} = \text{Relative Potency (cocaine) or Potency relative to cocaine.}$$

Then:

$$\frac{\text{Relative Potency (cocaine)}}{\text{Relative Toxicity (cocaine)}} = \text{Relative Rating (cocaine).}$$

LD50 values (by intraperitoneal injections in mice), tissue toxicities (by intradermal injections in guinea-pigs), and EC50 values for infiltration anæsthesia (by intradermal injections in guinea-pigs), and surface anæsthesia (of the guinea-pig cornea) were determined for procaine, metycaine, monocaine, naphthocaine, butacaine, octocaine, cocaine, amethocaine (pontocaine) and cinchocaine (nupercaine). The LD50 values decreased in magnitude in the order given. Adrenaline hydrochloride in solution with each anæsthetic increased the systemic toxicity of procaine and metycaine and decreased the toxicity of amethocaine and cinchocaine significantly, but had no effect on the toxicity of the other drugs. From these determinations Relative Rating indices for the 9 drugs are defined and estimated by the authors for infiltration anæsthesia, localised block anæsthesia, and corneal anæsthesia. The two relatively new drugs naphthocaine (β-diethylaminoethyl-4-amino-1-naphthoic acid) and octocaine (2-(1-methylheptyl)2:2-dimethylethyl *p*-amino-benzoate) have high Relative Ratings for all four types of anæsthesia. S. L. W.

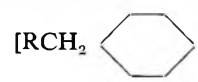
Orthoxine, Pharmacology of. B. E. Graham and M. H. Kuizenga. (*J. Pharmacol.*, 1948, **94**, 150.) In a study of a large series of phenyl propylamines, for the purpose of obtaining compounds more active than ephedrine

as bronchodilators and possessing little or no pressor activity, the methoxyphenyl*iso*propylamines appeared most interesting. The intravenous toxicities, pressor actions and bronchodilator properties of 14 of these amines are recorded. *ortho*-Methoxy-β-phenyl*iso*propyl methylamine hydrochloride (Orthoxine), because of its high activity and low toxicity, was investigated further. Perfusion experiments with constrictor agents on isolated lungs showed it to be a much more effective agent than ephedrine for relieving bronchoconstriction. Using isolated strips of jejunum or ileum, it was 4 to 8 times as effective as ephedrine in relieving intestinal smooth-muscle spasms induced by histamine, acetylcholine and barium chloride, and 5 times as effective in quieting the normal contractions of unstimulated intestinal muscle. On the non-pregnant uterus it was only half as active as ephedrine in stimulating the muscle to contraction. It produces little or no pressor response, and 4 times as much orthoxine as ephedrine must be administered to normal dogs to produce the bradycardia characteristic of the latter. Intestinal smooth-muscle tests indicate that it possesses anti-histaminic properties of the order of 1/20 that of benadryl but much greater than that of ephedrine. Its toxicity, chronic and acute (except for higher intravenous toxicity), is of the same order as that of ephedrine. The authors suggest that the drug may be of value in the treatment of asthma, and clinical trials are being carried out. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

Dithiocarbamic Acid Derivatives: Action Against Human Pathogens. C. R. Miller and W. O. Elson. (*J. Bact.*, 1949, **57**, 47.) The activity of compounds in this group against plant pathogens prompted the investigation of their *in vitro* activity against human bacterial and fungal infections. The substances tested included series of dithiocarbamates, thiuram monosulphides and thiuram disulphides, and a number of miscellaneous sulphur-containing compounds. The most active antibacterial compounds studied were tetramethylthiuram disulphide and sodium dimethyldithiocarbamate, their activity being greatest against *Streptococcus pyogenes*, much less against *Str. fæcalis*, *S. aureus* and *Eschericha coli*, and weakest against *Pseudomonas aeruginosa*. The same compounds were also the most active against the fungi tested, *Trichophyton gypseum* being most affected, *Epidermophyton floccosum*, *Microsporum canis*, *Sporotrichum schenckii*, *Blastomyces dermatitidis* much less, and *Candida albicans* least. H. T. B.

Quaternary Ammonium Sulphonamides, Antibacterial Actions of. C. A. Lawrence and G. R. Goetchius. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 424.) Six members of a series of compounds of the general structure



have been examined for antibacterial action against a number of organisms. The two most active compounds were those in which R = tetradecyldimethylamino-, R′ = R″ = H, and R = pyridine, R′ = dodecyl, R″ = H. These two compounds were highly active against both gram-positive and gram-negative organisms but not against *Pseudomonas æruginosa*. The antibacterial actions of these compounds are due to the quaternary ammonium component in the molecule and not to the sulphonamide radical.

A. L.

BOOK REVIEWS

PHARMAKOLOGISCHE METHODEN by L. Ther. Pp. 443 and Index. Wissenschaftliche Verlagsgesellschaft m.b.H. Stuttgart, 1949.

This book, a product of post-war Germany, should be of interest to those pharmacologists, physiologists, and others concerned with the techniques of experimental investigation of the properties of medicinal substances. The volume has many good features to recommend it as a practical book of reference, or a guide book to laboratory procedures, not least of which is the presence of some 244 line drawings of apparatus or techniques, and the citing of over 1,200 references to original literature describing technical procedures. The apparatus depicted is somewhat out of date, many of the blocks dating from the time when the favourite mode of heating depicted was a small Bunsen flame. The text deals mostly with continental methods with a preponderance of Teutonic origins. It is of interest that nothing much more recent than a decade ago is described. The descriptions apply briefly to a multiplicity of methods rather than provide an adequate description of any one procedure. The chapter on testing of vermicides is interesting, that on striped muscle singularly inadequate. The others vary in quality. There is no evidence of acquaintance with any of the recent advances in physics, biochemistry or operative procedures in this volume. The chapter on the handling of common laboratory animals is useful, though the rhesus monkey might have been included with advantage. The type and paper are good, the binding of a lower standard. The book might be described as a useful guide to the more classical methods of investigation of drug action, with a particular interest for the teacher of pharmacology on account of the description of several useful methods of demonstrating the action of drugs on tissues. J. D. P. GRAHAM.

A TEXT BOOK OF PHARMACOGNOSY, by G. E. Trease. Pp. VIII-811 and Index. Fifth Edition. Bailliere, Tindall and Cox, London, 1949, 30s. net.

A perusal of this text-book shows the wide field covered by modern pharmacognosy. Besides descriptions of a large number of vegetable and animal drugs, information is given on such varied materials as cotton, silk and surgical dressings, bacteria and fungi (including *Penicillium* spp. and yeast), chalk and kieselguhr, shellac, gelatin, beeswax and spermaceti, wool alcohols and gums; in short, the raw materials from the vegetable and animal kingdoms which go to furnish the pharmacist with his dressings, his vaccines and antibiotics, his plant insecticides and cosmetic creams, as well as the usual tinctures, infusions and tablets, pure alkaloids and crystalline products like picrotoxin and tubocurarine. The arrangement of the information is similar to that of the previous edition, the bulk of the book consisting of descriptions of crude drugs, etc., arranged in order of Phyla and families. Many of the descriptions include microscopical characters.

Besides this descriptive part there are short chapters on the history, commerce, cultivation, storage and evaluation of crude drugs. There are also chapters of a general nature on microscopical technique, constituents and extraction of crude drugs, and analysis (including fluorescence and chromatographic analysis). This information is necessary in order that the practitioner may be able to apply modern technique both to the description and evaluation of crude drugs.

As the author's aim is to cover the requirements of examination syllabuses

one should not perhaps expect much information on more recent work in pharmacognosy and additions to the materia medica such as alginates, *Ammi Visnaga*, the *Holarrhena* alkaloids, *Erythrina* alkaloids and rutin, but one would expect information on thyroid and pancreas, both of which are in the new syllabus for the degree of Bachelor of Pharmacy. Apart from these omissions the author's aim is well fulfilled in this book.

J. W. FAIRBAIRN.

PRECIS DE CHIMIE TOXICOLOGIQUE, by F. Schoofs. 2nd edition, 1948. Pp. 509 and Index. Les Presses Universitaires de Liege, Maison des Etudiants, Liege.

The scope of toxicology, and of toxicological chemistry in particular, is so wide that it is in the light of the author's statements that any volume on this subject must be considered. In the preface to the first edition it is stated that the book is intended for students as an introduction to the fundamental ideas of toxicological chemistry, not as an encyclopædic treatise but as a guide to those poisons which are more frequently encountered. As regards practical details it is said that these are given to enable "the more important methods used for the detection and determination of poisons" to be performed. It must be stated at the outset that, although the volume forms a useful introduction to the general principles of toxicological chemistry and deals with a wide range of poisons it would, in the opinion of the reviewer, be of limited value to anyone confronted with the actual task of toxicological analysis.

The work is divided into eight chapters: (1) general discussion; (2) gaseous substances; (3) volatile poisons and poisons isolated by distillation; (4) acids; (5) poisons extracted by immiscible solvents; (6) metallic poisons; (7) non-metallic poisons; (8) the purity of reagents. The first chapter on generalities is, to a chemist, one of the most useful in the book. It includes a discussion of the various routes of absorption and excretion of poisons, of the relation between molecular structure and toxicity, and contains some notes on the general precautions to be taken in toxicological analysis. It is to be regretted that the volume does not contain more references to the original literature. Most of the references are to text-books and little is given of recent analytical procedures and in particular of methods capable of detecting and estimating small quantities of organic and inorganic poisons. In dealing with lead, for example, useful notes are given on acute and chronic poisoning, on the toxic dose and on the method of elimination. It is difficult, however, to appreciate the value of the method given for the detection and estimation of lead when one considers that following this the use of a dithizone solution in chloroform is dismissed with the sentence "Cette solution verte, agitée avec un sel de plomb dissous dans l'acétate d'ammonium en présence de cyanure de potassium, donne une coloration rouge." Some sections, such as that on alcohol, are well written and fairly comprehensive, although that on barbituric acid derivatives occupies only six pages, a small number in view of the present day prevalence of this type of poisoning. The book is bound in paper covers and almost all of the pages require cutting. The editing and proof reading have been well done, although the formula for D.D.T. (p.396) is wrongly given. It is likely that this volume will be mostly useful in providing an account of the pharmacological action and general chemistry of a fairly wide range of poisons.

R. E. STUCKEY.

NEW REMEDIES

Dolcin* tablets contain calcium succinate 2·8 gr., and acetylsalicylic acid 3·7 gr. and are advocated for use in the treatment of rheumatic conditions. This succinate-salicylate therapy is based on the theory that the widespread systemic disturbance seen in some forms of arthritis is due to an alteration in tissue metabolism and respiration. Calcium succinate counteracts these effects by stimulating oxygen utilisation by the tissues; it also averts the depressant effect of salicylates on blood prothrombin. Dolcin is indicated in the treatment of rheumatic fever; articular rheumatism, including rheumatoid arthritis and osteoarthritis; non-articular rheumatism, including fibrositis, neuritis, lumbago and sciatica; arthritis associated with the menopause and gout. The initial dosage is 3 tablets four times daily, reduced to 2 tablets four times daily when the acute symptoms have subsided. The tablets are usually well tolerated and side-effects are rare. The tablets are issued in bottles of 100. S. L. W.

Folybden* Tablets each contain 1·7 mg. of folic acid and 3 gr. of molybdenised ferric sulphate. The addition to the iron of traces of molybdenum is stated to produce an increased rate of hæmoglobin formation. The tablets are suggested for use in the treatment of refractory normocytic anæmias when satisfactory hæmoglobin regeneration is not produced by the administration of liver extract or of folic acid alone. The dosage is 1 tablet three times daily after meals. S. L. W.

Genabrom* is a sedative preparation, one teaspoonful containing 7½ gr. of sodium bromide in concentrated yeast extract. When stirred with hot water it forms a palatable soup-like beverage. It is indicated in nervous conditions, such as hysteria, anxiety states, irritability, nervous vomiting, travel sickness, neurasthenia, and wherever a sedative is required. For insomnia, one or more small teaspoonfuls are dissolved in a cupful of warm water before retiring. Large doses may be given in epilepsy to lessen the frequency and severity of attacks. It is issued in jars containing 45 and 90 g. S. L. W.

Lantigen* is a desiccated bacterial vaccine for oral use containing antigenic substances prepared from a selected group of freshly isolated bacterial species chosen for their strong antigenic properties. The vaccine is presented as a slightly opalescent colloidal solution, containing as preservatives 0·1 per cent. of phenol and 0·01 per cent. of mercurithiosalicylate. It is claimed that the vaccines are absorbed by the alimentary mucosa and that their action is not impaired by digestion, as antibodies are produced before digestion begins. The vaccines should be taken daily, and the dose should be retained in the mouth as long as possible before swallowing, since the immediate local immunising response obtained is of additional benefit. Six Lantigen vaccines are prepared as follows:— "A," for coryza; "B," for nasopharyngeal catarrh and chronic bronchitis; "C," for rheumatic disorders of pathogenic origin; "D," for staphylococcal skin infections; "E," for hay fever and asthma; "F," for whooping cough. S. L. W.

Magsilate* tablets contain acetylsalicylic acid 5 gr., magnesium trisilicate 1½ gr., magnesium hydroxide 1½ gr., sugar and flavouring q.s. The aspirin is protected by a coating of sugar and flavouring agent, which, in turn, is

surrounded by layers of magnesium trisilicate, magnesium hydroxide, and sugar. The tablets are not intended to be swallowed but to be eaten like a sweet. The advantages claimed for this method of presentation are the absence of free salicylic acid, freedom from irritation of the gastric mucosa, and convenience of administration. Cartons are issued containing 12 tablets.

S. L. W.

Metheph* is a proprietary brand of methylephedrine hydrochloride suitable for oral administration. It is claimed to have the following advantages over ephedrine: (1) the blood pressor action is approximately only one-tenth that of ephedrine; (2) it does not stimulate the central nervous system; (3) it has a persistent slowing and deepening effect on respiration, and there is no secondary acceleration as with ephedrine. It is indicated for the relief of the bronchial spasm of asthma, especially in hypertensive asthmatics, the average dose being 1 tablet three times daily, with 1or 2 tablets at bedtime. Clinical trials have also indicated its value in the treatment of enuresis in children. It is supplied in tablets of 2/3 gr. in bottles of 25, 100 and 500.

S. L. W.

Tineafax* is a compound undecylenate ointment for the treatment of fungus infections of the foot. Undecylenic acid is not only effective against dermatophytic fungi but it possesses the blandness essential for prolonged prophylaxis and treatment, and being related to the normal constituents of sweat it does not cause irritation of the skin. It is also active over a wide *p*H range and is resistant to dilution by bathing and perspiration. In addition to the zinc salt of undecylenic acid, the ointment contains the fungicide zinc naphthenate, mesulphen, which possesses keratolytic properties, methyl salicylate, terpineol, and a small quantity of phenylmercuric acetate as preservative. Tineafax Powder, which is used in conjunction with the ointment, contains 10 per cent. of undecylenic acid, as the potassium salt, in an inert base. The ointment is applied night and morning, the powder being dusted on after the ointment has been rubbed in. The powder may also be used prophylactically by dusting into stockings, socks and shoes. The majority of cases of infection are cleared by this treatment in 7 to 21 days. The ointment is supplied in 1 oz. collapsible tubes, and the powder in 2 oz. tins.

S. L. W.

Trimetron: A New Antihistaminic Drug. I. W. Schiller and F. C. Lowell. (*New Eng. J. Med.*, 1949, **240,** 215.) Trimetron, 1-phenyl-1-(2-pyridyl)-3-dimethylaminopropane, has been shown in animal experiments to combine high antihistaminic activity with low toxicity. The authors now report the results of clinical tests on 84 allergic patients of either sex, ranging in age from 5 to 60 years. Doses of 25 to 125 mg. were given orally for several weeks. All of 12 patients with urticaria obtained satisfactory relief. Of 55 patients with perennial allergic rhinitis satisfactory or partial relief was reported by 47; the 3 reporting partial relief were not improved by increasing the dosage; 13 of 15 hay fever patients also obtained satisfactory or partial relief. Side reactions, chiefly drowsiness and dryness of the mouth, were mild; they occurred in only 10 patients. The drug is considered to be particularly useful in the treatment of perennial allergic rhinitis.

H T. B.

One of the latest products of CIBA research
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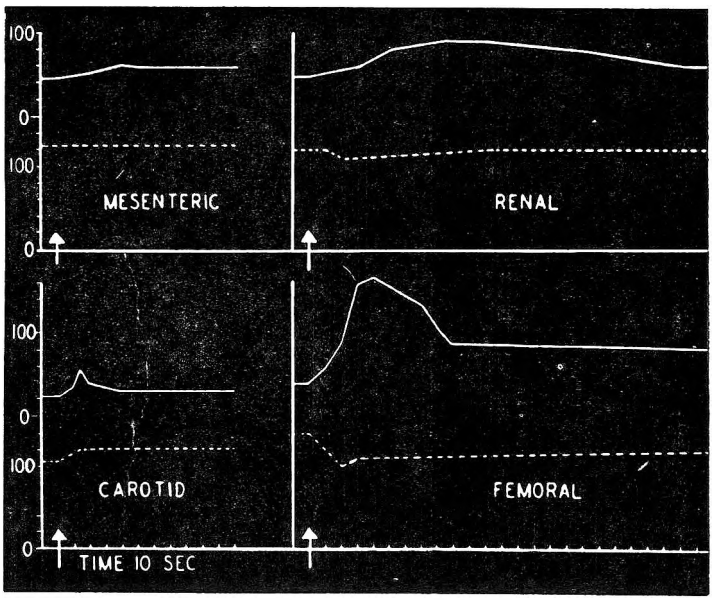

▲ Priscol 1 mg. intra-arterially — Blood Flow c.cm./min. - - - Arterial Pressure

THE ACTION OF PRISCOL ON BLOOD FLOW IN THE DOG

Priscol, 1 mg., was administered intra-arterially at each arrow. Active vasodilation as shown by the increased blood flow not due to increased arterial pressure occurs in all of the vascular beds. Note the variation in the degree of vasodilation ; the leg and kidney showing the greatest and the mesentery and head the least. These flow studies with intra-arterial injections prove that the vasodilating action of Priscol is peripheral and not central. (*J. Pharmacol. & Exper. Therap.* (1947) *89*, 274).

PRISCOL is proving to be of great value in the treatment of peripheral vascular disorders

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