REVIEW ARTICLE

THE DETERMINATION OF VITAMIN A

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At certain stages in vitamin studies biological assays on animals are indispensable but as knowledge increases the analyst strives to become independent of them. This is not merely because good analytical procedures are often quicker, cheaper and more trustworthy than biological assays (except perhaps for some microbiological methods) but also because the two approaches are complementary. The potency of a vitamin preparation when assessed by animal tests is the resultant of the vitamin content and a complex pattern of absorption and utilisation. If, let it be supposed, a fish liver-oil has a precisely known vitamin A content and gives in experimental rats a biological response indistinguishable from that elicited by a standard preparation providing the same amount of vitamin per rat per day, the pattern of absorption and utilisation must be the same for the liver-oil and the standard preparation. If, however, the biological responses are significantly different, important information will have been gained which could not have been obtained by either alone. This principle is very important in the vitamin A field for the following reasons:—

(i) Numerous carotenoid precursors of vitamin A exist, equal quantities of which (administered as pure substances) give different " yields " of vitamin, e.g., α-, β- and γ-carotene and their stereo-isomers.

(ii) The absorption of a given precursor varies with the form in which it is ingested, e.g., β-carotene in vegetable oil is more efficiently utilised than the β-carotene present in cooked carrots[1].

(iii) The absorption and utilisation of vitamin A or its precursors may be influenced by the nature of the vehicle, e.g., by the anti-oxidants or the vitamin E present in the diluent oil.

(iv) The biological test is not strictly specific for vitamin A_1 ($C_{20}H_{29}OH$), e.g., vitamin A_2 ($C_{20}H_{27}OH$), vitamin A_1 aldehyde $C_{19}H_{27}CHO$, vitamin A_2 aldehyde $C_{18}H_{25}CHO$, vitamin A_1 acid $C_{19}H_{27}COOH$ and vitamin A_1 methyl ether $C_{19}H_{27}CH_2OCH_3$ are all known to be highly potent.

(v) The problems of stereo-isomerism in vitamins A have not been fully worked out (see p. 142).

From the foregoing it may be seen that animal experimentation in this field is by no means obsolete.

The purpose of the present article is to deal with the narrower problem of determining the vitamin A content of oils, pharmaceutical preparations and tissues. Although the problem is admittedly rather complicated, all the major difficulties have been overcome.

Historical Background

Vitamin A does not occur in vegetable products, but herbivorous animals possess considerable liver stores of vitamin A esters. Carotenoid

precursors (provitamins) such as the β-carotene present in green foodstuffs are converted to vitamin A in the gut wall

$$C_{40}H_{56} \rightarrow C_{20}H_{29}OH + ?$$

and transported (in esterified form) to the liver to be stored partly in the Kupffer cells and partly in the true liver cells[2]. The latter contain an esterase (apparently lacking the former) and help to maintain a fairly constant plasma level of the functional vitamin A alcohol.

The carotenoid provitamins show selective absorption in the region near 450 mμ and vitamin A_1 shows a well marked ultraviolet absorption maximum near 328mμ (vitamin A_2, 350 mμ). With the antimony trichloride reagent (a saturated solution of anhydrous antimony trichloride in pure chloroform) blue solutions are formed with maxima at the following wave-lengths:—β-carotene, 590mμ; vitamin A_1, 617mμ; vitamin A_2, 693mμ., the extinction coefficients for provitamins A in general being very much lower than those for the vitamins A.

In principle, quantitative determinations of intensities of absorption can be used to ascertain provitamin or vitamin A content either by the ultra-violet maximum or the indirect colour test. The notation is as follows:—

If I_0 is the intensity of light incident on an absorption cell and I is the intensity of the light emerging after passage through a layer d cm. long of a solution of molar concentration c (pure solute) then

$\log I_0/I = E = \epsilon\ cd$ where ϵ is the molecular extinction coefficient. For vitamin A, $\epsilon_{max.}$ (at 328mμ) is 50,000, and for β-carotene $\epsilon_{max.}$ (at 455mμ) is about 130,800 (in *cyclo*hexane). An alternative way of expressing intensity of absorption is to give the $E\ ^{1\ \text{per cent.}}_{1\ \text{cm.}}$ value. For vitamin A, $E\ ^{1\ \text{per cent.}}_{1\ \text{cm}}$ 328mμ = 1750. (In practice this means, for example, that with a 1 cm. cell and a 0·0004 per cent. solution or with a 2 mm. cell and a 0·002 per cent. solution $E = 0{\cdot}7$.) For a fish-liver oil (or other preparation to be tested) $E\ ^{1\ \text{per cent.}}_{1\ \text{cm.}}$ is expressed in terms of the weight of material used. Thus, a fish-liver oil showing $E\ ^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 328mμ, 17·5 would contain 1 per cent. of vitamin A (subject to any correction).

The qualification *in principle* in the previous paragraph is necessary because the measured intensity of absorption at 328mμ is the sum of that due to vitamin A itself and a contribution due to other substances; in fish-liver oils the "irrelevant" absorption varies from sample to sample independently of the variation in vitamin A content. The gross $E\ ^{1\ \text{per cent.}}_{1\ \text{cm.}}$ value affords a first approximation to vitamin content but a correct value (in which due allowance has been made for irrelevant absorption) is now regarded as essential. The antimony trichloride test depends upon determinations of intensity of absorption by rather transiently blue solutions; and measurements must be made quickly. Some materials, however, contain colour test inhibitors which make estimates of vitamin A content too low. Since 1931[3] the aim has been to express vitamin A content and vitamin A potency in International Units. Carotene was already known as a crystalline substance but vitamin A had only been obtained as an "oil," and the first definition of an International Unit was the potency of 1μg. of a specimen of "carotene." By 1934 it had become clear that α-carotene and β-carotene differed quan-

titatively in their power to prevent or cure avitaminosis A. The first specimen of "carotene" was a mixture and the International Unit was redefined as 0·6μg. of pure β-carotene[4].

A range of fish-liver oils had been examined spectroscopically and the gross $E^{1\%}_{1\,cm.}$ value at 328mμ determined. Each oil had been assayed biologically against β-carotene. It was found empirically that the $E^{1\%}_{1\,cm.}$ values multiplied by 1600 gave estimates of potency agreeing with the biological values within the limits of error of the animal experiments. Subsequent work has shown that no better conversion factor (for fish-liver oils in general) could even to-day be chosen for expressing gross $E^{1\%}_{1\,cm.}$ values in terms of International Units, but oils showing little irrelevant absorption would be somewhat under-valued and oils exhibiting more irrelevant absorption would be over-valued. It was widely agreed by 1934 that relatively low-potency oils such as cod-liver oils exhibited considerable irrelevant absorption, most of which could be eliminated with the saponifiable fraction[4]. It has since been usual for such oils, to determine the intensity of absorption at 325 to 328mμ on the unsaponifiable fraction, but to express the result in terms of the weight of the original oil. Use of the unsaponifiable fraction also reduces the error caused by inhibitors of the antimony trichloride colour.

For a wide range of more potent fish-liver oils substitution of the unsaponifiable fraction for the whole oil resulted in little reduction in $E^{1\%}_{1\,cm.}$ values at 328mμ. and no enhancement of the colour test intensity. It is however now established that most liver oils contain materials which interfere with both tests and that many "impurities" (including vitamin A decomposition products) pass into the unsaponifiable fraction with the vitamin A.

The 1934 Conference recommended for adoption an International Unit for Vitamin A defined as the Vitamin A activity of 0·6 microgramme (0·6μg.) of pure β-carotene.

Further recommendations (1934) are worth quoting in full:—

"The Conference recommends that a sample of cod-liver oil, the potency of which has been accurately determined in terms of the International Standard Preparation of β-carotene shall be provided as a Subsidiary Standard of Reference.

"In view of the fact that the Reference Cod-liver Oil of the United States Pharmacopœia, which has been accurately assayed in terms of the provisional International Standard adopted in 1931, has been in effective use in the United States of America for some time, the Conference recommends that the Board of Trustees of the United States Pharmacopœia be approached and invited to place a quantity of their Reference Cod-Liver Oil at the disposal of the Health Organisation of the League of Nations with a view to its adoption for international use as a Subsidiary Standard for Vitamin A.

"In the event of the Reference Cod-liver Oil (of the U.S.P.) not being available for international adoption the Conference recommends that another sample of cod-liver oil be selected, its potency in terms of the International Standard Preparation of β-carotene accurately determined by biological comparison and independently by spectrophoto-

metric measurements, and that this selected sample be then adopted as a Subsidiary International Standard for Vitamin A.

"It has been found that, within certain defined conditions, measurements of the coefficient of absorption (E) at 328mμ., affords a reliable method for measuring the vitamin A content of liver oils and concentrates. As a means of converting values obtained for $E^{1\%}_{1\,cm.}$ 328mμ into a figure representing International Units of Vitamin A per gramme of the material examined the factor 1600 is recommended for adoption."[4]

In the U.S.A. the U.S.P. Reference Cod-liver Oil was much more freely employed than the β-carotene preparation and a conversion factor of 2000, deemed more appropriate than 1600, came into general use. (The U.S.P. authorities were, of course, under no obligation to conform to recommendations made by the Conference.)

The U.S.P. Reference Cod-liver Oil was not very stable. Two other U.S.P. Reference oils have since been issued and a considerable volume of work has appeared on their properties and stability. The upshot has been general agreement that $E^{1\%}_{1\,cm.}$ 328mμ values and the relative biological potencies of oils run very parallel. The conversion factor of 2000 applied to uncorrected *E* values received in the U.S.A. general and somewhat uncritical acceptance.

The β-carotene standard preparation could not be impugned on the score of instability, but it was impossible to remain permanently satisfied with a standard of vitamin A activity based on a substance other than the vitamin itself.

Experience with the U.S.P. Reference Oil tended to weaken initiative in providing a new Cod-liver Oil Subsidiary Standard for international use, and the outbreak of war temporarily put an end to the project.

For many purposes, however, it became tacitly accepted that the $E^{1\%}_{1\,cm.}$ value was the best criterion of vitamin A content, and that in translating such values into "units" 1 U.S.P. unit meant the same thing as 0·8 I.U.

The advent of pure crystalline vitamin A and vitamin A acetate changed the situation with regard to possible new standards, and developments in the production of photoelectric spectrophotometers increased the potential accuracy of the spectroscopic method and also of the antimony trichloride colour test.

The Present Position

The 1949 Conference (Expert Committee on Biological Standardisation, World Health Organisation) held in London made recommendations[5] (adopted in August, 1949) which constitute a real advance, in that they maintain essential continuity with the earlier international unit but provide for practical, and it is believed stable, International Standard Preparations acceptable everywhere. In the first place, the β-carotene Standard Preparation is retained, not as the Reference Standard for Vitamin A activity, but as the reference material for work on vitamin A precursors or, more specifically, carotenoid provitamins A.

By definition, then, 1 I.U. of provitamin A activity is 0·6μg. of

β-carotene, and pure β-carotene necessarily has a potency of $1{\cdot}66\dot{6} \times 10^6$ I.U./g.

In the second place, a new International Standard Preparation of Vitamin A acetate is now made available[6]. " The preparation is a solution in a suitable vegetable oil of such a strength that 0·1mg. of the solution contains 0·344μg. of vitamin A acetate. An oil suitable as solvent is one containing not less than 0·1 per cent. of tocopherol and not more than 32 parts per million of total peroxide oxygen."[7,8]

The International Unit of vitamin activity is defined as 0·344μg. of pure vitamin A acetate, corresponding stoichiometrically to 0·30μg. of vitamin A_1 alcohol. Necessarily, then, pure vitamin A_1 contains $3{\cdot}3\dot{3} \times 10^6$ I.U. per gramme. Spectroscopists are agreed (within very narrow limits of error) that the $E^{1\%}_{1\,cm.}$ value (at the ultraviolet maximum) for vitamin A_1 is 1750 and that of the acetate 1525 ($\varepsilon_{max.}$ 50,000). The " theoretical " or " true " conversion factor must, then, be near 1900 (i.e., $3{\cdot}3\dot{3} \times 10^6/1750$). The E values vary a little with the solvent.

In 1948 a new U.S.P. Vitamin A Reference Standard became official in the U.S.A.[9] It consisted of a solution of pure vitamin A_1 acetate in cottonseed oil (3·44mg. per gramme) and was labelled 10,000 U.S.P. units per gramme. Since the $E^{1\%}_{1\,cm.}$ value at 328mμ. for the preparation was stated, a conversion factor was implicit but " unofficial and given simply as information." The only official assay method (U.S.P. XIII p. 721) was biological, and it was the *Reference Standard Preparation* which became official on January 1, 1948, in the U.S.A. Of course, the Standard necessarily implied a " true " conversion factor near 1900, but as the U.S.P. had not recognised quantitative spectrophotometric assays it could ignore (officially) the difficulties attendant upon a change from 2,000 to 1,900 in the U.S.A. or a possible change from 1,600 to 1,900 elsewhere. This gave rise to some confusion[9]. The new U.S.P. Reference Standard was chosen and defined after very careful biological comparison of β-carotene and vitamin A_1 acetate, and the fact that the " unit " (0·3μg.) and the β-carotene " unit " (0·6μg.) are related as 1 : 2 is the result of experiment. After statistical examination of the protocols, the potency of the Standard Preparation (tested against β-carotene) is 9750 I.U./g. (between 9000 and 10,500 for $P = 0{\cdot}95$). If $E^{1\%}_{1\,cm.}$ 328mμ is 5·23 the conversion factor will be 9,750/5·23 = 1,864 (limits 1,721 and 2,007).

Earlier co-operative tests carried out in England under the auspices of the vitamin A Sub-Committee of the Medical Research Council had led to the following results :—

Biological tests	*Conversion Factor*
Cod-liver oil against β-carotene (*E* values on unsaponifiable fraction)	1,820
Vitamin A naphthoate against β-carotene	1,770
Halibut-liver oil against β-carotene :	
Uncorrected *E* values	1,570
Corrected *E* values	1,824

The British experiments taken together thus led to a conversion factor of 1,805 (limits not narrower than 1,700—1,900) and the American experiments to 1,864 (limits 1,721—2,007). It was almost inevitable that the 1949 London Conference should decide that the responses to 0·6μg. of β-carotene and 0·3μg. of vitamin A were indistinguishable by the accepted methods of biological assay carried out on any practicable scale.

The new U.S.P. Standard had, moreover, acquired momentum and great care had been devoted to issuing it in a convenient and stable form. There was, therefore, a strong case for recommending an International Reference Standard with the same specification.

The 1949 Conference recognised, however, that for much research and testing the spectrophotometric method was preferable to the biological assay, and it grasped firmly the nettle of the conversion factor. It accepted that if by definition the potency of vitamin A alcohol is 3·33 $\times 10^6$ I.U. per gramme and the $E_{1\text{ cm.}}^{1\text{ per cent.}}$ value is known to be 1,750, the conversion factor must be and can only be 1,900 within the limits of error of the spectrophotometric measurements. But the Conference also made it clear that the analyst must be satisfied that the effect of impurities in raising the $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 328mμ. above that due to the vitamin A content has been eliminated or allowed for before multiplying by 1,900. The Vitamin A acetate Reference Standard (dissolved in the solvent to be used for testing other materials, e.g., *cyclo*hexane, ethanol, *iso*propyl alcohol) can be examined by means of the analyst's own spectrophotometer, and the absorption spectrum may be plotted on a scale in which $E_{max.}$ is put at 1·00. The curve affords a criterion of "normality," i.e., of the absence of irrelevant absorption, especially if the compensating cell contains a solution of the diluent oil. If the absorption curve for a sample of oil, plotted in the same way, is appreciably distorted the $E_{1\text{ cm.}}^{1\text{ per cent.}}$ value at 328mμ must be corrected.

In effect, the true vitamin A content must be ascertained; the result could just as easily be expressed as mg. per g. or as percentage of vitamin A. The "potency in I.U. per g." is an alternative expression of the vitamin content, with the merit of preserving continuity with earlier work. The 1949 Conference broke new ground in recommending that the analyst as well as the bio-assayist should use the Reference Standard Preparation. It should be noted that spectrophotometers are best tested for adjustment by determining the absorption spectrum of a simple solution of potassium nitrate or chromate. The novel point in the use of a "biological" Standard is that the Reference Preparation will give the analyst an undistorted vitamin A absorption spectrum applicable to the performance of his own instrument. This is important because a definitive specification of the absorption spectrum of vitamin A determined under optimal conditions may well be less serviceable to the analyst than the curve he can himself obtain under ordinary working conditions. It is more important that he should be able to assess the degree of abnormality in an absorption curve than that he should be able to reproduce with extreme accuracy a "standard" absorption curve.

Bio-assays Compared with Spectrophotometric Assays

It is obviously necessary to know what can be expected of the two methods. Six recent biological assays of a fish liver-oil against the Vitamin A acetate Reference Standard[10] gave estimates of potency ranging between 46,290 and 86,350 I.U. per gramme (Coefficients of Variation ranging from 7·39 to 15·77 per cent). The weighted mean was 67,190 (C.V. 4·44 per cent.). Uncorrected $E^{1\%}_{1\,cm.}$ 328mμ. values (31 laboratories) gave 39·06 (Standard Deviation 0·405, C.V. 1·03); overall conversion factor, 67,190/39·06 = 1,720.

From this it must be concluded that, whatever the difficulties of the spectrophotometric evaluation, biological assays are only capable of advantageous use in determining vitamin A content if the number of animals used and the statistical treatment applied, are both on a scale prohibitive for any but the most important work.

The Need for Correcting Gross $E^{1\%}_{1\,cm.}$ Values

(i) Recent work by an important American group has shown[10]:—

(a) that using the normal biological technique, and testing seven oils or concentrates against the Vitamin A acetate Reference Standard, the weighted means of 6 assays gave potencies with coefficients of variation between 3·73 and 6·3 per cent.

(b) that the gross (uncorrected) $E^{1\%}_{1\,cm.}$ 328mμ values determined by photoelectric spectrophotometry gave C.V. values ranging from 1·03 to 2·25 per cent.

(c) that the apparent conversion factors (biological estimate in I.U./g. ÷ $E^{1\%}_{1\,cm.}$) were all less than 1,900 and ranged from 1,624 to 1,867.

This work makes it clear that to multiply all uncorrected $E^{1\%}_{1\,cm.}$ values at 328 mμ by 1,900 would result in an over-estimate of potency which would become progressively greater the worse the "spectroscopic" quality of the oil—a very discouraging situation to manufacturers able to avoid vitamin A decomposition.

(ii) Irving and Richards (at the Rowett Institute, Aberdeen) observed degenerative changes in the myelin substance of the central nervous system as a result of vitamin A deficiency. Coetzee[11] working under Irving at Cape Town has adapted a histological method (Marchi technique) to the quantitative determination of myelin degeneration for the purpose of a biological assay for vitamin A.

Comparison of the β-carotene Standard Preparation with a solution of crystalline vitamin A acetate gave a potency of 12,300 I.U./g. for $E^{1\%}_{1\,cm.}$ 328 mμ, 6·85 (gross) 6·80 (corrected). This corresponds with a conversion factor near 1,800. A vitamin A distillate, $E^{1\%}_{1\,cm.}$ 8·44 (gross) 8·15 (corrected) gave conversion factors of 1,957 and 2,028. For these two materials the ultra-violet absorption is little distorted and the average conversion factor is surprisingly near to 1,900.

On the other hand, the apparent conversion factors for 6 fish liver oils ranged between 1,411 and 1,817 (average 1,593, S.D., 134), using gross $E^{1\%}_{1\,cm.}$ values. Here, again, the need for correcting gross $E^{1\%}_{1\,cm.}$

values is manifest. By applying the correction procedure of Morton and Stubbs (see below) the average apparent conversion factor was revised to 1,768—a figure probably not significantly different here from 1,900.

The Effect of Irrelevant Absorption

The analyst's first aim is a true measure of the vitamin A contribution to the absorption at 328mμ and the determination of the whole absorption curve from say 250 to 380 mμ, is only necessary if it helps in correcting the gross $E_{1\ cm.}^{1\ per\ cent.}\lambda_{max.}$ value at 328mμ. Figure 1 shows the effect of irrelevant absorption; in a(i) the absorption curve is distorted on the short wave side of 328mμ and in a(ii) on the long wave side, but in neither case does the E value at 328mμ need correction; in b(i) the irrelevant absorption is the same over a range of wave-lengths, $\lambda_{max.}$ is not displaced from 328mμ but the E value needs to be corrected by the quantity corresponding to the double arrow, whilst in b(ii) and b(iii) $\lambda_{max.}$ is markedly displaced and the corrections needed are again shown by the arrows. The presence of irrelevant absorption is thus detectable by a displacement of the absorption maximum from 328mμ or a change in the ratio $E_{max.}/E_{\lambda_1}$ or $E_{max.}/E_{\lambda_3}$.

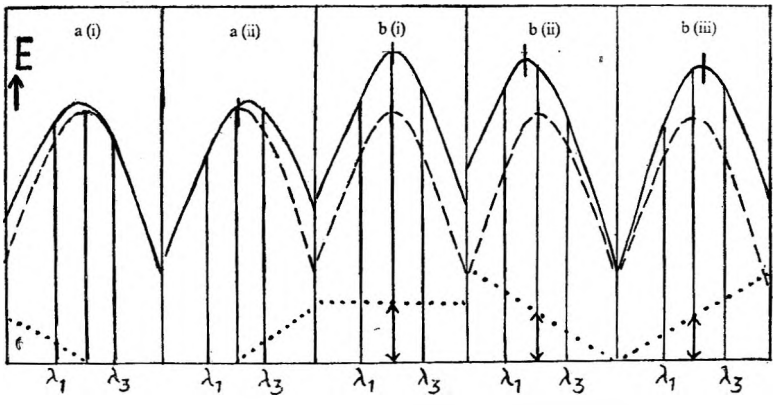

Fig. 1.—The effects of the presence of impurities exhibiting irrelevant absorption on the absorption spectrum of a substance showing selective absorption.
..... Irrelevant absorption.
— — — — — Absorption of a constant amount of selectively absorption material. e.g., Vitamin A.
———— Summation curves.

Correction For Irrelevant Absorption

Modern photoelectric spectrophotometers often permit considerably more accurate determinations of intensities of absorption than do the older photographic methods, and are particularly good for recording small differences over a restricted wave-length range.

Morton and Stubbs[12] discussed the general case where constituents other than the one which it is required to estimate make an unknown contribution to the measured absorption. Their general method of cor-

rection for unknown irrelevant absorption is based on (a) accurate measurements on the pure substance under study at three wave-lengths λ_1, λ_2 and λ_3 not very far apart and (b) similar measurements on the sample at the same wave-lengths. The assumption is made that the irrelevant absorption is linear over the narrow wave-length range chosen. Geometrical considerations lead to the formula

$$E_{\lambda_1} \text{ (corrected)} = AE_1 - BE_2 - CE_3$$

where E_1, E_2 and E_3 are readings at λ_1, λ_2 and λ_3 and A, B and C are constants calculated from the curve of the pure substance.

A much simpler method of approach is possible if the wave-lengths λ_2 and λ_3 can be chosen so that for the pure substance the extinctions at those wave-lengths are equal. The geometrical problem is illustrated in Figure 2 and it has been found convenient to choose λ_2 and λ_3 so that the intensities of absorption are 6/7 of that at λ_1 the absorption peak. [6/7 was chosen because the irrelevant absorption is only likely to be linear if λ_2 and λ_3 are not too far apart; if, on the other hand, λ_2 and λ_3 are very close together the differences in E values will be too small in

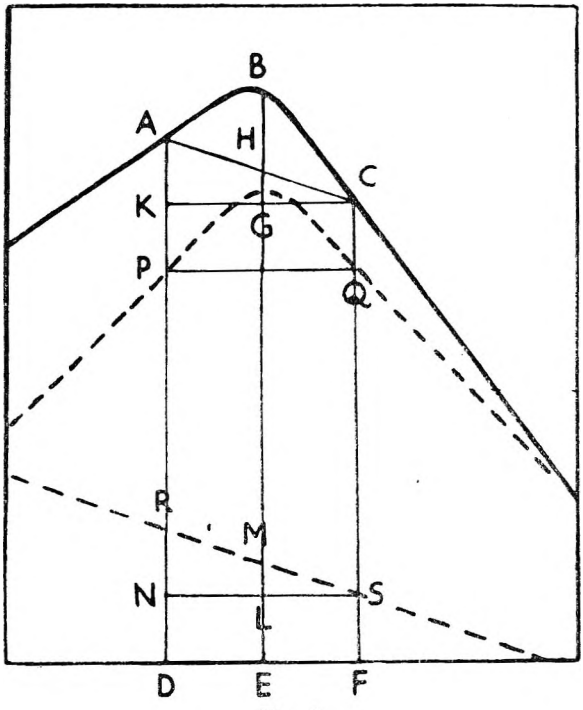

FIG. 2

relation to instrumental limitations.] The correction procedure then requires a knowledge of the absorption curve of pure vitamin A and the determination of ME in two steps ML and LE (Fig. 2).

The same authors have published[13] absorption measurements on the pure substances. It must be noted that the absorption curves for free

vitamin A and esterified vitamin A are not identical and the former curve is needed for studies on unsaponifiable fractions and the latter is suitable for work on most liver oils.

The intensities of absorption for vitamin A_1 (crystalline alcohol) and for pure vitamin A_1 acetate (cryst.) have been measured very carefully both in *cyclo*hexane and ethyl alcohol and E values at wave-lengths between 250 and 400 mμ are expressed as fraction of $E_{max.}$. Table I illustrates the most useful findings.

TABLE I

	Vitamin A Alcohol		Vitamin A Acetate	
Solvent	*cyclo* Hexane	Ethyl alcohol	*cyclo* Hexane	Ethyl alcohol
$\lambda_{max.}$ mμ	326	325	328	326·5
$\epsilon_{max.}$	48,310	51,100	48,460	50,020

Fixation points.

$E_{311m\mu} = E_{336m\mu}$ $E_{311m\mu} = E_{335\cdot5m\mu}$ $E_{313m\mu} = E_{338\cdot5m\mu}$ $E_{311\cdot5m\mu} = E_{337\cdot5m\mu}$

$\frac{E_{326m\mu}}{E_{311m\mu}} = \frac{7}{6}$ $\frac{E_{325m\mu}}{E_{311m\mu}} = \frac{7}{6}$ $\frac{E_{328m\mu}}{E_{313m\mu}} = \frac{7}{6}$ $\frac{E_{326.5m\mu}}{E_{311.5m\mu}} = \frac{7}{6}$

The usefulness of this kind of correction procedure is shown in a study of halibut liver oil[14].

In 1936 two halibut-liver oils were mixed and used by the Vitamin A Sub-Committee of the Medical Research Council in a large-scale co-operative study undertaken to ascertain afresh the conversion factor for expressing $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 328mμ in terms of International Units. The biological results were examined by statistical methods and a conversion factor of 1,570 emerged. A later co-operative experiment was carried out on vitamin A naphthoate (crystalline) and a conversion factor of 1,770 resulted. It was not easy to decide upon the significance of the difference.

Fortunately, however, samples of the two halibut-liver oils (in practically full, sealed, brown glass ampoules) had been kept at 0°C. from 1936 to 1946. The materials were re-examined in 1946 by photoelectric methods. One of them had kept perfectly, and the 1936 findings were reproduced almost exactly:—

		1936	1946	
$E_{1\text{ cm.}}^{1\text{ per cent}}$	617 mμ.	143	145	$SbCl_3$ colour test
	328 mμ.	47	46·2	ultra-violet test
			47·2	
			(two ampoules)	

The corrected $E_{1\text{ cm.}}^{1\text{ per cent.}}$ value at 328 mμ was 40·1, so that if 1570 was the conversion factor applicable to the gross E value, the factor applicable to the corrected E value would be $\frac{1570 \times 46{\cdot}7}{40{\cdot}1} = 1828$. Thus the three co-operative tests organised by the vitamin A Sub-Committee were

brought into line (see p. 133) and it may fairly be concluded that the conversion factor resulting from the labours of the Vitamin A Sub-Committee is not significantly different from 1900.

Many molecular distillates, vitamin ester concentrates and some samples of rich liver oils give absorption curves which need hardly any correction for irrelevant absorption, but other oils need substantial correction.

Cod-liver oils in general require correction if the *E* value is determined on the whole oil. It has, however, been customary for the last 15 years to measure the absorption on the carefully prepared unsaponifiable fraction[4]. If the fixation points given in Table I for the free vitamin are used, most fresh cod-liver oils give "unsap." fractions which need little correction. That this applies to a particular sample should, however, be ascertained and not assumed.

The availability of the Vitamin A acetate Reference Preparation enables the shape of the absorption curve and the fixation points[13] for esterified vitamin A (Table I) to be checked at any time. If the results in the Table are reproduced it will probably be safer to accept the fixation points[13] for the free vitamin A rather than to saponify the Standard Preparation. The figures given in the Table are based on very pure crystalline vitamin A.

Detailed Procedure for the Examination of Most Samples of Fish-Liver Oil

It is assumed here that the solvent is *cyclo*hexane, but ethyl alcohol or *iso*propyl alcohol could be used given the curves for pure vitamin A acetate in those solvents. The solution should contain an amount of oil such that $E_{328m\mu}$ for a 1 cm. cell is between 0·4 and 0·9 or at a value appropriate to the performance of the spectrophotometer in use.

The absorption curve may be measured at 5mμ intervals between 250 and 370mμ and at 313mμ, 328mμ and 338·5mμ (*E* values at the 3 last-mentioned wave-lengths provide the minimum information needed). The actual value of $\lambda_{max.}$ should be checked. Then the value of ML (Fig. 2) should be calculated by similar triangles. If LE is put equal to x,

$$\frac{E_{328\ m\mu.} - ML - x}{{}^{*}E_{313\ m\mu} - x} = \frac{7}{6}$$

*or $E_{338.5\ m\mu}$ whichever is the lower.

The total correction ME (ML + x) is subtracted from $E_{328m\mu.}$ The corrected $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 328mμ value is multiplied by 1,900 to give I.U. per g. Once $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ (corrected) at 328mμ has been ascertained, the entire curve for the vitamin A contribution can be reconstructed from the published data[13] on the pure substance. Subtraction of this curve from the observed curve will give a close approximation to the total impurity absorption curve. In some instances anhydrovitamin A is seen to be

the predominant source of irrelevant absorption. In others (e.g., ling cod-liver oils) vitamin A_2 with $\lambda_{max.}$ 350mμ is clearly revealed, and in some refined whale-liver oils the kitol spectrum is seen.

Determination of Vitamin A in Whale-Liver Oils

The geometrical correction procedure is based on the assumption that the irrelevant absorption is linear over a narrow range of the spectrum. As the correction becomes an increasing fraction of the total absorption, the validity of the method diminishes.

Whale-liver oil obtained by processing livers at sea is a very variable commodity, often containing phospholipids, free fatty acids, vitamin A decomposition products and kitol, a divitamin$A$,$C_{40}H_{60}O_2$, with $\lambda_{max.}$ at 286mμ). The kitol is biologically inactive and also interferes seriously both in the spectrophotometric test and in the antimony trichloride colour test. Until recently there has been for whale-liver oils no satisfactory alternative to the biological assay, itself of limited value since the fiducial limits of error (80 rats, $P = 0{\cdot}95$) may often be no better than 70 to 140 per cent. of the estimated potency.

With many whale-liver oils the correction procedure is pushed beyond the limit of its usefulness, and may still be unsatisfactory even when it is applied to the unsaponifiable fractions which show less irrelevant absorption than the crude oils.

The first trustworthy satisfactory analytical procedure to be published[15] was based on a preliminary chromatographic treatment of the unsaponifiable fraction so as to obtain a vitamin A-alcohol fraction giving an absorption curve either needing no correction or amenable to correction by the geometrical procedure.

The following modification[16] gives good results with perhaps less risk of manipulative losses. The adsorbent is alumina (Spence, Grade O) weakened by thorough admixture with 10 per cent. by weight of water. The column (12 × 1 cm.) is made by pouring the alumina into a tube as a slurry with light petroleum. The sample (0·1 to 0·2 g. in 25 ml. of light petroleum) is poured on the column and the chromatogram is developed with light petroleum. Passage of solvent through the column is hastened by pressure from above and the percolate is collected in 5 ml. portions in small test tubes. The first runnings contain anhydrovitamin A, detected by treating 1 or 2 drops of eluate with the antimony trichloride reagent. Several 5 ml. portions of eluate will then be obtained which give no blue colour with the reagent. Continued development with light petroleum results in the appearance in the eluate of vitamin A esters. The intensity of the blue colour obtained with one drop of eluate will increase steadily and then decrease until finally no colour is given. Development is continued with light petroleum containing diethyl ether (4 per cent. by vol., 50 ml.; 8 per cent., 50 ml.; 12 per cent., 50 ml.). The percolate now gives a purple colour (with the antimony trichloride reagent) and shows $\lambda_{max.}$ at 285 to 290 mμ (kitol esters). Further development results in a small fraction which gives little or no colour with the

antimony trichloride reagent, but continued development with light petroleum containing 16 per cent. and 20 per cent. of ether results in more or less sharply defined small fractions containing free vitamin A and free kitol respectively. Thus an oil with $E^{1\%}_{1\,cm.}$ 328 mμ 60·1 (gross) and a greatly distorted absorption curve gave a small anhydro-vitamin A fraction $E^{1\%}_{1\,cm.}$ 370 mμ 2·1, a vitamin A ester fraction $E^{1\%}_{1\,cm.}$ 328 mμ 37·9 (no correction needed), a kitol ester fraction $E^{1\%}_{1\,cm.}$ 286 mμ 24·35 and a free vitamin A fraction $E^{1\%}_{1\,cm.}$ 326 mμ 2·2 (corrected). The total $E^{1\%}_{1\,cm.}$ value at 328 mμ is therefore 40 and the potency 40 × 1900 = 76,000 I.U./g. In the above example only 0·125 g. of oil was used, and it cannot too strongly be stressed that much depends on good sampling of whale-liver oil at all stages; thorough mixing of the thick dark oil is essential. The chromatography must be controlled by testing each 5 ml. portion of eluate with the antimony trichloride reagent and using the colour test to distinguish the various fractions. The original papers should be consulted for further details.

Alternative methods of eliminating kitol[16,17] from whale-liver oil unsaponifiable fractions depend upon extraction with 50 per cent. aqueous ethyl alcohol in which the free vitamin A is preferentially soluble.

Some materials exhibiting distorted ultra-violet absorption curves may be studied before and after photo-chemical destruction of vitamin A by ultra-violet light, the decrease in absorption at 326 to 328 mμ being attributed to the vitamin A.

Vitamin A_2. Certain fish-liver oils, particularly those from fresh-water fishes contain a substance which with the antimony trichloride reagent gives a blue colour with $\lambda_{max.}$ 693 mμ and showing an ultra-violet maximum near 350 mμ. Salmon-liver oils, snoek-, red cod-, black cod- and ling cod-liver oils all contain appreciable quantities of the 693 mμ chromogen (Vitamin A_2), but in nearly all the liver oils of commerce vitamin A_1 preponderates over vitamin A_2. Thus in ling cod-liver oil the ratio vitamin A_1/vitamin A_2 is 8:1, whilst in many halibut-liver oils it is perhaps 20:1. The biological potency of vitamin A_2 is of the same order as that of vitamin A_1.

The presence in an oil of an appreciable proportion of vitamin A_2 distorts the absorption curve, particularly on the long-wave side of the maximum, and application of the correction procedure practically eliminates the vitamin A_2 contribution[13]. Hitherto most commercial and official testing of fish-liver oils has ignored the existence of vitamin A_2 and only in rather rare cases is there likely to be any significant error as a result of continuing to do so. There is no ruling on the matter, probably because of a lack of knowledge concerning the extent to which vitamin A_2 can replace vitamin A in human nutrition.

Synthetic preparations. Vitamin A acetate (synthetic) is now an article of commerce and there is no evidence that it can be distinguished from the acetate prepared from vitamin A of natural origin. It is, however, quite impossible to foresee the analytical difficulties which may arise from synthetic preparations in general, and the safest rule in cases

of doubt would be to carry out a comparison with the International Standard preparation using both the colour test and the ultra-violet absorption test.

The position of the colour test in quatitative work. For many purposes the antimony trichloride colour test is still very valuable, particularly in the determination of plasma vitamin A. An alternative colour test using activated glycerol dichlorhydrin has some advantages[18]. There are too many ways of measuring the colour intensity to justify giving details here but one general comment is necessary.

The International Standard Preparation of vitamin A acetate (or a secondary laboratory standard derived from it by comparison of $E_{328m\mu}$ values) should be used to calibrate the instrument for measuring the blue colour. This will lead to trustworthy results in testing unsaponifiable fractions, but the colour test is not recommended for unsaponified low-potency fish-liver oils because of the presence of the colour test inhibitors already referred to.

Provitamins A. The determination of carotenoid precursors of vitamin A depends upon preliminary chromatographic separation followed by spectrophotometry. The chromatography is used mainly to separate carotenes from "xanthophylls," but any accurate estimate requires a quantitative separation, identification and estimation of all the carotenoids present. As there are several provitamins A, each of which may occur as *cis-trans* isomerides the problem is sometimes very complicated.

As Zscheile and Porter[19] have said, "Different plants, plant parts or products made from plants require individual treatment and study, as extraction methods, identity of pigments and interpretation of both chromatographic columns (or other fractionation procedures) and characteristic absorption curves cannot be carried over arbitrarily from one material to another." Although perhaps over-cautious the above warning is salutary. The outstanding practical problem of this kind is the determination of β-carotene in dried grass—a matter of veterinary and agricultural importance, and it has a literature of its own which is outside the scope of this review. Figures giving the provitamin content of foodstuffs should be used with reserve and the temptation to equate or to treat as additive "provitamin" units and vitamin "units" should be firmly resisted.

Neovitamin A. Recent work tends to show that the vitamin A present in fish-liver oils consists mainly of all-*trans* vitamin A and *neo*vitamin A (a *cis*-form). Dr. P. D. Dalvi working in the writer's laboratory has isolated a very rich *neo*vitamin A ester fraction, the spectral absorption curve of which differs very little from that of vitamin A acetate. Although it is possible that further research may bring surprises there is so far little to indicate that any serious error in the determination of vitamin A will arise from neglecting the existence of *neo*vitamin A.

The satisfactory outcome of the 1949 Conference reflects consideration of great and sustained effort in several countries and the various experts

brought to the task knowledge of work done during the War when scientific communication had broken down. A full bibliography would now be very unwieldy.

Much excellent work, particularly the large American contribution, cannot be referred to here, but tribute must be paid to the labours of those who engaged in the various co-operative assays in academic institutions and industrial laboratories. In this country the brunt of the work fell on the Vitamin A sub-committee of the Medical Research Council. The present position is well summarised in the following quotations[5]:—

"The new conversion factor of 1900 cannot be applied indiscriminately because few of the materials commonly tested are free from irrelevant absorption in the ultra-violet region of the spectrum, including the significant region 325 to 328 mμ. It is therefore necessary (1) to specify the conditions under which the factor is applicable and (2) to indicate how, in principle, irrelevant absorption can be allowed for. The conditions for (1) are:—(a) that the absorption maximum shall be within the range 325 to 328 mμ and (b) that the shape of the absorption curve shall agree closely with that of the international standard measured under the same conditions and compensated with a solution of the diluent oil. Intensities of absorption in the region of 310 and 350 mμ expressed as decimal fractions of the maximum should not differ between sample and standard by more than 0·02 (for standard curves see Ref. 13). To make the adjustment needed under (2) absorption curves failing to meet the above requirements may be corrected to allow for irrelevant absorption, provided the maximum is not displaced in wavelength, by a geometric procedure[12]. Absorption curves in which the maximum occurs outside the stipulated wave-length range indicate a need for purification of the material prior to spectrophotometric analysis. For example, cod-liver non-saponifiable fractions[20,21] usually yield spectrophotometrically normal curves; whale-liver oils usually give a spectrophotometrically normal fraction after chromatography[15] and many fish-liver oils yield a fraction exhibiting a normal curve after selective solvent extraction[22]. Selective photochemical destruction of vitamin A has also been used with success[23,24]."

Experience may show a need for modifications in matters of detail, but the principle of vitamin A determination seem to be firmly laid down.

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RESEARCH PAPERS

ANTIMALARIALS : DERIVATIVES OF TETRAHYDROACRIDINE

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The activity of mepacrine in malaria infections has centred attention on the acridine nucleus, but the suggestion of Curd, Davey and Rose[1] that the activity of this type of compound depended upon the possibility of an electron displacement cannot be accepted, as compounds in which such a shift cannot take place—viz. 7-methoxy-1 : 2 : 3 : 4-tetrahydroacridone—were proved to be active as causal prophylactics by Stephen, Tonkin and Walker[2]. Several derivatives of tetrahydroacridine have been prepared and tested. Magidson and Travin[3] obtained compounds in which various basic side chains were attached to the meso carbon but even 2-chloro-5-(ω-diethylamino-α-methylbutyl)amino-1 : 2 : 3 : 4-tetrahydroacridine was found to be inactive. Sargent and Small[4] extended the series of this type of compound with no more success.

Consideration of the structure of the cinchona alkaloids led to the suggestion, by analogy with the quinuclidine fragment, that in derivatives of tetrahydroacridine the basic side chain should be incorporated in the reduced portion of the molecule. It was therefore decided to attempt the preparation of derivatives of 1 : 2 : 3 : 4-tetrahydroacridine in which the 1, 2, 3, or 4 position was substituted with a tertiary amino group, such compounds not having been previously reported.

The preparation by Glynn (unpublished report) of 1 : 5-dichloro-1 : 2 : 3 : 4-tetrahydroacridine provided an intermediate from which a start could be made. This intermediate was prepared by a modification of the original process by refluxing together 2-chlorocyclohexanone, anthranilic acid and phosphorus oxychloride. After decomposing with ice and basifying with ammonia the required compound was obtained in 95 per cent. yield. After recrystallisation from diethylamine the compound melted at 141°C. This compound proved to be very stable towards hydrolytic agents and was recovered unchanged after refluxing with (a) 10 per cent. hydrochloric acid (compare 5-chloroamino-acridines[5]); (b) concentrated hydrochloric acid for 3 hours (compare 5-chloroalkoxyacridines[6]); and (c) alcoholic potash for one hour. Heating with sodium carbonate decahydrate and with sodamide in boiling benzene were also without action.

Refluxing for 24 hours with 20 per cent. w/v sulphuric acid converted the compound into the monohydrate of 1-hydroxy-1 : 2 : 3 : 4-tetrahydro-acridone which lost the molecule of water on heating at 120°C. *in vacuo*. This product proved to be a typical acridone, being yellow in colour, high melting and soluble in N/1 potassium hydroxide in alcohol (50 per

cent.) giving a yellow solution from which it could be recovered on acidification or by dilution with water. Attempts to reduce this compound to the corresponding acridine by the classical methods[7,8] using sodium in alcohol or sodium amalgam gave uncharacterisable products.

On adding sodium carbonate decahydrate to a gently boiling mixture of 1:5-dichloro-1:2:3:4-tetrahydroacridine in methylaniline, 3:4-dihydroacridone was obtained as a viscid orange oil which finally crystallised from diethylamine and formed a hydrochloride which was quickly hydrolysed by water, depositing the free base. On refluxing the dichlorotetrahydroacridine with glacial acetic acid for 3 hours the mono-acetate of 1-hydroxy-1:2:3:4-tetrahydroacridone was obtained in the form of buff crystals readily soluble in alcohol, the solution exhibiting a bright blue fluorescence. The alcoholic solution became yellow with a green fluorescence on addition of potassium hydroxide and the change was reversed on acidification.

Reactions of the dichloro compound with amines with or without a catalyst proved to be equally difficult, attention being concentrated upon the reaction with ω-diethylamino-α-methylbutylamine. Eventually success was attained by heating a mixture of the dichlorotetrahydroacridine, excess of amine and anhydrous potassium carbonate at 150°C. with stirring. The main product gave analytical figures required by a chloro-(ω-diethylamino-α-methylbutyl)aminotetrahydroacridine and occurred as a deep red viscous syrup, soluble in benzene and alcohol with a slight green fluorescence. It was readily soluble in acids forming yellowish-brown solutions. The *p*Ka for the base obtained by potentiometric titration against N/10 sodium hydroxide after solution in a mixture of 20 ml. of N/10 hydrochloric acid, 20 ml. of water and 80 ml. of methyl alcohol was found to be 8·5. It formed a dipicrate and a water-insoluble methylenebisoxynaphthoate, and a very hygroscopic dihydrochloride separated from solution in alcoholic hydrogen chloride.

The relative positions of the chloro group and the alkylamino group have not yet been definitely established but fortunately the structure of two by-products of the reaction support the contention that the compound should be described as 1-(ω-diethylamino-α-methylbutyl)amino-5-chloro-1:2:3:4-tetrahydroacridine. These by-products were characterised as 3:4-dihydro-5-chloroacridine and 1-hydroxy-5-chloro-1:2:3:4-tetrahydroacridine.

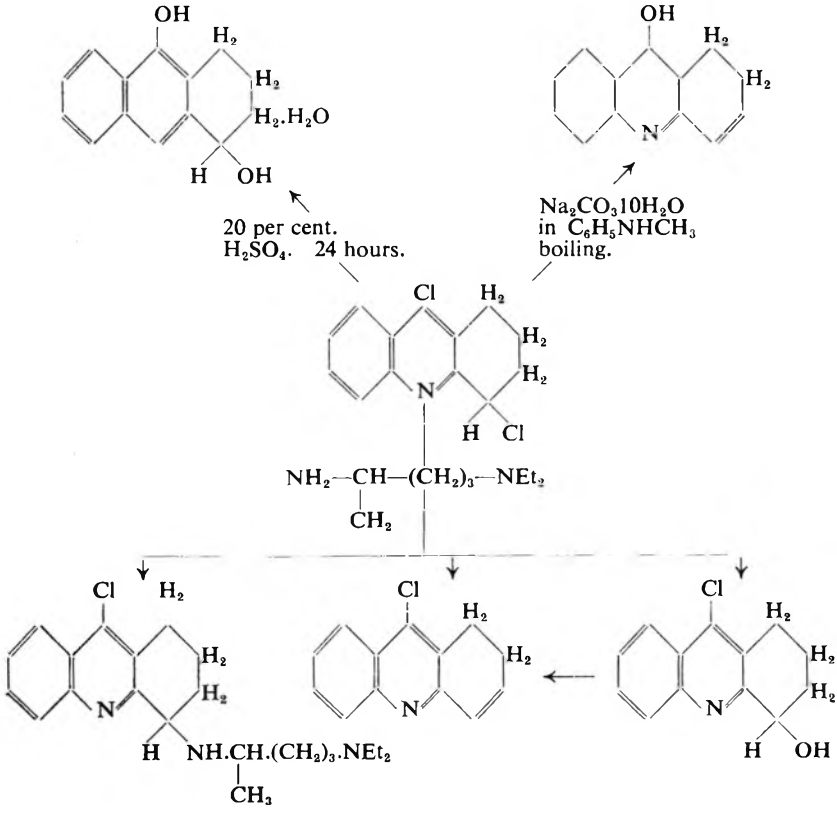
During these reactions exactly half the chlorine in the original dichlorotetrahydroacridine was obtained as ionisable chloride. Both the by-products possess a meso chloro group, thus indicating that under the conditions of this experiment the meso chloro group is the more stable. For these reasons the compound may be considered to be (1-(ω-diethylamino-α-methylbutyl)amino-5-chloro-1:2:3:4-tetrahydroacridine. The reactions may be summarised as shown on page 147.

Thanks are due to Professor Buttle of this School and to the Wellcome Laboratories of Tropical Medicine for the pharmacological tests.

After toxicity tests had been carried out on mice the hydrochloride of (1-(ω-diethylamino-α-methylbutyl)amino-5-chloro-1:2:3:4-tetrahydroacridine was administered at the rate of 100 mg./kg. to 10-day-old chicks

inoculated with 100 × 10^6 parasitised red cells. One dose was given on the day of injection, and then twice daily on 3 subsequent days. Blood smears were taken on the fifth day and the percentage of parasitised red cells found. Control birds were untreated. The compound was found to be inactive.

The inactivity may apply to all such derivatives of tetrahydroacridine but in this connection it should be remembered that acridine derivatives containing a 1-amino group are devoid of bacteristatic activity whereas amino groups in the other positions give compounds which exhibit activity[9].



Experimental

1:5-Dichloro-1:2:3:4-tetrahydroacridine was prepared by Glynn's method with important modifications. Freshly distilled (69° to 70°C. at 9 mm.). 2-chlorocyclohexanone (23 g.), anthranilic acid (23 g.), and phosphorus oxychloride (115 ml.) were refluxed together for 1½ hours. The dark liquid was poured, with vigorous stirring, on to ice and the whole left to stand for 2 hours with occasional stirring. The resinous precipitate described by the original author redissolved on standing and on carefully basifying the filtered liquid with strong ammonia in the

presence of ice dichlorotetrahydroacridine was precipitated in almost theoretical yield. It was washed with cold water, and dried.

Yield 40 g. (=95 per cent. of theory). M.pt. 135°C. On recrystallising from acetone the melting-point rose to 138°C. Yield 27 g. (=70 per cent. of theory). A small quantity recrystallised from diethylamine gave a constant melting-point of 141°C. Found C, 61·74; H, 4·24; N, 5·37; Cl, 28·00 per cent. $C_{13}H_{11}NCl_2$ requires C, 62·15; H, 4·38; N, 5·57; Cl, 27·89 per cent. Pale cream needles from diethylamine. Insoluble in water and in acetic acid (30 per cent.) but soluble to yellow solutions in mineral acids.

1 : 5-Dihydroxytetrahydroacridine (1-hydroxytetrahydroacridone). 1 : 5-dichlorotetrahydroacridine (2 g.) was refluxed for 24 hours with sulphuric acid (20 per cent. w/w) and the solution then poured on to ice, and basified with ammonia solution. The pale yellow precipitate, when washed and dried, melted over a range of 160° to 200°C. (Yield 1·7 g.). After removing unchanged starting material by refluxing with acetone the residual product melted at 226°C. and after twice recrystallising from alcohol a pale yellow substance of melting-point 280° to 284°C. (sealed tube) was obtained which proved to be the monohydrate of dihydroxytetrahydroacridine. Found C, 66·83; H, 6·33; N, 6·23; Cl, 0·65 per cent. $C_{13}H_{13}NO_2,H_2O$ requires C, 66·95; H, 6·44; N, 6·01 per cent. Loss in weight on heating at 120°C. *in vacuo* for 2 hours was 7·75 per cent. One molecule of water requires a loss of 7·72 per cent.

The yellow colour, high melting-point, and solubility of the product in 50 per cent. alcoholic potassium hydroxide (N/1) to form a yellow solution from which it is reprecipitated by acids or by water are together sufficient proof of the acridine structure. The loss of only one molecule of water on heating *in vacuo* favours the formulation of the product as the monohydrate of dihydroxytetrahydroacridine, as against the dihydrate of 3 : 4-dihydroacridone.

Reaction of dichlorotetrahydroacridine with methylaniline. Formation of 3:4-dihydroacridone. Dichlorotetrahydroacridine (1 g.) and methylaniline (2 ml.) were heated together until just boiling, and finely powdered sodium carbonate decahydrate added in small quantities over a period of 15 minutes. The contents of the flask were then subjected to steam distillation until no more methylaniline distilled and the flask left to cool. An orange brown residue was obtained which, after unsuccessful attempts to recrystallise from various solvents, was dissolved in benzene and poured on to an alumina column, followed by development with benzene. A broad orange-yellow band capped by a small pink band resulted, and development was continued until all the former had been removed from the column. Upon removal of the benzene the dihydroacridone was obtained as a viscous orange oil which refused to crystallise. Yield 0·6 g. (76 per cent. of theory). The hydrochloride was obtained as a blue hygroscopic solid by passing dry hydrogen chloride into an ethereal solution of the base. Found C, 66·0; H, 5·9; N, 6·0; Cl, 15·48 per cent.: $C_{13}H_{11}NO.HCl$ requires C, 66·8; H, 5·14; N, 6·00; Cl, 15·2 per cent.

The substance occurred as a bright orange oil becoming more viscous and finally recrystallisable from diethylamine to yield an orange powder melting at 192°C. and soluble in N/1 alcoholic potash. Solutions in organic solvents were bright orange with a green fluorescence. It formed a deep blue hydrochloride which was soluble in dilute hydrochloric acid, but hydrolysed in water to the orange oily base. Picrate, orange, m.pt. 205°C.

Both chlorine atoms of the original compound have been removed, and from the analytical evidence only one has been replaced by a hydroxyl group. Hence the other must have been lost as hydrochloric acid. This could only occur to the halogen in the 1-position. Furthermore the orange colour and the solubility in alcoholic potash are evidence for the acridine structure.

Preparation of 1-(ω-diethylamine-α-methylbutyl)amino-5-chloro-1:2:3:4-tetrahydroacridine. Dichlorotetrahydroacridine (12 g.), ω-diethylamino-α-methylbutylamine (24 ml. freshly distilled) and potassium carbonate (6 g.) (oven-dried at 110°C.) were heated together at 140° to 150°C. with stirring for 3 hours and, after cooling, the whole was extracted with an ether-water mixture and allowed to separate. The ionisable chloride content of the aqueous layer was determined gravimetrically, 7 g. of AgCl, equivalent to one chlorine atom, being obtained.

The dark ethereal layer was washed several times with water to remove the excess of water-soluble diamine, and then filtered to remove a little tar. The required base was then extracted by shaking with acetic acid (33 per cent.). The residual yellow ethereal solution was washed well with water and evaporated when a dark crystalline residue of 3:4-dihydro-5-chloroacridine (5·55 g.) was obtained (See A below). The deep-red acetic acid solution after being filtered from a little white solid (see B below) was basified with caustic soda and the bases extracted with ether. After washing the ether layer with water, drying with anhydrous sodium sulphate, and removing the ether, a deep red viscous oil was obtained (yield 4·05 g.).

This oil was dissolved in benzene and chromatographed through a column of alumina, followed by development of the column with benzene. Liquid chromatograms were collected in the following order and the solvent removed from each *in vacuo* to give the product and the yield indicated. (1) Deep red solution yielding 2·17 g. of red oil. (2) Orange solution yielding 0·4 g. of orange oil.

The development was now continued with 2 per cent. ethyl alcohol in benzene, when the following fractions were collected: (3) Pale straw solution yielding 0·13 g. orange oil. (4) Deep red solution yielding 0·7 g. red oil. (5) Brownish-straw solution yielding 0·13 g. dark solid, finally, on clearing the column with alcohol (97 per cent.) there was obtained (6) Orange yellow solution yielding 0·01 g. of solid. These six fractions accounted for 3·71 g. of the original 4·05 g. of red oil.

The 2·17 g. of red oil (1) was redissolved in benzene and passed through a second column of alumina. The column exhibited a 9-inch red band over a 2-inch bright orange band. After the latter had been washed

through with benzene and collected separately, the top of the column, showing a dirty brown zone, was removed mechanically and the bright red zone washed out with alcohol. On removal of the solvent *in vacuo* the bright red oily residue weighed 1·03 g., and after a final treatment on a fresh alumina column using benzene to develop, a homogeneous deep red treacle was obtained (yield 0·91 g.; 5 per cent. of theory, calculated on the acridine component).

The bright orange benzene solution, collected separately from the second column, on evaporation yielded a very viscous orange syrup (0·91 g.), which after treatment on a third column and evaporation of the benzene at a 100°C. *in vacuo* weighed 0·73 g. This substance has not yet been identified, but from the analytical evidence is very similar in composition to the red product. Found C, 71·41; 71·18; H, 7·23; 7·611; N, 9·60; 9·78; Cl, 7·0 per cent.

Identification and Properties of the red syrup. For analysis, the product obtained from the third alumina column was freed from benzene vapours by heating at 100°C. at 2 cm. pressure for half an hour. Found C, 72·53; 72·15; H, 8·30; 8·37; N, 11·2; 11·5; Cl, 7·55 per cent. $C_{22}H_{32}N_3Cl$ requires C, 70·68; H, 8·56; N, 11·24; Cl, 9·50 per cent. A picrate was obtained as a greenish yellow powder melting at 164°C. Found C, 48·91; H, 4·48; N, 14·6; Cl, 4·37 per cent. $C_{22}H_{32}N_3Cl.2C_6H_3N_3O_7$ requires C, 49·1; H, 4·57; N, 15·1; Cl, 4·27 per cent. None of the other products was characterised except A and B.

Methylene bisoxynaphthoate of the base. A 5 per cent. solution of ammonium methylenebisoxynaphthoate was prepared by boiling 5 g. of methylenebisoxynaphthoic acid with water and ammonia until the free acid began to precipitate. The yellow solution was then placed in a stoppered bottle and the clear liquid decanted when required. 0·2 g. of the red base was dissolved in 20 ml. of hydrochloric acid (N/10) and the calculated volume of caustic soda (N/10) added to give a solution containing the dihydrochloride of the base. 5 ml. of the clear solution of ammonium methylenebisoxynaphthoate was added with stirring and, after leaving a few moments, the pink precipitate was removed from the colourless solution, washed with water, and dried at 100°. Melting point, indefinite, about 260°C. Found C, 69·40; H, 6·08; N, 5·50; Cl, 4·61. $C_{45}H_{48}O_6N_3Cl$ requires C, 70·91; H, 6·30; N, 5·51; Cl, 4·672 per cent.

Examination of A. Yield 5·55 g. After recrystallising once from acetone and twice from diethylamine the melting-point was constant at 142°C., but a mixed melting-point with the original dichloro compound indicated a new substance. (Mixed m.pt.—122° to 123°C.) Analysis indicated that a chlorine atom had been lost from the original dichlorotetrahydroacridine, but had not been replaced by a hydroxyl group. This could only happen to the chlorine in the 1-position, hence the chlorine atom at position 5 is intact and the substance A is probably 3:4-dihydro-5-chloroacridine. Found C, 69·35; H, 4·83; N, 6·87; Cl, 15·55 per cent. CHNCl requires C, 72·4; H, 4·64; N, 6·5; Cl, 16·47 per cent.

Examination of B. This weighed only 0·12 g., and as it melted fairly sharply at 166°C. no attempt was made to recrystallise it. Analysis

showed it to be a chlorohydroxytetrahydroacridine, one of the chlorine atoms of the original dichlorotetrahydroacridine having been replaced by a hydroxyl group. As the substance has a low melting-point and is not appreciably more soluble in alcoholic potassium hydroxide (50 per cent., N/1) than in alcohol (50 per cent.) and was therefore not 1-chlorotetrahydroacridone, it must be 1-hydroxy-5-chlorotetrahydroacridine. Its very pale cream colour, not deepened to yellow by alcoholic potash, was further evidence for this formulation as was the occurrence of Compound A. Found C, 66·63; H, 5·18; N, 6·43; Cl, 15·52 per cent. $C_{13}H_{12}NOCl$ requires C, 66·83; H, 5·14; N, 6·0; Cl, 15·21 per cent.

Reaction between the same two components in glacial acetic acid and acetic anhydride. Dichlorotetrahydroacridine (0·5 g.) and ω-diethylamine-α-methylbutylamine (0·5 g.) were refluxed together in solution in glacial acetic acid (10 ml.) and acetic anhydride (5 ml.) for 3 hours, and the mixture poured into water. After standing for 3 days the buff precipitate was collected, dried and weighed. Yield 0·4 g.

After recrystallisation from alcohol (50 per cent.) the cream crystals melted at 276°C. (sealed tube). The compound was soluble in alcohol, even dilute, with a bright blue fluorescence. Readily soluble in alcoholic potash (50 per cent. N/1) with a bright green fluorescence and a yellow colour, the fluorescence being turned blue and the yellow colour discharged by acids. The high melting-point and behaviour with alcoholic potash indicated the presence of the acridone structure, and the analytical figures showed the substance to be 1:5-dihydroxytetrahydroacridine-1-acetate. Found C, 71·63; H, 5·86; N, 5·65 per cent. $C_{15}H_{13}NC_3$ requires C, 70·05; H, 5·84; N, 5·45 per cent. On repeating the experiment without the diamine, the same product was obtained. (Identified by melting-point and mixed melting-point with above.)

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THE COMBINED EFFECT OF PENICILLIN AND OF SULPHONAMIDES IN INFECTIONS WITH GRAM-NEGATIVE ORGANISMS. PARTS III AND IV

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Part III.

The Combined Effect on *Salmonella enteritidis*, *Shigella flexneri* and *Proteus vulgaris*

Similar experiments were done to study the effect of the combined action of penicillin and of the sulphonamides on these Gram-negative organisms. The experimental procedure was the same as that used with *Bact. coli* and *Salm. typhi*, and the results were equally similar[12].

In vitro, the results recorded in Tables VII, VIII and IX show that the inhibitory concentration of penicillin was reduced by 1/4th to 1/8th when the sulphonamide compounds were present in the culture medium as well; the concentration of the sulphonamide, however, was by itself non-inhibitory or only slightly bacteriostatic.

TABLE VII

The combined effect *in vitro* of penicillin and of some sulphonamides on the growth of *Salm. enteritidis*

Sulphonamide 0·1 mg./ml.	Penicillin concentration in units/ml.							
	20	10	5	2·5	1·25	0·6	0·3	0
———	0	4	4	4	4	4	4	4
Sulphathiazole	0	0	0	1	3	3	3	3
Sulphapyrazine	0	0	0	2	3	3	3	3
Sulphadiazine	0	0	0	4	4	4	4	4

TABLE VIII

The combined effect *in vitro* of penicillin and of some sulphonamides on the growth of *Shig. flexneri*

Sulphonamide 0·05 mg./ml.	Penicillin concentration in units/ml.							
	50	25	12·5	6·25	3·125	1·56	0·78	0
———	0	0	2	4	4	4	4	4
Sulphadiazine	0	0	0	0	0	2	2	2
Sulphapyrazine	0	0	0	0	0	1	2	2
Sulphathiazole	0	0	0	0	2	4	4	4

In vivo, there was a marked prolongation in the average survival period of mice treated with penicillin and sulphonamide combined as compared to that of animals treated by either chemotherapeutic substance alone. Table X shows this effect in mice infected with *Salm. enteritidis*.

TABLE IX

THE COMBINED EFFECT *IN VITRO* OF PENICILLIN AND OF SOME SULPHONAMIDES ON *PROTEUS VULGARIS*

Sulphonamide 0·2 mg./ml.	Penicillin concentration in units/ml.						
	100	50	25	12·5	6·25	3·125	0
———	0	0	4	4	4	4	4
Sulphadiazine	0	0	0	0	0	3	3
Sulphapyrazine	0	0	0	0	0	3	4
Sulphathiazole	0	0	0	0	0	3	4

TABLE X

THE COMBINED EFFECT OF PENICILLIN AND SULPHONAMIDES IN *SALM. ENTERITIDIS* INFECTION IN MICE.

Treatment		Number of mice used	Average survival period days
Penicillin	Sulphonamide		
———	Sulphathiazole 1 mg.	6	1·2
2000 units	Sulphathiazole 1 mg.	6	6·0
———	Sulphapyrazine 0·2 mg.	12	1·7
2000 units	Sulphapyrazine 0·2 mg.	12	4·9
———	Sulphadiazine 0·2 mg.	12	4·2
2000 units	Sulphadiazine 0·2 mg.	12	6·4
———	Sulphadiazine 0·1 mg.	6	3·0
2000 units	Sulphadiazine 0·1 mg.	6	4·7
2000 units	... ...	12	2·0
Untreated controls	... ...	18	0·4

2,000 units of penicillin caused an average survival period of 2 days; when this dose was combined with 1 mg. of sulphathiazole, the average survival period was markedly increased, to 6 days, 5 times as much as the survival period when the same dose of sulphathiazole was used alone. With sulphapyrazine, the average survival period was increased nearly 3 times more than that when 0·2 mg. of sulphapyrazine was given alone. When sulphadiazine was used, however, a less marked advantage was noticed from combining it with penicillin, probably because sulphadiazine was already very effective in a small dose against this infection when used alone.

Table XI shows that mice infected with *Shig. flexneri* and treated with the combined therapy enjoyed a survival time 2·6 and 4·4 times more than those treated with sulphadiazine or with sulphapyrazine alone respectively.

TABLE XI

THE COMBINED EFFECT OF PENICILLIN AND SULPHONAMIDES IN *SHIG. FLEXNERI* INFECTION IN MICE.

Treatment		Number of mice used	Average survival period days
Penicillin	Sulphonamide		
—— ——	Sulphadiazine 0·2 mg.	11	2·3
2000 units	Sulphadiazine 0·2 mg.	6	6·0
—— ——	Sulphapyrazine 0·2 mg.	6	1·3
2000 units	Sulphapyrazine 0·2 mg.	6	5·7
2000 units		17	0·8
Untreated controls		17	0·4

Table XII shows once more the advantage of the combined therapy, this time against *Proteus* infection in mice.

TABLE XII

THE COMBINED EFFECT OF PENICILLIN AND SULPHONAMIDS IN *PROTEUS* INFECTION IN MICE.

Treatment		Number of mice used	Average survival period days
Penicillin	Sulphonamide		
—— ——	Sulphadiazine 0·2 mg.	18	3·0
1000 units	Sulphadiazine 0·2 mg.	12	5·0
—— ——	Sulphapyrazine 0·5 mg.	6	3·0
1000 units	Sulphapyrazine 0·5 mg.	6	6·0
—— ——	Sulphathiazole 0·5 mg.	12	1·7
1000 units	Sulphathiazole 0·5 mg.	12	4·7
1000 units		18	1·6
Untreated controls		18	0·4

PART IV.

DISCUSSION AND CONCLUSIONS

1. *The nature of the combined action.* The combined effect of penicillin and of some sulphonamides on organisms known to be very sensitive to penicillin has been investigated by many workers[1-9]. Although they

agree that there is an advantage in such a combination, their conclusions differ in the interpretation of the nature of the combined action. Two terms were used by these authors; some described the combined effect as additive, while others used the term synergic. According to Gaddum[12], synergism means that two drugs which have the same effect are helping one another in their action; in this case the same effect which is produced by a certain concentration of each, can be obtained if fractions of the effective doses of either compound are used together when the sum of these fractions is less than two. If the sum of these fractions which give the same effect equals one, their effect is said to be additive; if on the other hand, the effect is produced by fractions of the two concentrations the sum of which is less than one, the drugs are said to potentiate the action of each other.

This terminology is now applied to the results of the combined action of penicillin and of the sulphonamides obtained in the *in vitro* experiments in the present investigation. It is seen that complete inhibition of the growth which was obtained by a certain concentration of penicillin or of the sulphonamides when used separately, was obtained when both substances were used together each in a concentration less than half the original one; e.g., if the inhibitory concentrations of penicillin and of the sulphonamides were 50 units /ml. and 1 mg./ml. respectively, the same effect was produced when 1/4th of the penicillin concentration (12·5 units /ml.) and 1/10th of the sulphonamide concentration (0·1 mg./ml.) were used together. This is, therefore, some evidence that this is a potentiation effect.

In vivo, the synergism between penicillin and the various sulphonamides has been also demonstrated; the results of the experiments show that this synergistic action is also of a potentiation character. A significant correlation between the doses of the drugs used and the resulting effect has been found, and the regression lines were calculated. In Part I of this paper[13] Figure 1 shows that in mice infected with *Bact. coli,* an average survival period of 5 days is obtained with 0·4 mg. of sulphapyrazine; this is 1/4th of the sulphapyrazine dose used in combination with 2,000 units of penicillin to produce the same effect (see Table III). 2,000 units of penicillin when used alone is definitely less than one half the effective dose (see Figure 2). When 0.5 mg. of sulphathiazole was used with 2,000 units of penicillin, the average survival period was found to be 5·7 days (see Table III); if sulphathiazole is to be used alone, 2·5 mg. is required to produce the same effect (see Figure 3); i.e., 5 times the dose used in combined therapy.

With *Salm. typhi,* the result of the synergistic action between penicillin and the sulphonamides[13] is recorded in Table VI. From Figure 4 it is seen that the same effect which was produced by 1 mg. of sulphadiazine when combined with 2,000 units of penicillin, can be obtained with sulphadiazine alone with 6·5 mg. are used. Also, Figure 5 shows that 8·9 mg. of sulphapyrazine are required to produce an average survival period of 6·1 days; this same effect however, was produced when 2 mg. of sulphapyrazine were used together with 2,000 units of penicillin. This dose of penicillin is less than one-half the effective dose (Fig. 6).

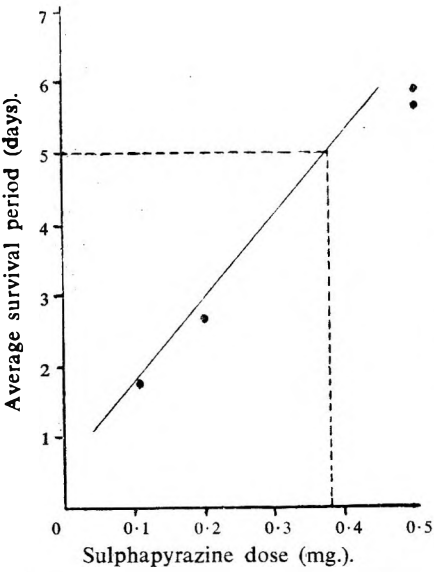

FIG. 1.—The average survival period per mouse infected with *Bact. coli* in relation to the dose of sulphapyrazine used in treatment.

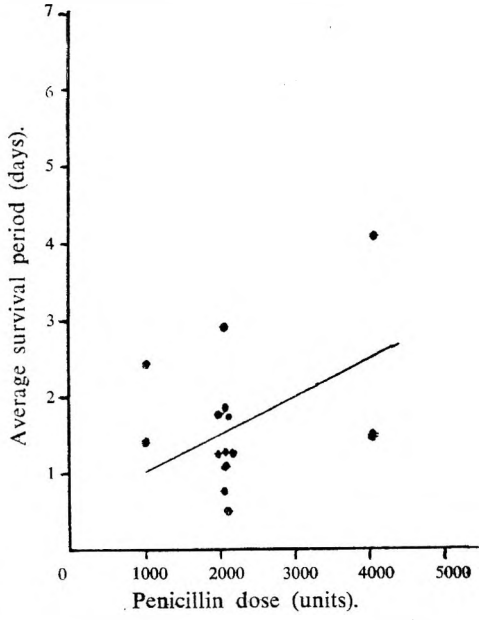

FIG. 2.—The average survival period per mouse infected with *Bact. coli* in relation to the dose of penicillin used in treatment.

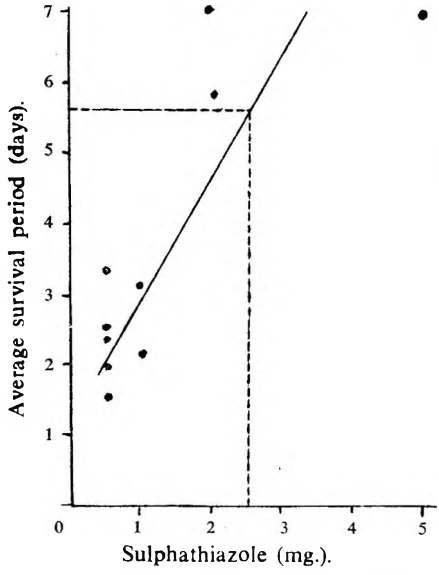

FIG. 3.—The average survival period per mouse infected with *Bact. coli* in relation to the dose of sulphathiazole used in treatment.

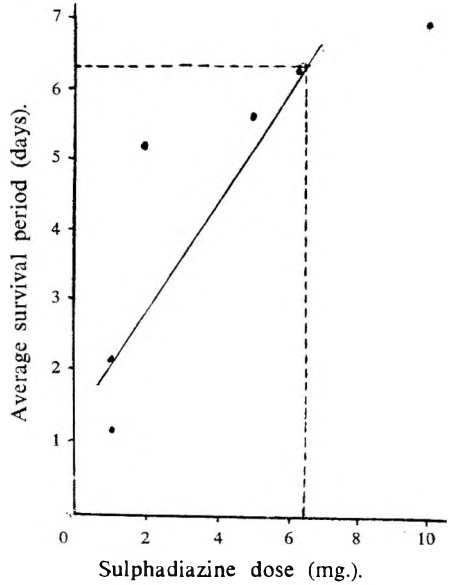

FIG. 4.—The average survival period per mouse infected with *Salm. typhi* in relation to the dose of sulphadiazine used in treatment.

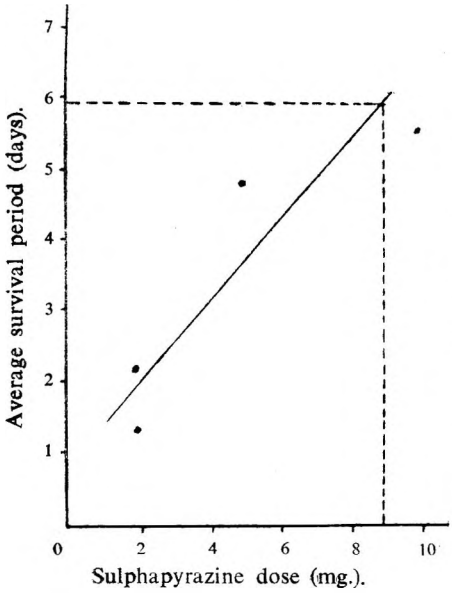

FIG. 5.—The average survival period per mouse infected with *Salm. typhi* in relation to the dose of sulphapyrazine used in treatment.

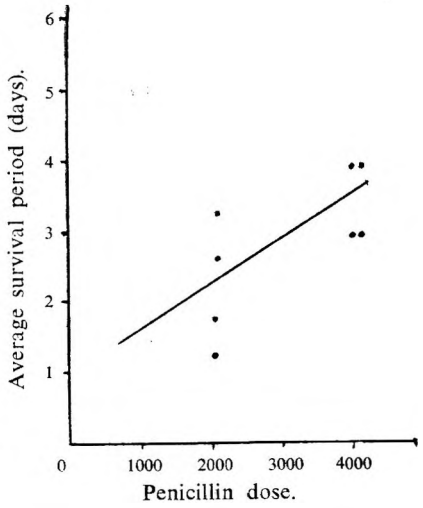

FIG. 6.—The average survival period per mouse infected with *Salm. typhi* in relation to the dose of penicillin used in treatment.

(2) *The mechanism of the combined action.* Klein and Kalter[5] found that the use of penicillin with sulphathiazole, or sulphadiazine or sulphapyrazine resulted in an increased antibacterial activity against staphylococci and streptococci *in vitro;* their explanation was that penicillin sharply reduces the total number of micro-organisms and thus permits the sulphonamides to be more active on the remaining small number of cells as these compounds are only partially bacteriostatic in the presence of large numbers of bacteria. This, however, does not explain why the synergism also occurs if the penicillin concentration was not bactericidal by itself.

Hobby and Dawson[6] explained the synergism observed between penicillin and sulphadiazine *in vitro* by suggesting that " the fact that sulphadiazine appears to increase the activity of penicillin against one strain resistant to penicillin alone suggests the possibility that penicillin may alter the bacterial cell so as to increase its sensitivity to sulphadiazine."

The work of Gardner[14,15] and of Fleming *et al.*[16] indicated that morphological changes occur in the Gram-negative bacilli when subjected to penicillin concentration inadequate for complete inhibition. The *in vitro* antibacterial activity, in the present investigation, was judged by the degree of turbidity in the medium caused by the growth of the organism; if the bacteria still grow fully in spite of the presence of a certain concentration of penicillin, it does not mean, therefore, that these bacteria were not at all affected by the antibiotic. It is suggested here that, as a non-inhibitory penicillin concentration affects the bacterial cells, although it does not kill them, this effect of the penicillin together with the partial

bacteriostatic action of the sulphonamide, may bring about complete inhibition of the growth; the two chemotherapeutic compounds thus co-operating in their action on the organism.

It is known that, although penicillin and the sulphonamides both produce inhibition of growth, they differ in the mechanism by which this end is achieved. The view mostly accepted is that penicillin is a bacterial agent[17,18], and that its acts on bacteria in the stage of active division[14,15,19,20,21]. Florey and Florey[22], however, state that penicillin is bacteriostatic and not bactericidal in its action, in concentrations likely to be produced therapeutically. According to Todd[23], the mechanism by which the organisms are destroyed is that they are first killed and then autolysed by autolysins. Chain and Duthie[5], however, stated that penicillin may abolish oxygen uptake by young organisms before cell division occurs. The sulphonamides, on the other hand, exert their bacteriostatic action by substrate competition[24,25], it is suggested that the sulphonamide molecule, being similar to that of paraminobenzoic acid, can replace it and thus deprives the bacterial cells of one of the essential metabolites. The two chemotherapeutic substances, administered at the same time, will, therefore, attack the bacterial cells from two different angles and thus interfere with their activity in a more efficient way.

(3) *Advantages of the combined therapy.* The following are the main advantages of the combined use of penicillin and of the sulphonamides observed in this study.

(a) The ultimate result obtained when the two chemotherapeutic substances were used together was an increase in the average survival period as compared to that resulting from the use of each substance alone in the same doses. It may be argued that the same therapeutic response may be obtained by increasing the dose of the sulphonamide compound, without using penicillin. However, it is definitely an advantage to use a small dose of the sulphonamide compound with penicillin than to use a much bigger dose of the sulphonamide alone, as this latter may prove to be too toxic to the host.

(b) In some conditions, the sulphonamides when used in ordinary therapeutic doses will not give a satisfactory therapeutic response, neither will penicillin even when used in very large doses. With penicillin, the difficulty is not with the size of the dose as, theoretically, this has no upper limit, but it is essential to maintain an adequate concentration in the blood and tissues during treatment. The presence of the sulphonamides in ordinary concentrations in the blood enables such a penicillin concentration as could be maintained there to exert an efficient antibacterial action, although it would exert no effect when present alone.

(c) In many instances, infection is not caused by one organism, but by a mixture of organisms which may vary widely in their sensitivity to the different chemotherapeutic substances. The use of one substance only, e.g., penicillin, may not be sufficient to eradicate the infection, and the combined therapy may be of great advantage in such cases.

(4) *Practical application of the combined therapy.* The value of the combined therapy has already been recognised in the treatment of infec-

tions caused by organisms which are more sensitive to penicillin. Dowling *et al.*[26] compared the mortality rate in two groups of patients suffering from pnuemococcal pneumonia; the first group was treated with sulphadiazine alone and the second treated with sulphadiazine and penicillin. Out of 94 patients in each group, the mortality was 9·6 per cent. in the first and 4·3 per cent. in the second. Waring *et al.*[27] treated 13 patients with pneumococcal meningitis with sulphadiazine and penicillin with one fatality only (7·7 per cent.). The authors compared these results with those of others before sulphonamide or penicillin therapy were introduced; mortality was 100 per cent. in one series of 29 patients before the introduction of sulphonamide therapy in 1936, this was reduced to 70 per cent. with sulphonamide treatment. Smith *et al.*[28] treated two groups of similar cases, 29 patients in each group; the first had penicillin only with 7 deaths and the second group was treated with penicillin and sulphadiazine with 2 deaths only, one of them was stated to be moribund on admission and the other died from an unrelated condition, fat embolism. Card *et al.*[29] observed that sulphathiazole and penicillin appear to enhance the effect of each other against *N. gonorrhœae* and that their combined used was a safe, rapid, efficient and economical method of treating gonorrheal urethritis.

Also with the more resistant organisms, the combined therapy has been applied and gave better results than single treatment. Levy and McKrill[30] compared the mortality rates in three groups of patients suffering from subacute bacterial endocarditis (due to *Streptococcus viridans*), in the first group treated with sulphadiazine alone, the mortality was 96 per cent. in the second treated with penicillin alone 40 per cent., but in the third when a combination of both substances was used, the mortality was only 28·6 per cent.

The results obtained in this experimental work suggest that a similar improvement in the prognosis of the diseases caused by the Gram negative organisms may be anticipated if they are treated with penicillin and with the sulphonamides together. The size and frequency of the doses to be used will depend on the particular infection in question. The organisms tested in this investigation cause a variety of local and systematic infections in the body which have been hitherto resistant to all forms of therapy.

Bacterium coli is a normal inhabitant of the intestines, but if it invades other systems of the body it causes various inflammatory conditions. The commonest sites affected by *Bact. coli* are the urinary tract, the gall bladder and the peritoneal cavity. Less commonly, *Bact. coli* is found in local suppurations, e.g., empyema cavities, in these there is no difficulty in maintaining the required penicillin concentration by local application. The urinary tract also is an easy situation where penicillin can reach in a high concentration; 40 units/ml. of urine is reached during treatment with 100,000 units daily and this is a very moderate dose[31]. This concentration may prove adequate to kill *Bact. coli* in the urinary tract, if not, the combination with the sulphonamides most probably will.

Salm. typhi causes a grave disease in man, the combined therapy with

penicillin and sulphathiazole has already been used with successful results[32,33,34].

Lastly, the combined therapy suggested may still prove its value in the resistant *Proteus* infection. *Proteus* is not considered as highly pathogenic, it may be found as a saprophyte in the nasal cavities and intestines. Pathologically, it is found in urinary tract infections in 6 to 13 per cent. of cases[35,36]. The fact that penicillin is found in high concentrations in the urine has already been referred to and it may prove useful in treating such cases. It has been found[37] that the average concentration required to inhibit the growth of strains of *Proteus vulgaris* obtained from infected urines was 8 units /ml. Wound infection with these organisms is occasionally met with and this is one of the conditions in which the local combined use of penicillin and of the sulphonamides should be tried. Less commonly *Proteus* septicæmia occurs, the primary infection may be in the urinary tract[38], or in local septic collection[39,40], in this condition effective treatment is more than ever needed, and the combined therapy may prove to be the answer.

Urinary tract inflammations are due, in many instances, to a mixed infection, the advantage of combined therapy in such conditions has already been referred to.

It is planned to apply this combined therapy to infections caused by other "resistant" organisms, e.g. *Streptococcus viridans*, the causative agent in the majority of cases of subacute bacterial endocarditis. Treatment of this condition with penicillin, although it has very considerably improved the prognosis in this fatal disease, gives a recovery rate of only 60 per cent[41,42]. This figure may be improved if combined therapy is instituted.

This synergistic action between penicillin and the sulphonamides is not only confined to these two drugs; it was shown, by many workers, that synergism also occurs between other pairs of chemo-therapeutic substances. Kolmer[9] demonstrated such an effect between penicillin and streptomycin against pneumococcal and anthrax infections in mice. Also, when penicillin was known to affect *Treponema pallidum*, synergism was found between penicillin and arsenic in treating experimental syphilis in rabbits[9,43]. Perhaps one of the most interesting results of the combination of two chemotherapeutic substances is its application to treatment of tuberculosis infection. Smith *et al.*[44,45], found that promin and other sulphone compounds when used alone were relatively ineffective in the treatment of experimental tuberculosis; better results were obtained when they were combined with streptomycin; the result of the combined therapy was better than the sum of the effects of the individual compounds. Callomen *et al.*[46] also showed that a much better therapeutic effect resulted by treating experimental tuberculosis in guinea-pigs with streptomycin and diasone together than by treatment with either substance alone. Brownlee and Kennedy[47] also showed that a combination of streptomycin and of various sulphone compounds produced a better effect than either agent alone.

Recently the present writers[48] demonstrated this synergism between

chloromycetin and sulphadiazine against *Salm. typhi.* infection in mice. With the rapid advance of the science of chemotherapy, the number of infections beyond control are progressively diminishing. Experimental results obtained *in vitro* or in animal experiments with a new chemotherapeutic substance are not always confirmed when practical application to human disease is attempted, nevertheless there seems to be a possibility that combined therapy with more than one therapeutic agent may be effective in conditions where one agent alone has not been successful, and it is likely that this combined therapy will be most successful when chemotherapeutic agents are employed which have a different point of attack on the infecting agent.

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A PHARMACOGNOSTICAL STUDY OF *DICHROA FEBRIFUGA* LOUR. A CHINESE ANTIMALARIAL PLANT

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PART I.—GENERAL

INTRODUCTION

THE roots of *Dichroa febrifuga* Lour.[1], family Hydrangeaceae*, known as Chang Shan, and the leafy tops, known as Shu Chi, have been used for the treatment of malaria in China for at least two thousand years[2,3,4,5,6]. Their use for medicinal purposes in China was first mentioned in the Herbal of the Emperor Shen-Nung, the written version of which dates back at least to the Han Dynasty B.C. 206 to 220 A.D.), and they have since been used along with several other medicinal substances such as betel nut, turtle shell and ginger for mitigating fever and for the treatment of malaria. During the last world war, owing to the scanty supply of quinine in the country, these preparations containing Chang Shan were used to take the place of quinine in some parts of China, and later, it was found that Chang Shan used alone was as efficacious against malaria as when used in combination with the other substances, except for attendant nausea[6,7]. The antipyretic and antiparasitic effects of Chang Shan were further proved by extensive pharmacological as well as clinical tests[6,7,8,9,10,11,12,13]. In China, Jang *et al.*[9,10] have isolated several constituents in pure form and they have shown that certain alkaloids present have a strong antimalarial action. They also found that Shu Chi, the leafy tops, was more effective against chicken malaria than Chang Shan, the roots. Workers in the United States of America also isolated two alkaloids from the roots, one of which had activity against *Plasmodium lophurae* in ducks 100 times that of quinine[13]. In Great Britain, the antimalarial activity of Chang Shan has been proved by Tonkin and Work[8].

The literature of these drugs contains only brief descriptions of the root[6,15] which would not be adequate to authenticate the material; and there is no description of the leaf part. It was decided therefore to make a thorough investigation of the characters of these two drugs in order that complete descriptions could be made.

CONSTITUENTS

Besides the active alkaloids already mentioned, the drug has been shown to contain 4-quinazolone and umbelliferone[10,11].

The chief interest has centred in the alkaloids which show antimalarial activity. However, it is reported that although the leaf material has a

* Older classifications put this plant in the family *Saxifragaceae*, subfamily *Hydrangeoidae*. According to Hutchinson's "Families of Flowering Plants," 1926, 204, this subfamily now has family status as *Hydrangeaceae*.

greater antimalarial activity than that of the root material, the alkaloidal content of the leaf is much lower than that of the root[12]. Therefore, it seems possible that active principles other than those so far investigated may be present in the leaf. Unfortunately, different workers in this field have given different names to the alkaloids they have isolated and Table I shows the results of these investigations.

TABLE I

CHARACTERS OF THE ALKALOIDS

Workers	Alkaloids	Formulæ	M.Pt. °C	Antimalarial Activity
Jang *et al.*[10, 11] ...	α-dichroine ... β-dichroine ... γ-dichroine ...	$C_{16}H_{21}O_3N_3$,, ,,	136 145 160	γ-dichroine the greatest, α-dichroine the least; curative dose for chicken malaria being 4 mg. of γ-dichroine per kg.
Kuehl *et al.*[12] ...	Alkaloid I ... Alkaloid II ...	$C_{16}H_{19}O_3N_3$,,	131-132 140-142	5 mg. of Alkaloid I or 2·5 mg. of Alkaloid II orally were equivalent to 40 mg. of quinine against chicken malaria.
Koepfli *et al.*[13] ...	Febrifugine ... Isofebrifugine ...	$C_{16}H_{19}O_3N_3$,,	139-140 154-156 129-130	Febrifugine has 100 times the activity of quinine against *P. lophuræ* in ducks or *P. cynomolgi* in monkeys, and 64 times the activity against *P. gallinaceum* in chicks.

Koepfli *et al.*[13] discuss this confusion in nomenclature and results and state that these alkaloids are easily interconverted and possibly some of them are formed during the process of extraction. The following conclusion, however, can be arrived at from the reports:—the alkaloids with the highest melting-points have the greatest anti-malarial activity. Two melting-points are given for the highly active febrifugine, and these two forms may be the same as β- and γ-dichroines, which also show high activity. Alkaloid II may also correspond to one of these forms of febrifugine. Similarly, *iso*-febrifugine, α-dichroine and Alkaloid I may be the same substance.

A further alkaloid, dichroidine, $C_{18}H_{23}O_3N_3$, m.pt. 212° to 213°C. has been reported [10,11], but its antimalarial activity has not been investigated.

HABITAT, CULTIVATION AND COLLECTION

The plant, which is a shrub with the habit of an hydrangea, is found in China (the middle, south-west and south-east provinces), the Himalayas, India, Indo-China, the Philippines, Malaya and Java.

The drug was collected chiefly from wild plants in Szechuan and Kweichow provinces of China until 1944 when scientific investigation showed the value of this drug for the treatment of malaria. The Chinese Government than started mass cultivation in Chin-fu-shan (Golden Buddha Mountain) in the district of Nanchuan, Southern Szechuan, which is a collecting centre of native medicinal herbs of the province[15]. The plant prefers fertile loam soils, a moist, warm climate, the temperature ranging from 15° to 20°C. and the altitude from 3,000 to 8,000 feet in the mountain valleys.

The plant is best raised from cuttings of young branches early in spring[15,16]. The cuttings may be first planted in a nursery covered by a rough roof and surrounded with sheltering-belts of trees or planted out directly to the fields in which castor-oil plants are grown for shelter. When the plants are 3 to 4 years old, they are dug up in fine weather during August or February. The roots are separated from the leafy stems and washed to remove soil and dried in the sun as speedily as possible. The dried root with a short portion of the stem attached is called " Chang Shan " and the leafy tops, after drying in the sun, are called " Shu Chi."

Materials

The question of the authenticity of botanical materials is always important especially when the active principles are being investigated; without this check much confusion arises. With *Dichroa febrifuga* this danger of confusion is very real as a number of drugs sold as Chang Shan are not derived from this plant. The following are some of the drugs which may be confused with genuine Chang Shan which is often distinguished as " Yellow Chang Shan " or " Chicken-bone Chang Shan."

White Chang Shan—the dried root of *Mussaenda divaricata* Hutchinson (Rubiaceae); Japanese Chang Shan—the dried root of *Orixa japonica* Thunberg (Rutaceae); Haichow Chang Shan—the dried root and leaf of *Clerodendron trichotonum* Thunberg (Verbenaceae); Native Chang Shan—the dried leaves of *Hyrangea opuloides* Steud (Hydrangeaceae).

Further, *D. febrifuga* Lour. has many metamorphosic forms[14] and it is possible to classify these into four " varieties " according to the differences in shape, size and trichomes of the leaf. We have confirmed this by examining a large number of pressed specimens of *D. febrifuga* Lour. in the Herbariums of the British Museum and the Royal Botanic Gardens, Kew. These specimens show variation in shape, size, venation, trichomes and margin of the leaf and the colour of the stem. It is not certain that all varieties are active.

In order to make certain that our description would be that of the active drug, we obtained some of the sample of *D. febrifuga* used by Tonkin and Work who had already shown this material was active[8]. Three further commercial samples were obtained (see below) and it was found that all four samples were identical in characters. In commercial roots there is a certain amount of aerial stem; the characters of this stem were compared with those of the stem present in the commercial leaf and thus the botanical identity of the commercial samples was established.

1. Sample of root and leaf from Dr. T. S. Work of the National Institute for Medical Research, Hampstead, London (root, 1945; leaf, 1947).

2. Sample of root from a drug store of repute in Chungking (1947).

3. Sample of leafy tops from a drug store in Chungking (1948).

4. Sample of root from Professor P. S. Liu of National Chekiang University, China (1948).

The characters of the leaves present in samples 1 and 3 were compared with the Herbarium specimens already mentioned and they were found

to be similar to the following specimens:—Fang 5720. (Nanchuan, Southern Szechuan, China); Fang 2010. (Kuanshien, Western Szechuan, China); Wilson 2956. (Western Hupeh, China); Wilson 1174. (Western Hupeh, China). It is interesting to note that these 4 specimens all come from the same district from which the commercial samples were obtained.

The description that follows is therefore based on the samples 1 to 4 and the four Herbarium specimens.

Part II. Chang Shan—The Subterranean Parts

Macroscopical and Sensory Characters

The subterranean structures of *Dichroa febrifuga* Lour. as sold under the name Chang Shan consist of dried roots, rhizomes and pieces of stems (Fig. 1 A,B,C). The bulk consists of roots which are usually attached in groups of 2 to 7 to a root-stock or crown which is about 2 to 5 cm. wide and bears short pieces of aerial stems. For retail trade the drug is usually chopped into thin transverse slices.

The *root* is usually about 0·3 to 2·5 cm. thick at its upper extremity and tapers slowly, being curved and contorted; up to 30 cm. long or even more. It is sometimes simple but often divides into spreading branches. The surface is yellowish brown and bears fine longitudinal striations; however, the cork is often exfoliated and then exhibits the yellow xylem with fine longitudinal striations. It is compact and hard, and breaks with a short and splintery fracture. The smoothed transverse surface shows the yellow xylem and the yellowish-white medullary rays of varying widths, each widening as it approaches the cork (Fig. 2A). Surrounding the xylem is a narrow band of phloem and a thin layer of brown cork. There is no pith in the centre.

The *rhizome* grows horizontally or, less usually, obliquely, and has a diameter of about 0·5 to 2·0 cm. The appearance of the internodes is very similar to that of the roots except for the presence of a pith. The root, root-stock and rhizome often merge into each other imperceptibly.

The *stem* is about 0·5 to 2 cm. in diameter and up to about 5 to 10 cm. long or even more. Its surface exhibits a greenish yellow to yellowish brown colour and bears pairs of opposite and decussate leaf-scars. The cork is often exfoliated and then shows the finely striated, yellowish xylem. The transverse section of the stem shows a large porous pith.

When exposed to screened ultra-violet light, all parts of the drug where the cork has been removed exhibit intense bright yellow fluorescence. In the transverse surface, only the phloem region and the central pith show strong bright fluorescence.

The drug has a slight odour and a bitter taste.

Microscopical Characters

The special features of the structure of the root, root-stock and rhizome are the presence of scalariform-perforated vessels, septate xylem-fibres, thick-walled pericyclic fibres, bundles of acicular crystals embedded in mucilage, and internal cork. Although pieces of stems occur

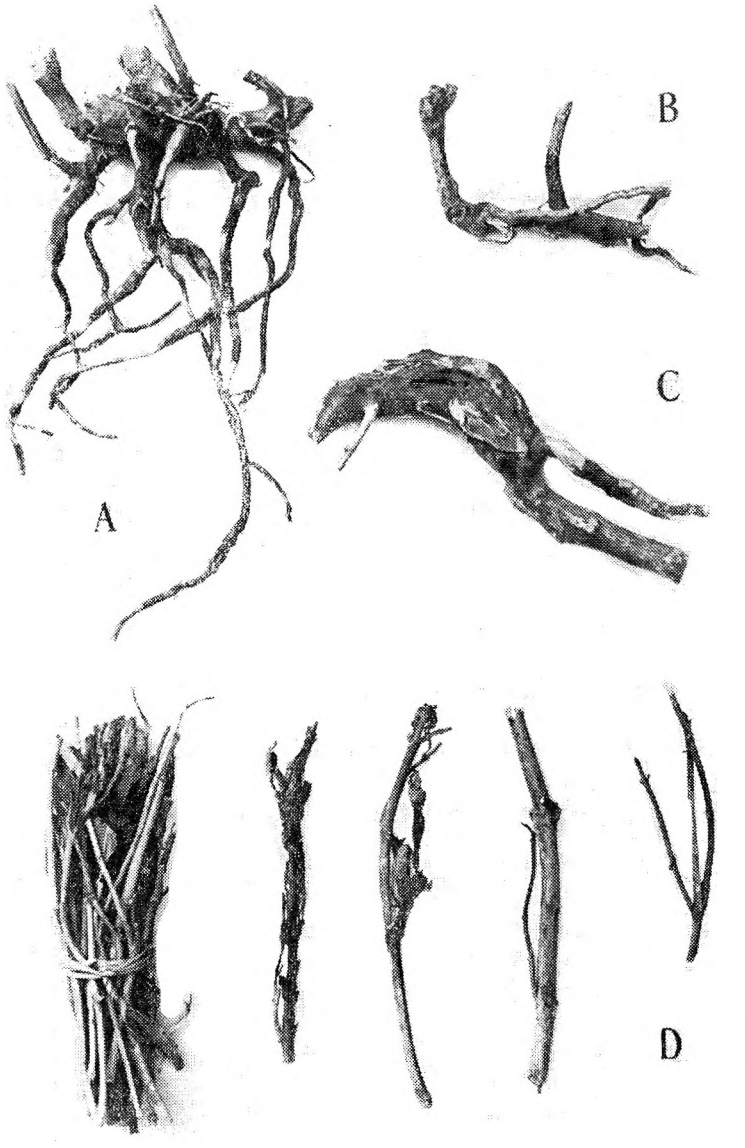

FIG. 1.—*Dichroa febrifuga* Lour. Photographs of commercial samples of the root, Chang Shan (A,B,C), and the leafy tops, Shu Chi (D). All × ⅓.

in commercial samples of the root, this structure will be dealt with in the section on aerial structures.

(1) *The Root.* At the centre of the root is a di- or tri-arch primary xylem surrounded by a secondary xylem with numerous medullary rays (Fig. 2). The elements of the *primary xylem* have spiral thickening and, unlike those of the secondary xylem, stain pink when allowed to stand

in a very dilute aqueous solution of ruthenium red. The *secondary xylem* consists of vessels, fibres, parenchyma and rays, all are strongly lignified; in older roots there are up to three or four annual growth-rings of varying width.

The *vessels* present an unusual appearance in transverse section, as frequently there appear to be pairs of semi-circular vessels with a delicate, bulging common wall (see Fig. 2D). A careful study of these vessels as seen in sections in all planes, and in macerated material showed that each *vessel-element* possesses unusually long, oblique end-walls which occupy about 1/6 to 1/4 of the length of the vessel-element. These end-walls are radially placed and have delicate scalariform thickenings (see Fig. 2E and 6C, D); the bars are only slightly lignified even in mature material. The middle lamina still remains in some of the immature materials as evidenced by the presence of a pectic substance between the bars which stains pink with ruthenium red, but in fully matured material the middle-lamina between the bars has disappeared. Hence, a transverse section of a vessel will often cut through the junction of two elements which will therefore have the appearance of two vessels with a delicate radial wall between them, as already described. The vessel-elements have the following dimensions: T = 10 to **35** to 50 μ, R = 20 to **35** to 50 μ, L = 350 to **1000** to 1700 μ. The lateral walls are comparatively thin, and show three types of pitting, viz.: (a) when in contact with another vessel they exhibit scalariform perforations; (b) when in contact with parenchyma or medullary-ray cells, bordered or simple pits occur and occasionally scalariform sculpture; (c) when in contact with fibres, the walls show small oblique pits. *Tyloses,* staining red with phloroglucin and hydrochloric acid, are frequently present in the vessels, sometimes in such large number as to block the lumen. These tyloses vary in colour from colourless to yellowish brown.

The *xylem-fibres,* T = 10 to **20** to 30 μ, R = 10 to **17** to 38 μ, L = 440 to **840** to 1350 μ, are usually arranged in radial rows. They have tapered ends which are sometimes forked (Fig. 6B). The walls bear oblique slit-like simple pits; the lumen is comparatively large and is divided by several very thin, pectosic septa; these stain pink with ruthenium red.

The *xylem parenchyma* consists of cells having the following dimensions: T = 10 to **17** to 27 μ, R = 10 to **17** to 35 μ, L = 78 to **120** to 380 μ. The walls bear numerous small, rounded, simple pits, but where they are in contact with vessels exhibit scalariform sculpture. Some particularly long parenchyma cells (about double the length of an average xylem parenchyma cell) have thin pectosic septa similar to those of the xylem-fibres (Fig. 6G).

The *medullary rays* are uniseriate or multiseriate. The multiseriate rays, 2 to **3** to **6** to 9 rows in width, 9 to **15** to **50** to 76 cells high, become wider and wider as they travel from the centre to the periphery. Individual cells, T = 20 to **30** to 45 μ, R = 13 to **30** to 50 μ, L = 28 to **60** to 120 μ, are rectangular to square prisms. The walls bear numerous

simple pits, but where they are in contact with vessels, bear bordered pits or occasionally scalariform sculpture.

Starch grains are present in all xylem elements except in the vessels and the newly formed xylem; they are particularly tightly packed in the

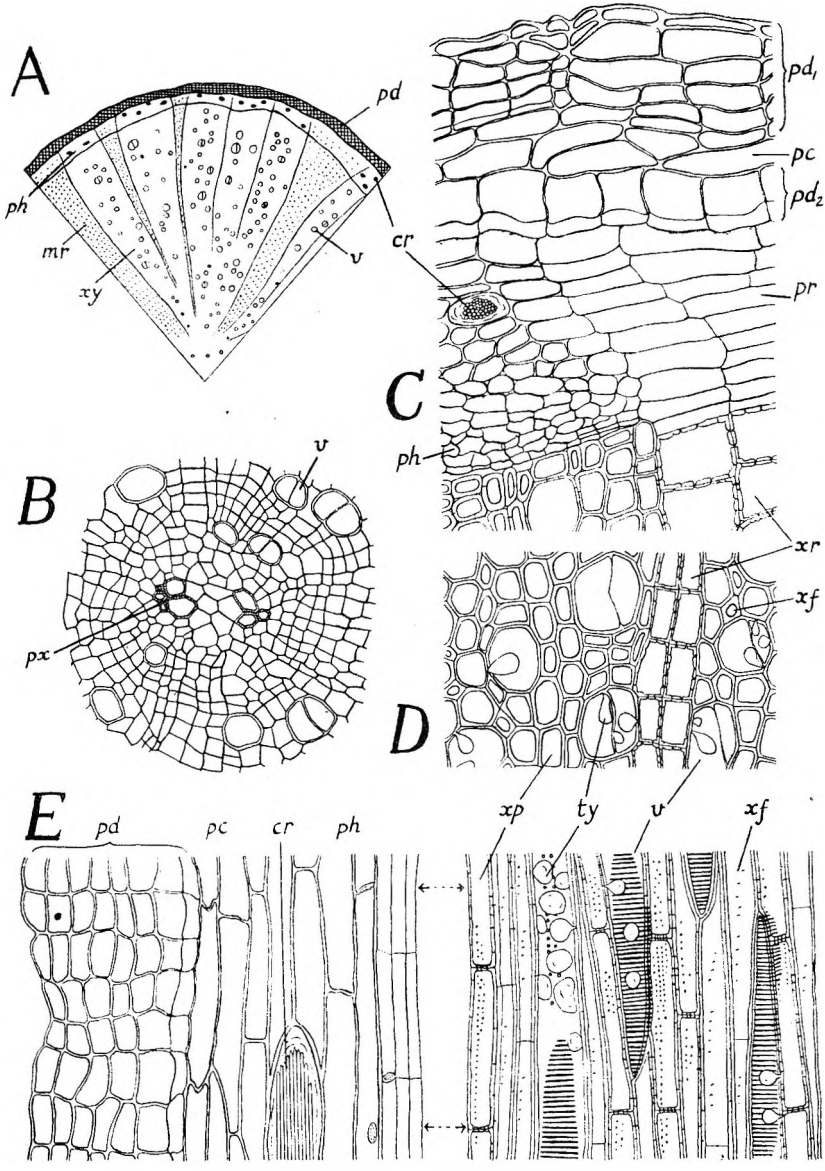

FIG. 2.—*Dichroa febrifuga* Lour. Root. A, diagrammatic transverse section × 20, B, central core of the wood showing primary xylem bundles ×200. C,D, transverse sections ×200. E, radial longitudinal section × 200. *cr*, acicular crystals; *mr*, medullary ray; *pc*, pericycle; *pd*, periderm; pd_1, old periderm; pd_2, new periderm; *ph*, phloem; *pr*, phloem ray; *px*, primary xylem; *ty*, tylose; *v*, vessel; *xf*, xylem fibre; *xp*, xylem parenchyma; *xr*, xylem ray; *xy*, xylem.

medullary ray cells. They are mostly single, but compound grains of 2 to 9 aggregation are also present. Individual grains are 2 to **8** to 20 μ in diameter, rounded or ellipsoidal in shape; compound grains are more or less roundish angular shaped; the hilum appears as a central or eccentric dark point, the striations are faint and often invisible (Fig. 6E).

The *cambium* is composed of 2 to 4 layers of thin-walled cells which are often not very apparent.

The *phloem* is comparatively small in roots with secondary development. It consists of sieve-tubes, phloem-parenchyma, crystal-idioblasts and phloem medullary-rays; all elements are cellulosic. The *sieve-tubes* are comparatively primitive in form: sieve-plates are either horizontally or obliquely placed, side-walls bear small sieve-areas. Companion cells are absent. The *phloem-parenchyma* is composed of thin-walled cells bearing a few simple pits. The *crystal-idioblasts*, T = 24 to **34** to 54 μ, R = 20 to **30** to 40 μ, L = 100 to **120** to 150 μ, only occur in the phloem parenchyma and are absent from the rays (see Fig. 2A). They contain vertically directed bundles of acicular crystals of calcium oxalate which are about 30 to **70** to 90 μ long and 0·5 to **1·5** to 3·5 μ in diameter and are embedded in mucilage which stains pink with ruthenium red. The *phloem medullary ray cells* are similar in size and shape to those of the xylem rays, but usually they are more tangentially elongated and the walls are thin and unlignified.

The *pericycle* is composed of several layers of collenchymatous cells; though in material with much secondary development this band of pericycle appears somewhat collapsed due to radial pressure. Pericyclic fibres are absent from the root.

Cork formation begins at a very early stage in root development so that only extremely young rootlets show cortical tissue. Normally even the thin roots of the commercial drug show cork development in the outermost layer of the pericycle. The *cork* is composed of 3 to 6 layers of polygonal-tabular cells, measuring about T = 18 to **48** to 64 μ, R = 7 to **15** to 28 μ, with thin walls which are lignified and suberised. Phellogen can be seen occasionally. Where the cork covers tissues containing crystal idioblasts the cork cells are longitudinally elongated as seen in surface view; otherwise they are isodiametric (see Fig. 3). In older roots, deeper seated bands of cork arise within the original band.

(2) *The Root-stock.* The structure of the root-stock resembles that of the root with the exception of possessing many outgrowths, which are the starting points of roots, rhizomes and stems. The differences in structure of these outgrowths are due to the organ which they are going to form; those which form roots contain neither pith nor pericyclic fibres, those which form rhizomes contain pith but no pericyclic fibres, those which form stems contain both pith and pericyclic fibres; details of these tissues are given under the headings, Root, Rhizome or Stem.

(3) *The Rhizome.* The structure of the rhizome is the same as that of the root except for the presence of a pith at the centre. The pith consists of two kinds of cells: those situated in the centre constitute the main portion and are very large, subspherical, parenchymatous cells,

about 65 to **170** to 300μ in diameter, with fairly thick walls; intercellular spaces occur in this region. The remainder forms a medullary sheath consisting of sclerenchymatous cells of varying size and shape, mostly polyhedral about 30 to **50** to 60μ in diameter. The walls of all the pith cells are lignified and bear large, rounded, simple pits.

(4) *The Epiphytes of the Root-stock.* The surface scrapings of several pieces of root-stocks were examined. Some golden coloured fungi with slender, multi-cellular, branched mycelium and a unicellular, or multicellular, uniseriate head were observed (Fig. 6H).

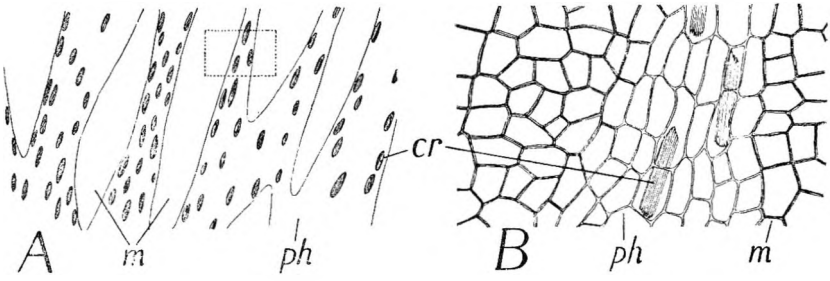

FIG. 3.—*Dichroa febrifuga* Lour. Root. A, diagrammatic surface view of the cork with crystal-idioblasts under it × 18. B, that portion of A enclosed in dotted lines, magnified to show details of the cells × 100. *cr*, acicular crystals in the sieve-tissue of the phloem; *m*, cork covering the medullary ray; *ph*, cork covering the sieve-tissue.

THE POWDER (CHANG SHAN)

Pale yellow to greyish-green. Diagnostic structures:—Cork consisting of pale brown tabular cells; vessels, up to 50μ in diameter, scalariformly perforated and often containing abundant tyloses; xylem-fibres, lumen septated; cells of the lignified medullary rays and xylem parenchyma containing starch; acicular crystals of calcium oxalate, 30 to 90μ long, scattered throughout the powder, sometimes in bundles and embedded in mucilage; starch abundant, single or 2 to 9-compound grains, individual grains 2 to **8** to 20μ in diameter. Less frequently-occurring structures are lignified pericyclic fibres with pointed or square ends, about 20 to 30μ in diameter, from the stem; large lignified pith cells from the rhizome and stem.

PART III. SHU CHI—THE AERIAL PARTS

The dried aerial part of *Dichroa febrifuga* Lour. is usually sold under the name of Shu Chi in the form of bundles about 15 cm. long each containing about 20 leafy stems (Fig. 1D). The stems which are up to 1 cm. in diameter are greenish-grey to pale greyish-brown and bear pairs of opposite and decussate leaf-scars; the surface has fine longitudinal striations; the fracture is splintery and shows a pale yellowish xylem and a large porous pith. The leaves which are usually shrivelled and partly broken are yellowish-green to greenish-grey as seen in bulk.

Macroscopical and Sensory Characters of the Leaf

The leaves (Fig. 4A) are usually 4·0 to **10** to 14 cm. long and 1·5 to **3·6** to 5·6 cm. wide, with a ratio of length to width 2·2 to **2·8** to 4·0; the lamina is narrowly to broadly elliptical, simple and entire; the margin is finely serrate, the teeth at the basal part of the lamina being less distinct; the upper surface is deep greyish green, almost glabrous to slightly pubescent; the lower surface is pale green to pale greyish green, pubescent; the midrib projects on both surfaces, the venation is pinnate, lateral veins, 5 to 8 pairs, leaving the midrib alternately or oppositely at an angle of about 45° to 50° curving towards the apex and anastomosing near the margin; a veinlet ends in each tooth of the margin and the veinlets are usually prominent on the lower surface of the leaf only; the texture is papery; the apex is acuminate; the base tapers symmetrically into a petiole; the petiole is 0·7 to **1·0** to 2·0 cm. long, slightly grooved on the upper surface; very young leaves are almost sessile. The odour is slight but characteristic; the taste is bitter.

Microscopical Characters of the Leaf

The midrib (Fig. 4B) contains a meristele in the usual position with a second smaller one above it; between them is a narrow band of parenchyma. This *upper meristele* is flat in transverse section and shows the phloem occurring above the xylem. The *lower meristele* is gutter-shaped and shows the usual arrangement of the xylem above and the phloem below. The vessels consist of slender elements with spiral or annular thickenings. Both meristeles are surrounded by a common starch sheath outside of which is parenchymatous tissue containing a few starch grains. There are 3 to 5 layers of collenchyma situated immediately beneath both the upper and lower epidermises of the midrib region. Transverse sections of the midrib or petiole may show variations in the arrangement of the meristeles from that just described. This is because there are three vascular strands entering the petiole; these strands fuse and subdivide along the length of the leaf, so that the lower meristele may appear as three separate meristeles and the upper one as two separate meristeles according to the position at which the section is made.

The *upper epidermis* (Fig. 4C) is composed of tabular cells with straight anticlinal walls; these walls contain oval to elongated pits so that the walls appear beaded in surface view. The outer periclinal walls are papillose and finely striated.

The *lower epidermis* (Fig. 4D) is composed of tabular cells with interlocking, sinuous, pitted anticlinal walls, except over the midrib where they are elongated and have straight walls. The outer periclinal walls are also finely striated like those of the upper epidermis but they are not papillose.

Epidermal trichomes are present on both surfaces but are much more numerous on the lower surface. The trichomes are unicellular, conical, and often curved near the base so that the limb is appressed to the epidermis. The walls are fairly thick especially at the apex and covered with very prominent warts. The trichomes are usually 97 to **140** to 200μ long

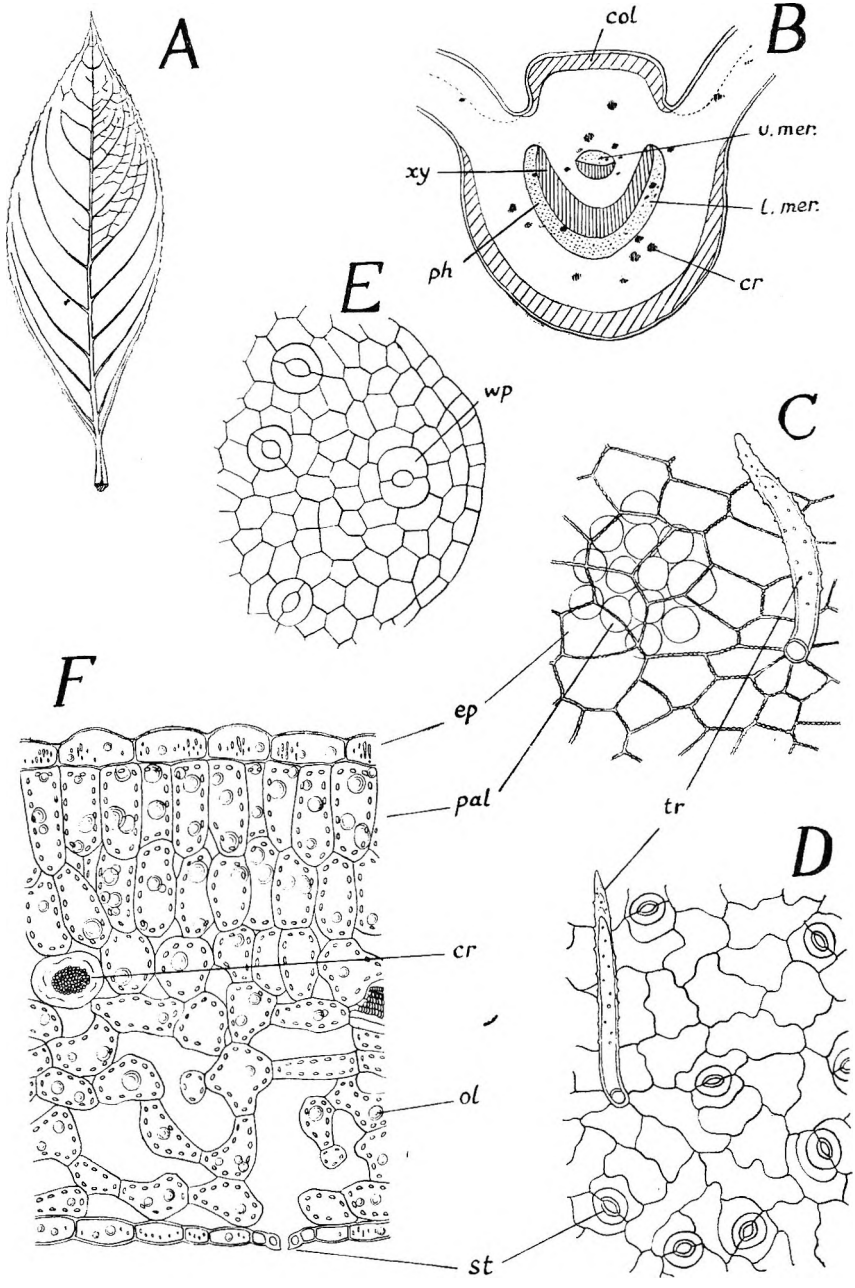

FIG. 4.—*Dichroa febrifuga* Lour. Leaf. A, sketch of a leaf $\times \frac{1}{2}$. B, diagrammatic transverse section of the midrib $\times 40$. C, upper epidermis $\times$ 200. D, lower epidermis $\times$ 200. E, upper epidermis of a tooth showing the water-pores $\times$ 200. F, transverse section of a portion of lamina $\times$ 160. *col*, collenchyma; *cr*, acicular crystals; *ep*, epidermis; *l.mer.*, lower meristele; *u.mer.*, upper meristele; *ol*, oil globules; *pal*, palisade; *ph*, phloem; *st*, stoma; *tr*, trichome; *wp*, water-pore; *xy*, xylem.

and 15 to **19** to 30μ in diameter, but sometimes they may be as long as 420μ, especially those over the veins.

Stomata of the paracytic* (rubiaceous) type are present on the lower epidermis only; they are absent however from the marginal region and those parts of the lower epidermis under which large veins lie. The stomata, 35 to **38** to 52μ long and 22 to **27** to 33μ wide, are usually slightly raised above the level of the epidermis. *Water-pores* (Fig. 4E) in groups of 3 to 7 are present on the upper epidermis of each tooth near the end of a vascular bundle; there is usually a slightly larger one situated near the top of the tooth. There is no special arrangement of the subsidiary cells around the water-pores.

The *mesophyll* (Fig. 4F) is sometimes undifferentiated but usually has 2 layers of palisade cells under the upper epidermis with spongy mesophyll in the lower half; the palisade is not continuous over the meristele. Numerous globules of oil occur in the cells of the leaf; the oil stains with Sudan III solution, but not with osmic acid solution. It is insoluble in alcohol (95 per cent.) and when the leaf is distilled with water, no volatile oil comes over. These tests indicate the globules are those of a fixed oil. Idioblasts containing bundles of acicular crystals of calcium oxalate, embedded in mucilage, are present throughout the lamina and are especially numerous in the region near the meristeles. Each crystal is about 30 to 90μ long.

Numerical values :—

Palisade Ratio	(upper surface) =	1·8 to **2·5** to 3·5.
Stomatal Index	(lower surface) =	12·3 to **17·2** to 21·3
Stomatal Number	(lower surface) =	110 to **186** to 290 per sq. mm.

Microscopical Characters of the Stem

The structure of the stem is similar to that of the rhizome except for the presence of thick-walled *pericyclic fibres*. These fibres are strongly lignified and highly refractive and bear small, simple pits. They have pointed or square ends, and the transverse section appears somewhat angular and shows a small lumen (Fig. 5 and 6A). Individual cells measure T = 17 to **27** to 45μ, R = 10 to **20** to 27μ, L = 60 to **120** to 700μ.

From the outermost layer of the pericycle arises the *periderm* which is similar to that of the root and rhizome. In young stems, the two layers of cells—phellem and phellogen—are usually visible outside the pericycle. The walls of the phellem are lignified, suberised and pitted. In older stems, the several layered phellem cuts off entirely the cortical tissues and later, deeper seated bands of periderm arise in the pericycle and phloem.

The *cortex* is composed of fairly thick walled, pitted and lignified parenchyma in the inner part, and collenchyma in the outer part. The *epidermis*, which is present in the very young stems only, is composed of a single layer of polygonal, tabular cells with cellulosic walls. *Starch grains* are most numerous in the cells of the xylem medullary rays and of the medullary sheath. *Crystal-idioblasts* are present in the pith, phloem, pericycle and cortex.

* This term *paracytic*, which was devised at the Royal Botanic Gardens, Kew, will be incorporated in their book on the anatomy of the Dicotyledons.

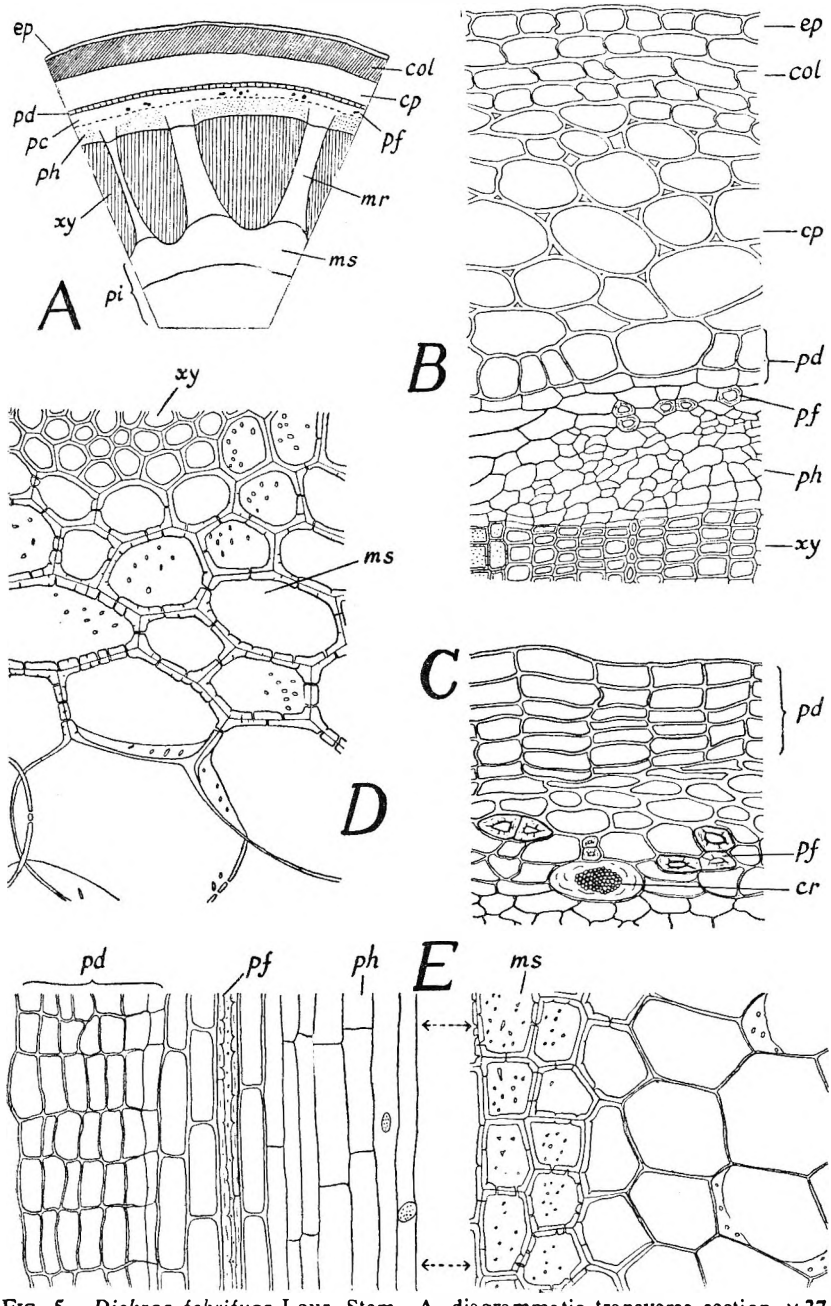

FIG. 5.—*Dichroa febrifuga* Lour. Stem. A, diagrammatic transverse section × 37. B, transverse section of a young stem (outer region) × 200. C, transverse section of an older stem (outer region) × 200. D, transverse section of a portion of the central core showing the medullary sheath and large pith-cells × 200. E, radial longitudinal section (outer and inner regions only, xylem being omitted) × 200. *col*, collenchyma; *cp*, cortical parenchyma; *cr*, acicular crystals; *ep*, epidermis; *mr*, medullary ray; *ms*, medullary sheath; *pc*, pericycle; *ped*, periderm; *ph*, phloem; *pi*, pith; *pf*, pericyclic fibre; *xy*, xylem.

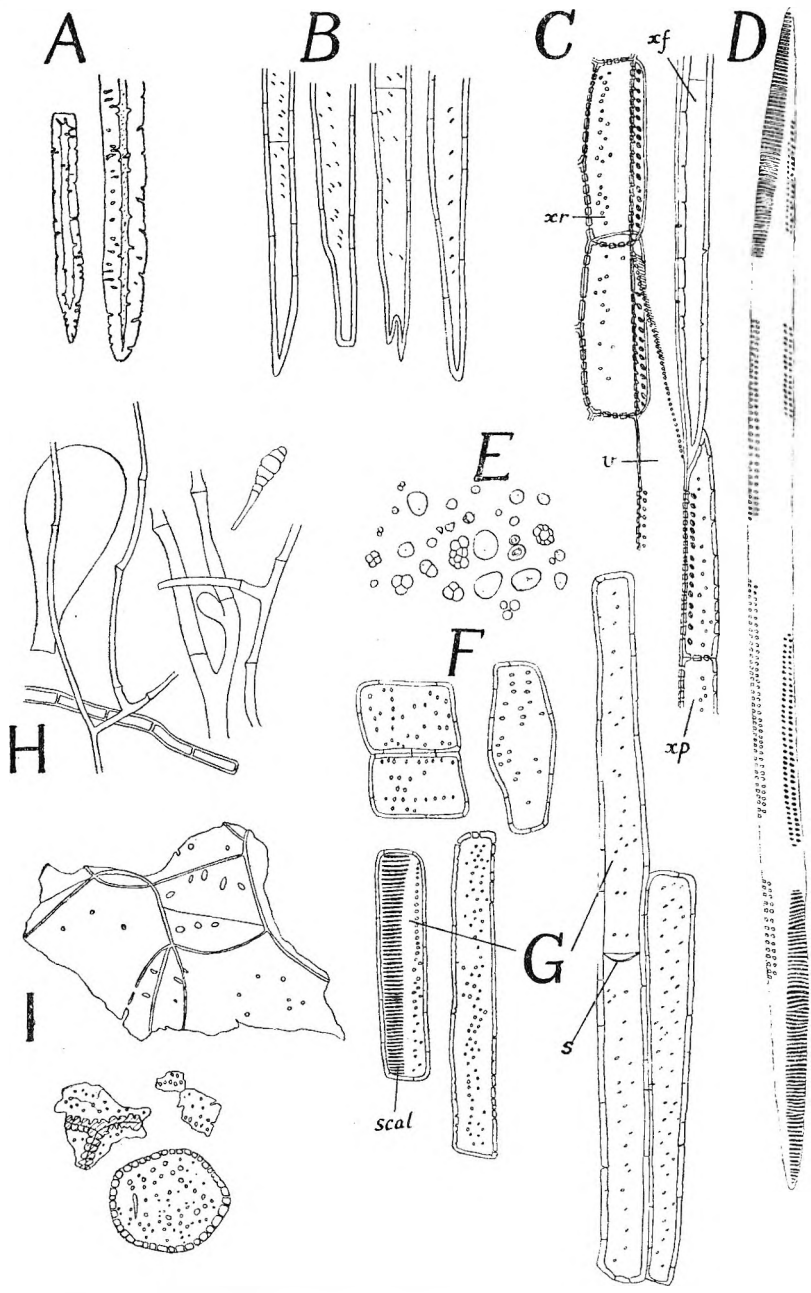

FIG. 6.—*Dichroa febrifuga* Lour. Isolated tissues and epiphytes. All ×200. A, pericyclic fibres; B, portions of xylem-fibres; C, portion of xylem tissue from the root showing the perforation of the vessel and the pitting of various xylem elements; D, an isolated vessel-element; E, starch grains; F, xylem-ray cells; G, xylem-parenchyma cells; H, epiphytes from the root-stock; I, portion of large pith-cells and cells of the medullary sheath. *s*, septum present in an abnormally elongated xylem-parenchyma cell; *scal*, scalariform pitting of a xylem-parenchyma cell; *xf*, xylem-fibre; *xp*, xylem-parenchyma; *xr*, xylem-ray cell; *v*, vessel.

The Powder (Shu Chi)

Green or greyish-green. Diagnostic structures:—Upper epidermis with straight and beaded anticlinal walls; lower epidermis with sinuous walls; stomata of the paracytic (rubiaceous) type present on the lower epidermis only; covering trichomes unicellular with very prominent warty walls; mesophyll with two rows of palisade cells and occasionally bundles of acicular crystals of calcium oxalate; vessels, mostly spiral or scalariform, a few annular: tyloses few; acicular crystals of calcium oxalate, 30 to 90μ long, scattered throughout the powder, sometimes in bundles and embedded in mucilage; starch granules 2 to 8 to 20μ in diameter, like those of the root and rhizome but not so numerous; lignified pericyclic fibres with pointed or square ends, about 20 to 30μ in diameter; cells of the lignified medullary rays and xylem parenchyma and of the sclerenchymatous medullary sheath containing abundant starch.

Discussion

The anatomical features of *Dichroa febrifuga* Lour., described in this paper, fit in well with those of the family Hydrangeaceae as given by Solereder[17], Thouvenin[18] and Holle[19]. Of the four commercial "Chang Shans" mentioned earlier which may be confused with the genuine Chang Shan from *Dichroa febrifuga*, three are members of unrelated families and will therefore show different anatomical features. The fourth, "Native Chang Shan," *Hydrangea opuloides* Steud., is a member of the Hydrangeaceae and therefore will show many features similar to those of *Dichroa febrifuga* as can be seen by a study of the paper of Ueno and Nakaoki[20]. This drug, however, only occurs in the form of leaf and has a sweet taste (one of its native names means "sweet tea"). These features serve to distinguish the two drugs in the unbroken condition but it would be necessary to investigate *Hydrangea opuloides* more thoroughly than is done in the paper mentioned above in order to distinguish the drugs when in powder or when accidentally admixed. As far as is known, intentional admixture does not occur.

Summary

1. The plant *Dichroa febrifuga* Lour. yields the two following drugs, both of which are antimalarials: Chang Shan, which consists of the subterranean portion, and Shu Chi, the leafy tops.

2. A brief account of the history, constituents, cultivation and collection and a detailed description of their sensory, macroscopical and microscopical characters are given.

3. The important diagnostic features of Chang Shan are:—vessels with long oblique end-plates having well-marked scalariform perforations and often containing tyloses; septate xylem-fibres; idioblasts containing bundles of acicular crystals embedded in mucilage; thick-walled pericyclic fibres; the deep-seated origin of the cork. A well-marked medullary sheath of thick-walled, lignified and pitted parenchyma occurs in the pith of both the stem and rhizome.

4. The important diagnostic features of Shu Chi are:—thick-walled,

warty, unicellular trichomes; paracytic (rubiaceous) stomata; epidermal cells with pitted anticlinal walls and finely striated cuticle; water-pores in the teeth of the leaf; idioblasts with bundles of acicular crystals embedded in mucilage; droplets of fixed oil in most of the cells of the leaf; thick-walled pericyclic fibres from the stem as well as deep-seated cork and characteristic vessels as seen in the subterranean organs.

We wish to thank Dr. T. S. Work, National Institute for Medical Research, London, for sample No. 1; Dr. F. C. Lou, People's Medical College, Shanghai, for samples No. 2 and 3; Mr. G. E. Trease, University of Nottingham, for sample No. 4; and Dr. T. E. Wallis for kindly advising us on certain anatomical problems.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***Erythrina thollonia*, a Curare-like Compound from.** C. Lapière and G. Coppée. (*Experientia*, 1948, **4**, 387.) From *Erythrina thollonia* (from the Belgian Congo) one of the authors succeeded in isolating an alkaloid, $C_{16}H_{19}NO_3$, with a powerful curare-like action on the frog. At a concentration of less than 1 in 10^6, it produces a complete neuro-muscular block; and on electrical stimulation of the motor nerve, there is no contraction but the muscle reacts by giving an electrical wave having exactly the same characteristics as those of a similar preparation made with curare or with quaternary ammonium derivatives, while a similar parellelism is found in the decurarisation by means of veratrine. The alkaloid has little curariform activity on mammals; with the isolated phrenic diaphragm preparation of the rat, incomplete block was produced at a concentration of 1 in 5000.

G. M.

Quinine, Extraction from Cinchona Barks. N. B. Bhunvara and M. L. Khorana. (*Indian J. Pharm.*, 1949, **11**, 148, 152.) Alkaloids may be extracted from cinchona bark by the use of dilute acid. A suitable method is: 30 g. of bark in No. 60 powder is stirred for 40 minutes with 400 ml. of 0·5 per cent. hydrochloric acid, filtered with suction and the residue washed with hot water. About 85 per cent. of the total alkaloidal content of the bark is removed by the first extraction, and the residue is treated with a further 200 ml. of the acid for 20 minutes to give a total extraction of 94·2 per cent. The acid solution is made just alkaline to phenolphthalein. For this purpose, slaked lime is preferable to sodium hydroxide, which yields too fine a precipitate. The alkaline mother liquor contains a little of the alkaloids, and may be acidified, filtered and used to extract a further quantity of bark, enabling 93 per cent. of the total alkaloids of the bark to be recovered at this stage. The precipitate can be treated by 4 hot extractions with alcohol. Alternatively, 4 cold extractions with turpentine can be used and in this case it is not necessary to evaporate the solvent, as the alkaloids can be removed by shaking with dilute sulphuric acid. Using either alcohol or turpentine, about 91 to 93 per cent. of the total alkaloids of the bark is extracted. Turpentine can also replace benzene or mineral oils in the processes of extraction already in use, and extraction of a mixture of powdered bark and soda lime can be carried out at room temperature instead of the higher temperatures usually used when benzene or mineral oil is employed. The ratio of total solids to alkaloids in the extract is lower for turpentine than for benzene. The alkaloid is removed from the solvent by shaking with 0·5 per cent. sulphuric acid. The powdered drug, after treatment with soda lime, may be packed into a percolator and immediately percolated with turpentine, the bark usually being exhausted when 10 ml. of percolate has been collected for each g. of drug. Some of the solvent retained by the drug may be recovered by pressing the marc,

and a further quantity by warming the marc with water, although a better yield is obtained by distillation, and solvent recovered in this way is cleaner

G. B.

ANALYTICAL

Amidone, Microchemical Identification of. R. C. Watson and M. I. Bowman. (*J. Amer. pharm. Ass. Sci. Ed.*, 1949, **38**, 369.) The reactions of amidone (6-dimethylamino-4:4-diphenyl-3-heptanone) with 197 reagents including many of the common so-called "alkaloidal reagents" were examined. Of these, 43 gave crystals, the remainder giving only amorphous precipitates or none at all. Only 3 reagents, palladium chloride, potassium ferrocyanide, and bromine water yielded crystals which gave a positive identification of amidone; these reagents give relatively few crystalline precipitates with other common alkaloids and synthetic drugs. The tests were made by placing the reagent drop on a microscope slide adjacent to the sample drop and allowing the two drops to flow together; the slide was allowed to stand for a short time and was then examined under a microscope (magnification × 100) for crystal formation. The slides were not scratched to aid crystallisation and cover glasses were not used. Photomicrographs are given of the crystals obtained with palladium chloride solution (approximately 1 per cent. in dilute hydrochloric acid), with potassium ferrocyanide solution (approximately 5 per cent. in water) and with a dilute solution of bromine water (3·5 ml. of saturated bromine water in 100 ml. of water).

R. E. S.

Antihistamines, Identification of. T. J. Haley and G. L. Keenan. (*J. Amer. pharm. Ass. Sci. Ed.*, 1949, **38**, 384.) Methods have been investigated for the identification and differentiation of 7 new anti-histamines namely : neoantergan (N′-*p*-methoxybenzyl-N′-2-pyridyl-N-dimethylethylenediamine), neohetramine (N′-*p*-methoxybenzyl-N-2-pyrimidyl-N′-dimethylethylenediamine), linadryl (β-morpholinoethylbenzhydryl ether hydrochloride), decapryn (α-[2-dimethylaminœthoxy]α[methylbenzyl]-pyridine, No. 204 (2-imidazoline-2-methyl benzhydryl ether), antistine (N′-phenyl-N′-benzylaminomethyl imidazoline), and dramamine, (Sc. 1694, N-dimethylethylbenzhydryl ether-8-chlorotheophyllinate). Tables of the melting points of the compounds and of their salts are given. The reactions obtained with concentrated sulphuric acid, concentrated nitric acid, Mandelin's reagent, Marquis' reagent, Frohde's reagent, Buckingham's reagent, chloroplatinic acid, chloroauric acid and picric acid solution are also listed. The precipitation reagents used were of little value due to the formation of amorphous precipitates, although decapryn succinate formed a crystalline precipitate with chloroplatinic acid. The colorimetric tests can be regarded as useful in any general classification, but a melting-point determination is necessary for an accurate identification.

R. E. S.

Benzene in Presence of Homologues, Polarographic Determination of. A. S. Landry. (*Anal. Chem.*, 1949, **21**, 674.) The determination is accomplished by nitration of the aromatic compounds present in the atmosphere by aspirating the air for 10 minutes through a U.S. Bureau of Mines type of bubbler for nitrating benzene. The toluene and xylene nitration products are then selectively oxidised with chromium trioxide and the dinitrobenzene is isolated using the differential solubility in light petroleum. Details of procedure for the nitration and extraction are given and polarograph curves, under varying conditions, are included. The

curves for dinitrobenzene, dinitrotoluene and dinitroxylene show the effect of the selective oxidation procedure, as the treated compound produces a curve that has a very small step in comparison to the untreated nitrated compound. Calibration curves are given and it can be deduced that concentrations of toluene above a certain amount will introduce an error; such an error is not large but further work is in progress to establish a correction factor. A colorimetric method based on the selective oxidation-differential solubility procedure is also being developed. R. E. S.

Benzoic, Salicylic and Acetylsalicylic Acids, Colorimetric Determination of. C. Lapière. (*J. Pharm. Belg.*, 1948, **3**, 123.) The method is based on the colouration given by these acids with copper sulphate and pyridine. Five ml. of a solution of 2·5 to 20 mg. of the acid in pyridine (10 per cent.) is placed in a separating funnel, and exactly 5 ml. of chloroform, and 1 ml. of a solution of 0·5 ml. of pyridine and 0·4 g. of copper sulphate in 5 ml. of water are added. After shaking, the chloroformic solution is run off into a stoppered tube, dehydrated with sodium sulphate, and transferred to a 1 cm. cell. The colour is determined using filter S66·6. The quantity of the acid, in mg., is given by the formula E/0·048 (for benzoic acid), E/0·064 (for salicylic acid), or E/0·033 (for acetylsalicylic acid). The colour may also be measured in ammoniacal solution, by shaking 4 ml. of the chloroformic solution with 5 ml. of a solution of 50 g. of ammonium sulphate in 100 ml. of 2 per cent. ammonia. In this case filter S61 is used, and the factors are E/0·026 (for benzoic acid), E/0·0275 (for salicylic acid) or E/0·0185 (for acetylsalicylic acid). The latter method is sometimes of advantage when it is necessary to eliminate the effect of coloured substances which are soluble in chloroform.

G. M.

Bromine, Iodimetric Microdetermination of. J. F. Alicino. A. Crickenberger and B. Reynolds. (*Anal. Chem.*, 1949, **21**, 755.) A sample of 3 to 6 mg. was analysed by the catalytic combustion method of Pregl with certain modifications. A combustion time of 20 to 30 minutes was necessary and the contents of the absorbing spiral (N sodium hydroxide solution) were transferred to a flask with water. After the addition of 5 ml. of a 20 per cent. sodium dihydrogen phosphate solution, 5 ml. of a commercial sodium hypochlorite solution (approximately 5 per cent.) was added and the solution was heated just to boiling; 5 ml. of a 50 per cent. sodium formate solution was added (with accompanying vigorous effervescence), and after cooling to room temperature 10 ml. of sulphuric acid (9N), 1 drop of ammonium molybdate solution (0·5N) and potassium iodide (approximately 1 g.) were added, the liberated iodine being titrated within 1 to 2 minutes. A blank determination on the complete procedure was necessary with each new sample of bleaching solution, the blank ranging from about 0·2 to 0·4 ml. of sodium thiosulphate solution. Satisfactory agreement with the calculated values was obtained with a wide variety of bromine compounds and although no improvement in accuracy and precision over existing methods was claimed, the method was quick and an average analysis required only 1 hour. R. F. S.

Camphor, in Galenical Preparations, Determination of. J. Julien. (*Trav. Lab. Mat. Med.*, 1943-5, **32**, Part 5.) Although a large number of methods have been suggested for the determination of camphor in galenical preparations, few are satisfactory. Polarimetry cannot be used for the determination of synthetic camphor. Other physical methods lack specificity, they are dependent on the alcohol concentration, or are insufficiently delicate.

Colorimetric methods are not considered on account of the special apparatus required. Volumetric methods, dependent on the formation of oximes, are numerous, but do not give satisfactory results. The best methods are those based on weighing camphor as the 2:4-dinitrophenylhydrazone. For tincture of camphor the process of Janot and Mouton (*J. Pharm. Chim.*, 1936, **23,** 547) is recommended: for other preparations (including camphorated oil and ammoniacal camphorated liniment that of Leonard and Smith (*Analyst.*, 1898, **23,** 281). No satisfactory methods were found for paregoric elixir and for compound ammoniacal camphorated liniment. G. M.

Cyanides, Argentometric Determination of. N. Serries. (*Trav. Soc. Pharm. Montpellier,* 1948, **8,** 71.) For accurate determination of cyanides by the method of Denigès it is essential to avoid too high a concentration of ammonia, to use sufficient iodide, and not to dilute too much. When the conditions described below are observed, the volume of silver nitrate solution is proportional to the quantity of cyanide at any concentration. Ten ml. of a solution containing 10 ml. of ammonia, 6 g. of potassium iodide, and water to 100 ml., is mixed with the solution of cyanide or of hydrocyanic acid, and the volume is made up to about 100 ml. The mixture is then titrated with 0·02N or 0·05N silver nitrate until there is a slight permanent turbidity. In order to allow for the amount of silver nitrate required to produce the turbidity, 0·05 ml. is subtracted from the reading. G. M.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Ouabain, Microscopical Characterisation of the Polymorphic Forms of. G. L. Keenan. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 355.) Ouabain crystallises in two forms. (1) Quadrilateral plates, obtained by crystallisation from water at room temperature. The crystals show very weak refraction in polarised light (crossed Nicol prisms) and apparently belong to the tetragonal system. The refractive indices ($\omega = 1{\cdot}525$; $\epsilon = 1{\cdot}523$) are similar to those of immersion fluids, and consequently the borders of the plates are almost invisible in ordinary light. (2) Rod form, obtained by crystallisation from alcohol (95 per cent.). The crystals show strong double refraction with parallel extinction and positive elongation, when viewed with crossed Nicol prisms supplemented by the selenite plate. The refractive indices are $\alpha = 1{\cdot}533$, $\beta = 1{\cdot}547$, $\gamma = 1{\cdot}580$. G. B.

Rutin, Occurrence of, in *Sambucus canadensis*. B. C. King and A. E. Schwarting. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 531.) Rutin was isolated from the leaves and flowers. Extraction studies did not reveal its presence in the stem, nor in the immature or mature fruit. Leaves were collected at 1-week to 10-day intervals from the time of early leaf development until after fruiting. Flowers were collected at immature and mature stages; a commercial sample of elder flowers was also used in the study. The material was extracted with ethyl alcohol in a Soxhlet extractor, the extract concentrated under reduced pressure and allowed to crystallise after addition of water. The rutin was identified by the melting-point. 186° to 192°C., by spectrophotometric examination, and by chemical tests. The purified aglycone obtained melted at 313°C. and an acetyl derivative melted at 196°C. These figures corresponded with quercetin. Phenylosazones fractionated with acetone gave soluble and insoluble fractions with m.pts. corresponding to those of rhamnosazone and glucosazone respectively. Results showed that rutin occurs in amounts up to 0·52 per cent. and that there is

variation in the amount in plants growing under different conditions. A progressive rise in content during the growing season until the fruiting stage was shown. G. R. K.

PLANT ANALYSIS

***Digitalis purpurea*, Chromatographic Examination of.** F. Ulrix (*J. Pharm Belg.*, 1948, **3**, 2.) Chromotography of a solution of digitalis extract in a mixture of (exactly) 1 part of methyl alcohol and 15 parts of chloroform, on alumina, gave a column showing three definite zones. Of these the lower zone (III) may be separated by washing through the tube; zone II is eluted by absolute alcohol and zone I with dilute alcohol. Purification of these zones was then continued as follows. Zone II: by dilution with water and partial evaporation of the alcohol a precipitate was formed which, after washing with methyl alcohol to remove pigments, was identified as gitoxin. Zone III: chromatography from ethyl acetate gave three zones containing respectively digitoxin, gitoxigenin and digitoxigenin. Zone I: it was not found possible to identify the third heteroside, gitalin, in this fraction, which appeared to contain the two purpurea glucosides described by Stoll. Chromatography from ethyl alcohol of various strengths gave six distinct zones giving, with Kiliani's reagent, alternatively purple and brown colorations. Two of these appear to be due to the purpurea glucosides A and B of Stoll, the others to four heterosides not previously described—purpurea glucosides A_2, B_2, A_3 and B_3. The experiments thus fail to confirm the presence of gitalin or of the hypothetical purpurea glucoside C, while the compounds actually identified were gitoxigenin, digitoxigenin, gitoxin, digitoxin, the purpurea glucosides A and B, and the four new glucosides. When chromatography is applied to commercial preparations of digitalin and digitoxin, they are found to be mixtures. G. M.

Ergothioneine Content of Ergots from Different Plant Hosts. G. Hunter, S. G. Fushtey and D. W. Gee. (*Canad. J. Res.* (*E*), 1949 **27,** 240.) The ergothioneine content was determined as follows. 30 to 50 mg. of sample was placed in a 15-ml. centrifuge tube with 4 ml. of water and 1 drop of 2 N acetic acid, and the tube put in boiling water for 10 minutes. The contents of the tube were transferred to a mortar, the ergot finely ground, and the mixture returned to the tube with the aid of 5 to 6 ml. of water. The tube was replaced in boiling water for 5 minutes and then centrifuged. The supernatant liquid was treated with a slight excess of uranium acetate and again centrifuged. The excess of uranium was removed with sodium dihydrogen phosphate and the supernatant liquid decanted. Aliquot portions of this solution were assayed by the diazo method of Hunter (*Canad. J. Res.* (*E*), 1949, **27,** 230); at least two aliquots should show strict linear transmittance. The analyses were carried out usually on a single sclerotium in triplicate and the results expressed as percentage of air-dried material. Five specimens of ergot of barley showed an overall average of 376 mg. of ergothioneine per 100 g., with a range of 231 to 531 mg. For 5 specimens of ergot of rye, the average was 336 mg./100 g., and the range, 183 to 474 mg. Ergots from *Bromus* and *Agropyron* contained about the same concentration as barley and rye ergots. The lowest values were obtained from ergots from *Calamagrostis*, but the sample may have contained extraneous material.

G. R. K.

Plant Constituents, a Proposed Method of Isolating. G. D. Curtis and L. E. Harris. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 468.) Ethoxyethanol

(Cellosolve) will extract all plant constituents that are normally extracted by the use of light petroleum, ether, chloroform, and alcohol. A scheme of isolating plant constituents is presented involving their extraction with ethoxyethanol and fractional separation by the addition of water. It is estimated that the extraction and separation of plant constituents by this method can be accomplished in about one-third the time required for the traditional four-solvent method, at only about half the cost. S. L. W.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Heparin, Notes on Fractionation and Colorimetric Assay of. O. Snellman, R. Jensen and B. Sylven. (*Acta chem. scand.*, 1949, **3**, 589.) By means of electrophoresis, commercial heparin was separated by these authors into two distinct fractions, both of which exerted anticoagulant and metachromatic activities (*J. biol. Chem.*, 1948, **174**, 265). This paper is a report of attempts made to separate heparin by means of serial precipitation, using the organic precipitants acetone, alcohol and dioxane. The experiment was unsuccessful, both components precipitating concurrently in a characteristic two-step fashion. No reasonable explanation of this fact can so far be advanced. During the first phase of precipitation a remarkable discrepancy was observed between the anticoagulant and metachromatic activity of the remaining solute material. This may be due to the possible precipitation of some impurity which might hamper the metachromatic reaction. This unexplained phenomenon constitutes a source of error in colorimetric assay of heparin. S. L. W.

Salicylic Acid, Metabolism and Toxicity of, in Combination with Various Drugs. G. Cranheim, J. Wiland and M. Ehrlich. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 451.) Combinations of sodium salicylate with various antacids (sodium bicarbonate, aluminium hydroxide gel, and magnesium trisilicate) and with sodium or calcium ascorbate were investigated for toxicity and salicylate absorption and excretion. The drugs were administered to rabbits and dogs, in water (10 to 15 ml. respectively) by stomach tube, followed by 5 and 10 ml., respectively, of plain water. The results of acute toxicity tests while not very definite on the basis of a statistical analysis, seemed to indicate that both sodium bicarbonate and ascorbic acid reduce to some extent the acute toxicity of salicylic acid, while magnesium trisilicate is without any effect. An investigation of blood levels in rabbits after single doses showed that, except for large doses of sodium salicylate, all the antacids used reduce the concentration of salicylic acid in the blood. With repeated doses of sodium salicylate the addition of magnesium trisilicate has no effect on the salicylate blood level while sodium bicarbonate reduces it. Data are presented showing the urinary excretion of free and conjugated salicylic acid in dogs and free salicylic acid in rabbits. No salicyluric acid could be detected in rabbit urine. S. L. W.

Vitamin A, Examination of Indian Shark Liver Oils for. S. M. Bose and V. Subrahmanyan. (*Ind J. med. Res.*, 1949, **37**, 1.) The analytical characteristics of a number of commercial shark liver oils obtained from Madras, Travancore and Bombay were determined using standard methods. For 26 representative samples the saponification values varied from 140 to 190, the acid values from 0·46 to 26·05, the acetyl values from 12·1 to 38·2, the iodine values from 140 to 163, the unsaponifiable matter from 1·55 to 13·27 per cent., the iodine values of the unsaponifiable matter from

132 to 192, the peroxide number from 0 to 6·2, the induction period at 65°C. from 3·5 to 8·8 hours, the moisture content from 0·26 to 2·09, and the iodine values (calculated) of the glycerides from 141 to 161. The vitamin A potency of 35 samples varied from 656 to 21,984 I.U. per g. of oil; values were determined by the Lovibond Tintometer method, the Pulfrich photometric method using filter S.61 ($B^{1\ per\ cent.}_{1\ cm.}$), and by $E^{1\ per\ cent.}_{1\ cm.}$ 328 mμ determination. The $B^{1\ per\ cent.}_{1\ cm.}$ values for the unsaponifiable fractions were invariably found to be higher than those for the whole oils. The ratio, $B^{1\ per\ cent.}_{1\ cm.}$ value (unsap. matter)/$B^{1\ per\ cent.}_{1\ cm.}$ value (whole oil), ranged between 1·02 and 1·72 for 28 samples analysed, the average being 1·34; the ratio tended to be lower for the richer oils and higher for the poorer oils, suggesting the presence of some saponifiable substance in the oil which inhibited the development of maximum blue colour. The $E^{1\ per\ cent.}_{1\ cm.}$, 328 mμ values, however, were found to be lower for the unsaponifiable portions than for the whole oils. The ratio, E (whole oil)/$E^{1\ per\ cent.}_{1\ cm.}$ (unsap. matter), was found to range between 1·03 and 1·34, the average being 1·17, this ratio being lower for the high potency oils than for the low potency oils. The ratio, $B^{1\ per\ cent.}_{1\ cm.}$ $E^{1\ per\ cent.}_{1\ cm.}$ for the unsaponifiable portion of the oil, was found to vary from 1·84 to 2·68 (neglecting only one lowest value 1·22 and one highest value 2·97), the average being 2·31 for 28 samples. The factor for converting $B^{1\ per\ cent.}_{1\ cm.}$ into I.U./g. varied from 763 to 1616, the average being 945 for 28 samples; the range of variation was, however, narrower when the unsaponifiable fractions were used and was found to be between 597 and 870, the average being 713. R. E. S.

BIOCHEMICAL ANALYSIS

Benzylpenicillin, Infra-red Assay of Procaine Salt of. N. H. Coy, C. W. Sabo and B. T. Keeler. (*Anal. Chem.*, 1949, **21**, 669.) Infra-red spectra studies showed that all the different types of penicillin possessed a characteristic band at 5·6μ which was not present in any degradation product of penicillin, the band being attributed to the presence in the molecule of the β-lactam carbonyl group. Chloroform, which is transparent in the region 5·0 to 5·2μ, was found to dissolve sufficient benzylpenicillin to produce a strong absorption band at 5·6μ; the procaine moiety of the penicillin salt did not interfere with the strength of the 5·6μ band. There was a straight line relationship between optical density and concentration over a range of concentrations from 0·2 to 0·8 per cent. Fourteen commercial samples of procaine penicillin were examined and the results are given, together with the biological values; the test was further applied to miscellaneous samples of the procaine salt of benzylpenicillin, including aqueous suspensions and also mixtures in oil with aluminium stearate. In the case of the aqueous suspension, the water was first removed by spreading the sample on a watch glass and drying in a vacuum desiccator. For samples containing oil, those compounds which interfered with the infra-red absorption at 5·6μ were removed by treating the sample with light petroleum and centrifuging. The solvent was decanted and the residue taken up in chloroform and tested. Results indicated that various procaine penicillins, including those which had deteriorated on storage, could be tested with accuracy by the infra-red method. R. E. S.

Lactose in Urine, Detection of. A. A. Ormsby and S. Johnson. (*J. Lab. clin. Med.*, 1949, **34**, 562.) Mix 5 ml. of urine with 1 ml. of a 0·2 per cent. aqueous solution of methylamine hydrochloride and 0·2 ml. of a

10 per cent. aqueous solution of sodium hydroxide, cover the tubes with glass bulbs or marbles and heat on a water-bath at 56°C. Urine containing 0·5 per cent. of lactose produces a red colour after heating for 20 minutes, and 0·05 per cent. of lactose can be detected after heating for 30 minutes and allowing to stand at room temperature for 30 minutes. Maltose gives the same colour, but other sugars give no reaction or only a yellow colour. Higher temperatures cannot be used on account of caramelisation, and violent shaking or exposure to air during the test should be avoided. The sensitivity of the test decreases if the quantity of sodium hydroxide used is outside the limits of 0·2 to 0·4 ml. of 10 per cent. solution. Ammonia in quantities up to 10 mg. of nitrogen increases the sensitivity of the test, but greater amounts inhibit the formation of the colour. For quantitative work, the temperature and alkalinity must be carefully controlled. The absorption is measured using a filter having its maximum transmission at 540 mμ and a cell containing urine as a blank to compensate for the colour of the urine. An allowance has to be made for the concentration of ammonia present, unless the ammonia is first completely removed with permutit and then 5 mg. of N ammonia is added to increase the sensitivity of the test. G. B.

Vitamin B_{12}: Microbiological Assay using *Lactobacillus leichmannii*, H. T. Peeler, H. Yacowitz and L. C. Norris. (*Proc. Soc. exp. Biol. N.Y.*; 1949, **72,** 515.) The organism used is *Lactobacillus leichmannii* ATCC 4797, but ATCC 7830 has also been used successfully. It is grown in a synthetic medium containing nearly 50 ingredients including numerous amino acids, with mineral salts, canned tomato juice and vitamins, and 0·3 unit of 15 unit U.S.P. injectable liver extract per 10 ml., and adjusted to *p*H 5·5. The optimum concentrations of several of the constituents for growth of the organism were worked out in numerous trials. Inoculation and incubation are in accordance with the technique of Daniel *et al.* (*J. biol. Chem.*, 1948, **174,** 71). Five ml. of double strength medium and the sample to be assayed are placed in the assay tubes and inoculated with the organism, and the tubes are then incubated at 37°C. for 16 hours, or until the tube containing 0·1 mg. of vitamin B_{12} gives a reading of 30 on the galvanometer scale of a Coleman spectrophotometer at wave-length 650 mμ. The turbidity of the unknown is read by placing the blanks at 100 on the galvanometer scale. A standard curve is prepared from known amounts of vitamin B_{12}, from which quantities can be read off according to the galvanometer reading. Vitamin B_{12} was found to have 24,000 times the activity of thymidine, and the latter can therefore be ignored. When the method was applied to U.S.P. liver extracts, ranges 87·1 to 2170 mμg. were found to correspond to 1 so-called U.S.P. unit. The method was also applied to the determination of B_{12} in various substances used in chick diets. Wilson's liver L (389 mμg., crude casein (104 mμg.), white fish meal (98·3 mμg.), condensed fish solubles (92 mμg.) and red fish meal (111 mμg.) were found to be particularly rich in B_{12}. H. T. B.

PHARMACY

DISPENSING

Ascorbic Acid Solutions, Deterioration and Stabilisation. S. K. Ganguly. (*Indian J. Pharm.*, 1949, **11,** 145.) Ascorbic acid solutions deteriorate rapidly at a low *p*H in the presence of air and copper. Stabilisation of the solutions may be achieved by buffering to about

*p*H 6 and storing under carbon dioxide or nitrogen. It is an advantage if the solutions are prepared free from copper. The following method is given. Dissolve 60 g. of trisodium phosphate (Na_3PO_4,$12H_2O$ of reagent purity) and 1 g. of chlorocresol in 100 ml. of freshly distilled, pyrogen-free water, add 15 ml. of pure calcium carbonate and place in a 2-l. pyrex flask closed with a cotton plug wrapped with cellophane paper freed from soluble electrolytes and copper by repeated boiling with water. Shake thoroughly and autoclave at 15 lb. for 20 minutes, cool and filter through sintered glass into a sterilised pyrex flask. Heat to boiling, pass nitrogen through the solution and saturate with nitrogen at 0°C. The removal of copper should be confirmed by the dithizone test. To the buffer solution add 55 g. of ascorbic acid, saturate with nitrogen at 0°C., filter through sintered glass into resistant glass containers and seal under nitrogen. The *p*H of the resulting solution is 6·0 to 7·0, and deterioration on storage of the sealed containers at 25° to 30°C. for 3 months is of the order of 4 per cent. G. B.

NOTES AND FORMULÆ

Methapyrilene Hydrochloride (Thenylene Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **140,** 1097.) Methapyrilene hydrochloride is N, N-dimethyl-N′(α-pyridyl)-N′-(α-thenyl)-ethylenediamine hydrochloride $(CH_3)_2N.CH_2.CH_2N(C_5H_4N)(C_5H_5S)$, HCl. It is a white crystalline powder with a faint odour, m.pt. 159° to 162°C., soluble in water, alcohol and chloroform, and slightly soluble in ether and benzene; a 5 per cent. aqueous solution has a *p*H of 5·9 to 6·4 Addition of alkali to an aqueous solution liberates the base as an oil. With sulphuric acid it gives an orange-red solution, which becomes reddish brown (distinction from pyribenzamine hydrochloride). Methapyrilene hydrochloride gives a pink precipitate with Reinecke's salt and in alcoholic solution a yellow precipitate with trinitrophenol. It is assayed for chloride content, and also for base by liberation with alkali, extraction with ether, shaking the ether with a known excess of hydrochloric acid, extracting with water and titrating the excess of acid. It contains 12·1 to 12·4 per cent. of hydrogen chloride and 87·0 to 88·5 per cent. of base. A 0·001 per cent. alcoholic solution exhibits ultra-violet absorption maxima at 2400 and 3050 Å and a minimum at 2740 Å. Methapyrilene hydrochloride is an antihistamine substance with a moderate tendency to gastrointestinal irritation. The average adult dose is 50 to 100 mg. G. R. K.

Thonzylamine Hydrochloride (Neo-hetramine). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **139,** 1148.) Thonzylamine hydrochloride is N, N-dimethyl-N′-(*p*-methoxybenzyl)-N′-(2-pyrimidyl)-ethylenediamine hydrochloride, $(CH_3)_2N.CH_2.CH_2.N(C_4H_3N_2)(CH_2.C_6H_4.OCH_3)$,HCl. It is a white, crystalline powder with a faint odour, m.pt. 173° to 176°C., soluble in water, alcohol and chloroform, and almost insoluble in ether. A 2 per cent. aqueous solution has *p*H 5·1 to 5·7, and addition of alkali liberates the base as an oil. It gives a pink precipitate with Reinecke's salt, a yellow dipicrate which melts at 141° to 145°C. and with sulphuric acid a pink colour which becomes red. A 0·001 per cent. alcoholic solution exhibits an ultra-violet absorption maximum at 2440 Å. It contains 17·0 to 17·6 per cent. of nitrogen and 98·5 to 101·5 per cent. of thonzylamine hydrochloride, determined by extracting the free base from alkaline solution with ether, recombining with hydrochloric acid and titrating the excess of

acid. Thonzylamine hydrochloride is an antihistaminic substance less efficacious than most of the other members of the group, but much better tolerated. The average adult dose is 100 mg. G. R. K.

Zincundecate (Desenex). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **140**, 21.) Zincundecate is a preparation containing undecylenic acid and zinc undecylenate and used as powder or ointment in the treatment of fungal infections of the skin. Undecylenic acid, 10-hendecenoic acid, or 10-undecenoic acid (CH_2:CH.$(CH_2)_8$.CO_2H) is a yellow liquid soluble in alcohol but almost insoluble in water. It has a refractive index at 25°C. of about 1·4486, a specific gravity at 25°C. of about 0·911, and an iodine value of 131 to 138, and contains 95 to 108 per cent. of undecylenic acid when titrated with potassium hydroxide. Zinc undecylenate, $(C_{10}H_{19}CO_2)_2Zn$, is a very fine white powder insoluble in water and alcohol. The content of zinc undecylenate, determined by ashing, is 98 to 102 per cent., calculated on the dried material; the loss on drying at 105°C. for 2 hours is not more than 1·25 per cent. Desenex is supplied as a powder containing zinc undecylenate 20 per cent., and undecylenic acid, 2 per cent., in talc, and as an ointment containing zinc undecylenate, 20 per cent., and undecylenic acid, 2 per cent., in a water-miscible base. G. R. K.

PHARMACOGNOSY

Fluorescence of Vegetable Drugs. C. R. Chase, Jr., and R. Pratt. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 324.) Powdered drugs (a) mounted in a solution of nitrocellulose in amyl acetate (b) treated with methyl alcoholic N sodium hydroxide, dried and mounted in solution of nitrocellulose in amyl acetate, and (c) treated with methyl alcoholic N sodium hydroxide and used while still wet, were examined by radiation from a mercury arc with a filter passing the 365 mμ band. 93 per cent. of the drugs examined showed fluorescence after treatment by at least one of these methods. An identification key based on the colour of the fluorescence has been worked out for 151 drugs and some further distinctions can be made by comparison of the intensities. For example, China and Saigon cinnamon do not fluoresce when mounted by methods (a) and (b), and may be distinguished from the Ceylon variety which shows fluorescent spots. Treatment (c) gives a blue colour with Ceylon cinnamon; China and Saigon give a green colour, but these varieties can be distinguished by the intensity of the fluorescence. In order to identify certain leaves (e.g. belladonna, hyoscyamus and stramonium) which are not readily distinguished by the above treatment, further tests have been devised which also serve to distinguish the different varieties of certain drugs. Alcoholic extracts of the drugs are treated (1) with silver nitrate, followed by 0·1 N sodium hydroxide and (2) with 0·1 N sodium hydroxide followed by mercuric chloride. The different varieties of benzoin or cinchona, for example, can be distinguished by the colour of the fluorescence produced by this treatment. The colour of the fluorescence of an alcoholic extract is sufficient to distinguish long buchu from short buchu. G. B.

Gum Arabic from Tanganyika. H. E. Coomber and F. J. Coomes. (*Bull. imp. Inst.*, 1948, **46**, 231.) Three samples of gum collected in September, 1947, have been examined and compared with samples of *Acacia senegal* gum from Tanganyika, and Kordofan cleaned gum. The results were considered encouraging since figures for ash, matter insoluble in cold water, colour and acidity were rather better than those of a sample of

commercial Kordofan cleaned gum previously examined. On the other hand the solutions were much less viscous than that of the Kordofan gum, a fact which may limit the value of the Tanganyika product.

	Samples			*Acacia senegal* from Tanganyika	Kordofan cleaned
	1	2	3		
Moisture, per cent.	14·4	14·6	14·5	15·9	14·0
Ash, per cent.	1·3	1·2	1·1	2·3	3·0
Acid value, mg. KOH/g. gum	1·6	1·8	1·6	2·5	2·9
Matter insoluble in cold water	0·24	0·13	0·04	0·11	0·24
Viscosity of 10 per cent. w/w sclution at 20°C. relative to water	4·35	4·0	4·3	5·21	13·50
Dynamic centipoises pH (50 per cent. w/v solution)	4·78	4·39	4·72	—	—
Colour (50 per cent. w/v solution) Lovibond scale :—	4·43	4·27	4·31	—	—
Yellow	1·3	2·0	1·0	—	6·7
Red	0·5	0·8	0·5	—	1·7
Black	Nil	Nil	Nil	—	1·3

G. R. A. S.

Opium Poppy, Dutch Culture of. W. R. Becker and A. W. M. Indemans. (*Pharm. Weekbl.*, 1949, **84,** 669.) The investigations, previously reported, of the alkaloidal content of a number of varieties of the opium poppy, grown in Holland, were continued during 1948. The wet season resulted in a considerable drop in alkaloidal content, and this was most marked with the foreign varieties. Since the capsule is only a by-product in the production of poppy seed, it would hardly be profitable for the Dutch grower to attempt to develop varieties rich in morphine and suitable for the climate. Moreover the result of such attempts is problematic, since it is just those varieties with a high morphine content which, in a normal wet summer, show the greatest drop in content to a level comparable with that of the native varieties. The same considerations apply to German and French varieties, Dutch poppy seed is of very good quality, and the introduction of foreign strains would probably lead to a reduction in quality. G. M.

Pyrethrum Flowers from Ceylon. A. E. Chittenden and H. E. Coomber. (*Bull. imp. Inst.*, 1948, **46,** 230.) Chemical examination of a sample of Ceylon flowers, which was of very good appearance, showed that it was similar in total pyrethrin content to Kenya-grown flowers.

	Ceylon Sample per cent.	Kenya Sample per cent.
Pyrethrin I	0·74	0·89
Pyrethrin II	0·70	0·64
Total Pyrethrins	1·44	1·53

G. R. A. S.

PHARMACOLOGY AND THERAPEUTICS

***nor*Adrenaline, Liberation from the Suprarenal Gland.** E. Bulbring and J. H. Burn. (*Brit. J. Pharmacol.*, 1949, **4,** 202.) The object of this investigation was to discover what substance was released by the normal suprarenal gland during splanchnic stimulation. In the spinal cat the ratio of contraction of the denervated nictitating membrane to that of the normal membrane is very much greater when noradrenaline is injected than when adrenaline is injected. If the cat is eviscerated and the splanchnic fibres to one suprarenal gland stimulated, the effect on the membranes is inter-

mediate between that of adrenaline and that of noradrenaline, and can be matched by infusing a mixture of the two. Such a match cannot be made with a mixture of adrenaline and dehydroxynorephedrine (corbasil) and other reasons exclude epinine or hydroxytyramine as substances which might be released. If the splanchnic is stimulated repeatedly a gradual decline in the proportion of adrenaline secreted was observed in normal cats, but this decline seemed absent in cats fed on a diet rich in methionine. The authors conclude that the evidence indicates the lease of noradrenaline as well as of adrenaline from the suprarenal gland in amounts varying from 20 to 80 per cent. of the total. S. L. W.

***p*-Aminosalicylic Acid, Increase in Resistance of Tubercle Bacilli to.** A. Delaude, A. G. Karlson, D. T. Carr, W. H. Feldman and K. H. Pfuetze. (*Proc. Mayo Clin.*, 1949, **24**, 341.) Cultures from 71 patients who had not been treated with *p*-aminosalicylic acid, including 10 cultures which were resistant to streptomycin, were found to be resistant to only 0·006 or 0·012 mg. of the sodium salt per 100 ml. of egg-yolk agar medium. Eighteen of these patients were treated with *p*-aminosalicylic acid alone for 94 days or less and at the end of therapy cultures were still resistant to these concentrations. Cultures isolated from 4 of 5 patients treated with *p*-aminosalicylic acid for periods of 157 to 251 days were able to grow in concentrations of the sodium salt of 1·6 to 6·4 mg. per 100 ml. of medium, but cultures from 4 patients who received approximately the same amount of *p*-aminosalicylic acid therapy in combination with promin and streptomycin did not show this resistance to the sodium salt *in vitro*. S. L. W.

Ammonium Compounds: Intravenous Toxicity. N. W. Karr and E. L. Hendricks. (*Amer. J. med. Sci.*, 1949, **218**, 302.) The toxicity of ammonium chloride solution when administered intravenously in the treatment of alkalosis is dependent mainly on the rate of administration and is virtually independent of the total amount given. The clinical effect is dependent mainly on the amount given. Toxic effects are due to the ammonium ion and not to the acidifying action of the compound. H. T. B.

Antabuse: Formation of Acetaldehyde in Relation to Dosage and to Alcohol Concentration in Blood. J. Hald, E. Jacobsen and V. Larsen. (*Acta Pharmacol. Toxicol.*, 1949, **5**, 179.) An increased amount of acetaldehyde has been identified in the expired air of rabbits treated with antabuse and alcohol. Increasing doses of absorbed antabuse, up to 1 g. result in increasing amounts of acetaldehyde in the blood of rabbits when the alcohol concentration in the blood is kept constant. When this saturation limit is reached the absorption of more antabuse has no further effect on the acetaldehyde level. On the other hand, in animals saturated with antabuse, increasing concentrations of alcohol in the blood result in increasing concentrations of acetaldehyde. While the reason for this is not clear it is of great importance in connection with the clinical use of antabuse, since it means that higher doses of alcohol will give more marked symptoms and that a single heavy dose may perhaps result in alarming symptoms. It is highly advisable therefore to observe caution in respect of the alcohol dosage to antabuse-treated patients. S. L. W.

Antabuse, Sensitising Effect to Ethyl Alcohol. J. Hald, E. Jacobsen and V. Larsen. (*Acta Pharmacol. Toxicol.*, 1948, **4**, 285.) The toxicology and pharmacology is reviewed and a study of its absorption and elimination rate in man is reported. The ingestion of even moderate amounts of alcohol on the day following oral administration of 1 g. gives

rise to the following symptoms. Within 5 to 15 minutes after taking the alcohol there is a feeling of heat in the face, followed a few minutes later by vasodilation, which reaches its maximum intensity within about 30 minutes, by which time the face is scarlet with a slight tinge of blue. The sclerae are also involved, causing a "bull eyed" appearance, and there is slight oedema in the loose tissue under the lower eyelids. Simultaneously with the flushing, palpitations start, with a feeling of intense pulsation in the lower neck, sometimes accompanied with a pulsating headache. The pulse is accelerated but at this stage the blood pressure is generally unaltered. There may be a feeling of constriction in the neck, with a sensation of dyspnœa; more frequently there is mild irritation in the throat, causing coughing. After larger doses of alcohol, 40 to 50 g. or more, especially when taken with food, nausea may occur, beginning 30 to 60 minutes after the onset of the cardiovascular symptoms; when nausea is felt the intense flushing disappears and a considerable fall in blood pressure takes place. After a shorter or longer time the nausea results in copious vomiting. The intensity and duration of the symptoms varies with the individual and with the dose of alcohol; about 5 g. will produce mild symptoms in sensitive individuals and after 10 g. symptoms always occur. The symptoms last from 30 to 60 minutes in slight cases to several hours in more pronounced ones. When the symptoms fade the patient feels exhausted but feels completely well after a few hours' sleep. No signs of habituation have been observed; indeed, it seems that the tolerance for alcohol is often lowered in repeated experiments. S. L. W.

Anthisan and Phenergan, Comparison of as Histamine Antagonists. W. H. Bain, J. L. Broadbent and R. P. Warin. (*Lancet*, 1949, **257**, 47.) These two histamine antagonists were compared quantitatively as regards their weight-for-weight potencies, durations of action and therapeutic efficacies in chronic urticaria. The relative potencies were compared by estimating the oral doses of each required to produce the same degree of reduction of the intradermal histamine weal response. Phenergan was shown to be about 7 times as potent as anthisan. The relative durations of action were estimated from the times taken for the maximum effect of approximately equipotent doses of the drugs to be reduced by half. The mean half-action time for phenergan was $19\frac{1}{2}$ and for anthisan $5\frac{1}{6}$ hours. The two drugs were compared therapeutically in 20 cases of chronic urticaria, anthisan being given at the usual intervals but phenergan, because of its long duration, in a single dose at night. Of the 20 patients 14 preferred phenergan because of the fewer side-effects. The relative therapeutic potencies were estimated from the individual ratios between the daily dose of anthisan and the daily dose of phenergan required to produce the same therapeutic effect. Phenergan was found to be, on an average, 14 times as potent as anthisan. Thus, a patient with chronic urticaria whose condition was controlled by 350 mg. of anthisan a day, is likely to show a similar clinical response to a single daily dose of 25 mg. of phenergan.

S. L. W.

Atropine, Pethidine, Procaine and Quinidine; Common Pharmacological Properties. N. K. Dutta. (*Brit. J. Pharmacol.*, 1949, **4**, 197). This paper describes the action of these substances on the perfused superior cervical ganglion of the cat, on the phrenic nerve-diaphragm preparation of the rat, and on the bronchioles of the guinea-pig. Thus they were examined for curariform activity, both in the ganglion and at the motor end-plate, and also for antihistamine activity. The experiements provided further evidence that no sharp line of distinction is possible between the actions of these four

substances though they are classified quite differently for medical purposes. All of them depress the response of the perfused cervical ganglion to preganglionic stimulation, acting in this way like *d*-tubocurarine. They all augment the contractions of the isolated rat diaphragm, both when stimulated through the nerve and when stimulated directly after curarisation; very high concentrations depress the contractions. They all depress the bronchoconstrictor action of histamine in the guinea-pig; atropine and pethidine have about one-tenth, procaine about one-hundredth, and quinidine about one four-hundredth the potency of antistin. S. L. W.

Aureomycin in the Treatment of Penicillin-resistant Staphylococcal Bacteræmia. D. R. Nichols. (*Proc. Mayo. Clin.*, 1949, **24,** 309.) Of 50 strains of *Staphylococcus aureus* recently isolated from various clinical materials 34 were penicillin-resistant, and of 15 strains isolated from patients with bacteræmia 12 were penicillin-resistant. Growth of all the strains of staphylococci isolated was inhibited by aureomycin, and 4 of 6 patients suffering from penicillin-resistant staphylococcal bacteræmia recovered when treated with aureomycin. In 4 of the patients the drug was given by intermittent intravenous administration, a dose of 200 to 500 mg. in 250 ml. of normal saline solution being administered 4-hourly every 6 or 12 hours, 10 to 15 minutes being allowed for injection. The drug was given orally in 2 cases and to complete the course of treatment in the other 4 a dose of 500 mg., 750 mg., or 1 g. was given every 4 or 6 hours. Vitamin supplements were given either orally or intravenously with prolonged aureomycin therapy. Of the 2 patients who died one had received oral therapy only and the other the combined intravenous and oral therapy. Use of aureomycin is not advocated as a routine in the treatment of staphylococcal bacteræmia but it would appear to be the drug of choice in penicillin-resistant cases. S. L. W.

Cinchona Alkaloids, Toxicity of. C. C. Johnson and C. F. Poe. (*Acta Pharmacol. Toxicol.*, 1948, **4,** 265.) The toxicity of quinine, quinidine, cinchonine and cinchonidine, for white rats (injected intraperitoneally) and for micro-organisms has been studied. The study of the effect on micro-organisms was divided into two parts: (1) an investigation of the effect of the alkaloids on the normal fermentative action of bacteria of the genus *Escherichia* and of the genus *Aerobacter;* (2) a comparison of the germicidal and antiseptic values of the alkaloids with phenol, using *Eberthella typhi* and *Staphylococcus aureus* as the test organisms. In general, the experimental results with cinchonine and cinchonidine showed the *d*-isomer to be more toxic for white rats and for bacteria than the *l*-isomer; quinine on the other hand was found to be more toxic than quinidine. Quinine, quinidine and cinchonidine showed greater toxicity for *Aerobacter,* whereas cinchonine showed slightly greater toxicity for *Escherichia.* Slight antiseptic or germicidal action was shown by the alkaloids on *E. typhi* (a phenol co-efficient of 0·03 for cinchonidine and of about 0·10 for quinine), but little action was shown against *S. aureus.* The relative degree of toxicity of alkaloids for animals and bacteria correlated fairly well. S. L. W.

Compound E, Effects of on the Acute Phase of Rheumatic Fever. P. S. Hench, C. H. Slocumb, A. R. Barnes, H. L. Smith, H. F Polley, and E. C. Kendall. (*Proc. Mayo. Clin.*, 1949, **24,** 277.) In each of 3 patients with acute rheumatic fever the intragluteal administration of 17-hydroxy-11-dehydro-corticosterone (compound E) was followed by the rapid disappearance not only of the fever, tachycardia and polyarthritis but also the elevated sedimentation rates and abnormal electrocardiographic

changes. The compound E acetate was administered in the form of a suspension, in solution of sodium chloride, of finely ground crystals in a concentration of 25 mg./ml. The total daily doses generally were 200 mg. (100 mg. twice daily) at first for several days, and 100 mg. (50 mg. twice daily) for a few more days. No definite toxicity or mental depression was encountered. It is not yet possible to draw conclusions as to the effect of compound E on the rheumatic hearts of these 3 patients, but in view of the markedly beneficial effect which this compound has on the skeletal muscles and fibrous tissues of patients with rheumatoid arthritis, it is hoped that it will exert a similar effect on the cardiac muscle. S. L. W.

Decamethonium Iodide as a Muscle Relaxant in Anæsthesia. A. J. H. Hewer, B. G. B. Lucas, F. Prescott and E. S. Rowbotham. (*Lancet*, 1949, **256**, 817.) The effect of decamethonium iodide as a muscle relaxant in surgical anæsthesia was investigated in a series of 85 unselected patients, whose ages ranged from 13 to 79 years. The surgical procedures varied from simple orthopædic manipulations to major thoracic surgery. All patients were induced with thiopentone, and the majority were anæsthetised with intermittent thiopentone-nitrous oxide-oxygen. The total dose of decamethonium iodide varied from 3 to 15 mg., given intravenously. The onset of curarisation began after 2 to 3 minutes and reached a peak within 4 to 8 minutes, the duration of relaxation being 15 to 25 minutes. Relaxation comparable to that obtained with *d*-tubocurarine could only be obtained with doses which paralysed the muscles of respiration, though very adequate operating conditions were present during the phase of respiratory recovery. In some cases this state of muscular relaxation with respiratory paralysis was obtained with the initial dose of 3 mg. Complete curarisation was produced by a dose little above the sub-threshold one. The return of muscular tone and respiration was equally abrupt, the tidal air returning to normal within 3 minutes of the onset of recovery. Repeated doses produced no cumulative effect even when given to maintain relaxation for as long as 3 hours. No significant side-effects were noted and bronchial or laryngeal spasm was not observed in any case. In none of the 85 cases was there sufficient respiratory depression to cause alarm. The authors conclude that decamethonium iodide is a satisfactory relaxing agent with a wide margin of safety, provided adequate pulmonary ventilation is maintained, and that the antagonist, pentamethonium iodide, has no useful place in anæsthetic practice because of its autonomic blocking effect, which might be a contributory factor in operative shock. S. L. W.

Dibromoprocaine Hydrochloride, Radioactive, as Spinal Anæsthetic, Tissue Distribution of. F. Howard. (*Nature*, 1949, **163**, 679.) In the first series of experiments doses of the anæsthetic varying from between 0·007 and 0·03 g. were given to cats, by lumbar puncture. Autopsies were performed at periods of 1 to 39 hr. after administration of the anæsthetic and specimens taken from the organs, the sample being broken down in lithium hydroxide solution under reflux and the resulting fine suspensions estimated by means of scaling unit and "liquid" counter. In a second series the anæsthetic was given intravenously, in order to study the effects on tissue concentration of higher blood levels. In the intrathecal series the blood levels were between 5·4 and 1·3 μg. per ml. as against levels of 44 μg. per ml. in the intravenous series. Apart from spinal cord and spinal root only the kidney and liver showed a concentration above that obtaining in the blood, namely, 9 times and 3 times the concentration respectively, while their secretions,

urine and bile, showed maximal concentrations of 133 times and 27 times that of the blood respectively; the concentration in the duodenal contents followed that of the bile. Of the remaining tissues, the lung showed the highest drug concentration (0·8) and muscle tissue the lowest (0·1). With increased time between administration and autopsy a slight increase in the blood-tissue ratios was observed, the effect being maximal about 20 hours after administration. When the drug was given intravenously the cerebrospinal fluid in the lumbar theca showed a barely estimable amount of tracer, while the cord showed 0·05 to 0·1 times, and the roots 0·3 to 0·4 times, blood concentration. At high blood levels the concentrating activity of liver and kidney appeared to decrease, but variations in blood levels had little effect on the blood-tissue ratios elsewhere. S. L. W.

Diethyl nitrophenylthiophosphate (DPTF), Poisoning by. A. H Anderson and T. Jersild. (*Acta Pharmacol. Toxicol.*, 1949, **5**, 129.) This is one of a new group of insecticides, the so-called alkylpolyphosphates and their related compounds, which are being used in horticulture and are particularly effective against aphides; other members of the group are hexathyltetraphosphate (HTF) and tetrathylpyrophosphate (TPF). They were originally believed to be almost non-toxic to man and animals but it is now known that DPTF is absorbed through the skin, as well as through the lungs and after subcutaneous injection. The effect is qualitatively the same for all alkyl phosphates and depends on the fact that these substances inhibit cholinesterase. Hence the signs of poisoning resemble those following injection of large doses of acetylcholine, namely fibrillar contractions of the striated muscles, convulsions, pilomotor contractions, increased salivation, peristalsis, bronchial secretion and miosis. Treatment is mainly focused on the pulmonary œdema and cessation of respiration. Atropine is the rational antidote. S. L. W.

Emetine, Distribution of in Tissues. L. G. Parmer and C. W. Cottrill. (*J. Lab. clin. Med.*, 1949, **34**, 818.) The tissue distribution of emetine in rabbits after a single injection was determined. Within half an hour the highest concentrations of drug were found in the lung, kidney and spleen. The liver and heart contained much less, and the intestinal level was relatively very low. In striated muscle of the leg it was found in very low amounts up to 2 hours after injection. Significant amounts of the drug were not detectable in the brain. In most organs the maximum concentration of drug occurred within 12 to 24 hours after injection, this level persisting with slight change through the second day and then gradually declining, so that by the fourth day the concentration in the major organs was about half what it was at the maximum. Some organs were free of emetine after 2 weeks, but lymph node and kidney contained significant quantities for as long as 4 weeks, and the spleen did not become free of the drug for between 6 and 9 weeks. The authors note that while almost all toxic symptoms from emetine in man and animals are associated with the heart, muscles and intestine, it was in just these organs that the lowest concentrations of the drug were found. In other words only those organs having contractability as their most important function are adversely affected even though the concentration in these organs is not very high. They suggest as a possible explanation that emetine interferes with a chemical function of the cell which converts glycogen into contractile energy. S. L. W.

***l*-N-Ethylephedrine and *l*-Ephedrine: Comparison of Pharmacological Actions.** A. Åström. (*Acta Pharmacol. Toxicol.*, 1948, **4**, 53.) *l*-N-ethyl-

ephedrine and *l*-ephedrine seem to have the same qualitative effect, the differences observed being mainly quantitative. The pressor effect of *l*-ephedrine was some 10 to 15 times stronger than that of *l*-N-ethylephedrine when doses of 0·5 to 1 mg./kg. were used. The effect of adrenaline on the blood pressure was not diminished by the two amines in chloralosed cats; cocaine depressed the effect of both substances. The effect of small doses on normal rabbit intestine is often a stimulation, and of larger ones always an inhibition, of the rhythmic contractions, the effect on the tone being very slight. After increasing the tone with acetylcholine or other compounds an inhibitory action was observed. The spasmolytic action of *l*-N-ethylephedrine was about twice that of *l*-ephedrine. Small doses of the ephedrines abolished the usual inhibitory effect of adrenaline on isolated strips of rabbit intestine and the action of electrical stimulation of the periarterial nerves to the intestine. Non-pregnant uterus of rabbit and cat were stimulated by the two amines, but the rhythmic contractions were diminished at the same time; in this respect *l*-N-ethylephedrine was somewhat more active than *l*-ephedrine. S. L. W.

Norisodrine Sulphate Dust, Inhalation of in Asthma. L. R. Krasno, M. J. Grossman and A. C. Ivy. (*J. Allergy*, 1949, **20,** 111.) A group of 24 asthmatic patients with symptoms ranging from 3 to 28 years, and not satisfactorily controlled with the usual drugs, were treated by the inhalation of norisodrine dust. The dose consisted of the amount of drug (3 to 5 mg.) released by one inspiration from an inhaler, the patient being instructed to take one inhalation of the dust during an impending attack of asthma and to repeat this within half to one hour as necessary. Nineteen of the patients responded satisfactorily and were brought completely under control with this treatment, 4 showed a good response and 1 was uninfluenced. Sixteen patients were controlled by the inhalation of norisodrine dust alone, 8 required norisodrine plus aminophylline and iodides, and/or an antihistamine, a combination of drugs appearing to be effective when each drug by itself was ineffective. In asthma with bronchitis a combination of penicillin dust and norisodrine dust therapy appears very effective. The inhalation of norisodrine dust is a safe procedure, no serious side-reactions being observed. Transitory palpitation and/or dizziness were noted in only 4 of 30 subjects. Continued use of the dust does not give rise to diminution in its effect. S. L. W.

Piperidine, Pyrrolidine and Pressor Concentrates from Dog Urine, Pharmacology of. M. J. Lockett. (*Brit. J. Pharmacol.*, 1949, **4,** 111.) Pressor concentrates from both normal dog and normal human urine were prepared by the author (*J. Physiol.*, 1944, **103,** 185; 1946, **105,** 138) and were referred to as base B concentrates. After incomplete oxidation of these concentrates with potassium permanganate the following substances can be identified in pharmacologically significant amounts: piperidine, pyrrolidine, dimethylamine and ammonia. These four bases were absent in the original concentrates but can be obtained as degradation products from base B concentrates. This paper is concerned with a comparison of the pharmacological action of base B concentrates with that of suspected degradation products. Of the four degradation products piperidine and pyrrolidine exhibited approximately equal pressor activity. The amounts of piperidine and pyrrolidine derived from base B concentrates might account for rather less than half the pressor action of the original base B concentrates, but for the dissimilarity in the actions of the base B concentrates and these two bases. The main features of this dissimilarity are that the pressor action of base B concentrates is not reversed by tetraethylammonium chloride, and is only very slightly reduced

after acute sympathectomy, denervation, or removal of the suprarenal glands. Piperidine and pyrrolidine cause the liberation of acetylcholine into the effluent perfusate from the superior cervical ganglion and of a pressor compound into the adrenal venous blood; base B concentrates do not. These dissimilarities indicate that piperidine and pyrrolidine were not present as such in the original base B concentrates, though they can be obtained from these concentrates by oxidative degradation, and that the pressor activity of these concentrates is due to some compound other than piperidine or pyrrolidine.

S. L. W.

Streptomycin and *p*-Aminosalicylic Acid, Additive Effect of, in Experimental Tuberculosis Infection. R. J. W. Rees and J. M. Robson. (*Nature*, 1949, **164,** 351.) By using the cornea of the rabbit as the site of infection it has been found possible by a method based on that described by Robson (*Brit. J. Ophthal.*, 1944, **28,** 15) to produce a standard tuberculous lesion reliable enough for studying the effect of anti-tuberculous substances *in vivo*. Twelve rabbits were used ; in one group of 6 both eyes, and in the others the right eyes only, were infected, by intracorneal injections, with approximately 300 tubercle bacilli. After a mean incubation period of 8 days all infected eyes developed early small tuberculous lesions. The animals were then divided into three groups and treated as shown in the table.

Group	No. of Rabbits	Right Eye	Left Eye
A	6	Streptomycin and *p*-aminosalicylic acid ...	Streptomycin
B	3	*p*-aminosalicylic acid	Streptomycin
C	3	Untreated controls	Streptomycin

Treatment by intravitreous injections was started on the 9th day after inoculation, streptomycin being given in 10 mg. doses twice weekly and *p*-aminosalicylic acid in 10 mg. doses three times weekly. Group C all developed rapidly progressive tuberculous lesions. In group B, while the lesions were active and progressive, those treated with *p*-aminosalicylic acid were smaller than in the control group. In group A, after 4 weeks' treatment, the left eyes all showed very small but active lesions whereas the right eyes, on combined therapy, all appeared inactive and showed fading lesions, and when therapy was stopped all the lesions in both eyes rapidly spread. The additive effect with streptomycin and *p*-aminosalicylic acid demonstrated in this experiment was striking enough to suggest that, if confirmed in man, it might add considerably to the chemotherapy of acute tuberculosis, particularly in reducing the tuberculous flora before resistance to streptomycin developed. S. L. W.

Thiomerin: A New Diuretic. A. R. Feinberg, J. H. Isaacs and W. S. Boikan. (*Amer. J. med. Sci.*, 1949, **218,** 298.) Thiomerin is the disodium salt of N(γ-carboxymethylmercaptomercuri-δ-methoxy)propylcamphoramic acid and is a mercurial diuretic which is active when given subcutaneously. In an investigation on 409 patients, receiving a total of 2069 injections, it was found that thiomerin is virtually non-toxic, painless when given subcutaneously and equally or more effective than other mercurial diuretics. The usual dose was 0·14 to 0·3 dissolved in 1 to 2 ml. of water.

H. T. B.

Trichloroethylene, Toxicity of. A. R. Hunter. (*Brit. J. Pharmacol.*, 1949, **4,** 177.) A batch of 10 mice was exposed for 1 hour to 1 per cent.

[*Continued on page* 198

PHARMACOPŒIAS AND FORMULARIES

THE BRITISH PHARMACEUTICAL CODEX, 1949

A Review of the Analysis of Organic Compounds and Pharmaceutical Preparations

By CARL OLOF BJÖRLING

Professor in the Chemical Department of the State Pharmaceutical Laboratory, Stockholm

The British Pharmaceutical Codex, 1949, presents many new features compared to the issue of 1934 and its supplements. As an analyst I am pleased to record that most of the drugs have been exactly defined, either by reference to the British Pharmacopœia or by a description on pharmacopœial lines. The B.P.C. has become a book of standards in the same way as the B.P.

Organic Compounds. Nothing revolutionary has been introduced in the tests for identity. These tests seldom give complete identification; no drug is exactly defined until it has passed all the tests. The identifications mainly include qualitative reactions for elements, radicals, etc. Often a characteristic derivative is prepared, isolated, and identified, e.g., by its melting-point or optical activity. Properly, the melting-point or any physical constant of the substance itself should be put under the heading identity, but this is merely a question of convenience. Three of the four barbiturates are chiefly characterised by their melting-points, cyclobarbitone also by the m.pt. of its *p*-nitrobenzyl derivative. As the m.pts. of many of these drugs lie close together it would be advisable to differentiate them, *e.g.*, by derivatives or by titrations, argentometric or bromometric (the allyl compound).

Among the tests for purity the dangerous contaminations with arsenic and lead are traditionally estimated, as are the content of moisture and ash. Also, more quantitative or semi-quantitative determinations of impurities are prescribed than before. But in many cases the tests for purity still are qualitative. Now that the trend in all countries—not least in the U.K.—is to use instruments which permit the estimation of even traces of impurities, is not the time approaching when all pharmacopœias will introduce quantitative methods even for impurities? Such a transition certainly implies much work, but it would be worth while. The control tests for Congo red seem to be insufficient; deaths have followed the injection of impure material.

In this edition assays for organic compounds are more frequently required than formerly. Assays are not included for drugs which are easily defined in other ways—*e.g.*, acetanilide—or which are difficult or impossible to determine. Among the alkaloids—some 20—the titrimetric procedure is required for 10, but the base is extracted and titrated in only half that number, though it is possible in at least 10. Drying of the chloroform extract so frequently directed is unnecessary, even if ammonia is the alkalising agent. Pholedrine sulphate can be easily determined bromimetrically.

The compilers of the Codex have not had the courage to introduce chromatographic methods, which are so convenient for many alkaloidal salts (with Al_2O_3) and salts of metals with inorganic or some organic acids (with cation exchange resins). Nitrobenzene is used to enclose the

AgCl in Volhard titrations, but not consistently. As often as possible a drug is determined by its optical activity.

The photometric or fluorimetric assays are very few, though such methods often are rapid, easy, and specific. Also, it seems feasible to state a value for the molecular or specific extinction at a certain wave-length instead of referring to a standard curve of an auxiliary substance (folic acid). The use of Lovibond units for burnt sugar is certainly practical, but might have been completed by figures more generally applicable. Among the relatively few gravimetric assays in the monographs the determination of methylamphetamine is worth noticing. It is not clear why, when a weighing should be accurate this is always mentioned but never for an accurate measuring (*e.g.*, phenoxyethanol, where the pipetting of the acetic anhydride-pyridine solution is extremely critical).

Formulary. Much work has been done to provide determinations for so many of the preparations. The principle seems to be to provide an assay of the active ingredients of all stored preparations. But when known methods are too inaccurate, or too difficult, none has been included, and the monograph left incomplete. I think this is the way to proceed the first time a new scheme is tried. However, very often no assay is given, though it would have been easy. In the next edition the list of assayed preparations will presumably be longer.

It must be emphasised that the determinations appear to be reliable, especially considering the complex nature of many of the formulæ. Contrary to the Monograph part, many of the quantitative analyses in the Formulary are gravimetric, perhaps in order to give the analyst an opportunity of identifying and testing the ingredient thus isolated. Yet, this is seldom mentioned in the text. The gravimetric procedures are sometimes unnecessarily long and less exact than other methods possible. A typical example is the determination of phenolphthalein in emulsion of liquid paraffin with phenolphthalein, where the weighing of 17 mg. concludes a complicated analysis. Here a photometric estimation can be done, starting with one fortieth of the quantity of emulsion now required. The weights per ml. are stated for all liquid preparations, for tinctures, etc., also the alcohol content. No biological method is described, which no doubt is very sensible. Finally, some notes on special items. The analysis of liniment of methyl salicylate is a real writing desk procedure, impossible to perform. Mercury is assayed by an amalgam method which is very satisfactory, arsenic (in Blauds pills) by distilling $AsCl_3$, a method nowadays abandoned for better ones. Tablets of erythrityl tetranitrate are determined by a simplified colorimetric method, comparing two solutions.

To sum up, in spite of some criticisms, the chemists in the British Empire and all over the world will use the new B.P.C. with confidence. The analytical new look of the issue will, no doubt, be permanent.

LETTERS TO THE EDITOR

The Cephaëline Content of Emetine Hydrochloride

SIR,—The B.P. 1948 requires emetine hydrochloride to contain not more than 1 per cent. of cephaëline and the U.S.P. XIII not more than 2 per cent. The cephaëline content of emetine hydrochloride may conveniently be determined by the well-known process for the separation of neutral and basic substances from phenolic bodies. A solution of the salt is treated with sodium hydroxide solution, and the emetine is extracted with an organic solvent after which the aqueous layer is made acid and then made alkaline by the addition of a slight excess of dilute ammonia solution. Any cephaëline is then extracted with an organic solvent and the extract, after washing, evaporated to dryness when the residue is weighed. The essential features of this process have been adopted both by the B.P. 1948 and the U.S.P. XIII but whereas the former uses chloroform as solvent the latter employs ether. It has been found in our laboratories that when emetine hydrochloride is examined for cephaëline by using chloroform none or practically none is ever found, but that the presence of cephaëline is nearly always indicated when ether is used. To investigate this discrepancy, we added 10 per cent. of cephaëline hydrochloride [Found on dried material C, 61·99, 61·91; H, 7·57, 7·68; MeO, 17·24, 17·34; $C_{28}H_{38}O_4N_2$, 2HCl. C, 62·3; H, 7·5; MeO, 17·25 per cent. $[\alpha]_D^{20°C.}$ +27·1° (*c.*, 4·496 in water). Loss at 120°C., 12·70; 12·50 per cent.] to emetine hydrochloride and estimated the cephaëline in the mixture by the two processes. The results were—using chloroform, cephaëline nil; using ether, cephaëline 80·5 per cent. recovery.

The experiment was then repeated using pure cephaëline hydrochloride when the following results were obtained:—using chloroform, cephaëline 1 per cent. recovery; using ether 94·6 per cent. There is no doubt that chloroform extracts cephaëline from solutions made alkaline with sodium hydroxide, and ether is a much more satisfactory solvent for this test.

F. ASHWORTH,
G. E. FOSTER.

The Wellcome Chemical Works,
Dartford, Kent.
November 1, 1949.

ABSTRACTS (*Continued from Page* 195)

trichloroethylene vapour in air daily, except on Sundays for 12 days. Three animals died during the first inhalation of the mixture but the remainder survived except that during the 9th, 11th and 12th exposures one mouse died. A similar experiment was carried out with 1·5 per cent. trichloroethylene, and in this experiment none of the animals survived the eighth inhalation. Trichloroethylene is thus shown to be a potential poison to mice; further, their sensitivity to the drug seems to increase with repeated exposure. The animals appear to die because the drug increases the rate of respiration until ventilation becomes inefficient. In the doses used in this study there was no toxic action on the liver or kidneys. The importance of these findings to clinical anæsthesia is that they emphasise the desirability of giving plenty of oxygen to patients who develop tachypnœa as a response to trichloroethylene, and that they confirm the previously made observations that the drug has no serious toxic effects on the parenchyma of liver and kidneys of healthy mice even when administered repeatedly in anæsthetic concentrations for an hour. S. L. W.

BOOK REVIEWS

NATURAL PRODUCTS RELATED TO PHENANTHRENE, by L. F. Fieser and M. Fieser. Pp. XII + 704. 3rd Edition. Reinhold Publishing Corporation, 330, West Forty-Second Street, New York 18, U.S.A., 1949. £4 0s. 0d.

This book was first published early in 1936, and a second edition followed within twelve months. No substantial changes were made in the text, but data published during 1936 were summarised in an appendix of 90 pages. A simple calculation shows that if new knowledge had continued to accumulate at the same rate, an additional 1,000 pages would have been required to provide an adequate appendix in the 1949 edition! Needless to say, Dr. Fieser, now joined by his wife, has not adopted such an expedient, but has written what is virtually a new book, and the authors are to be congratulated on having restricted the increase in the number of pages to just over 50 per cent. Three of the chapters in the second edition, dealing with the chemistry of phenanthrene, carcinogenic hydrocarbons and triterpenoid sapogenins, have now been omitted, as the substances discussed were, strictly speaking, either not natural products or not true phenanthrene derivatives. The chapter on sterols and bile acids has been expanded from 100 to 208 pages, whilst new chapters have been added on Adrenal Cortex Hormones, Steroid Metabolism and Steroid and Terpenoid Alkaloids. The fact that the chapters on sex hormones and cardiac glycosides have increased by only 10 pages each, although a large number of new compounds of both types are referred to, illustrates the drastic pruning that the older material has undergone in order that more recent discoveries may be adequately reviewed. Primarily, this is a text-book of advanced organic chemistry in a restricted branch of the subject which has grown so quickly that only the specialist can be expected to be fully conversant with the latest developments. Because it reviews the chemistry of so many substances of physiological significance and actual or potential therapeutic value, and because this information can only be obtained otherwise by a search of the original literature, the book is of outstanding importance to pharmacists and to chemists engaged in the pharmaceutical industry. They will appreciate particularly the sections on the therapeutic value of the different sex hormones, the pharmacological properties of the cardiac glycosides and aglycones and the physiological actions of the various cortical hormones; this last section is to be especially commended for its lucid treatment of a difficult subject and because it is of such topical interest. British readers may cavil at the price now asked for American text-books, and the Treasury at the dollar expenditure involved, but a book of this type must be regarded by the chemist as an essential tool of his profession and accorded a correspondingly high priority.

F. A. ROBINSON.

NEW APPARATUS

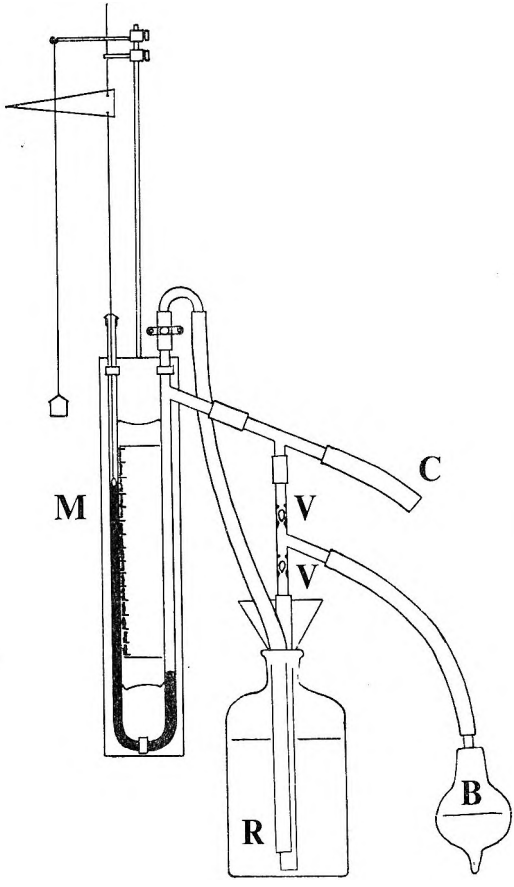
A THREE WAY VALVE FOR MANOMETRIC BLOOD PRESSURE RECORDS

By B. Basil and G. F. Somers

From Glaxo Laboratories, Ltd., Greenford, Middlesex.

Received January 4, 1950.

Traditional methods for recording the blood pressure of anæsthetised animals employ a mercury manometer and a bottle containing an anticoagulant fluid. The pressure in the manometer is raised, either by elevating the bottle or by increasing the pressure in the bottle—e.g., by means of a bicycle pump and valve—but the bottles tend to be cumbersome.



A simple apparatus for raising the pressure in the manometer and for washing out the arterial cannula is illustrated in the figure. It consists of two glass valves (V) and a small rubber bulb (B). When the bulb is pressed and released, anticoagulant fluid is drawn from the reservoir (R). When the bulb is again pressed, either the fluid is forced through the cannula (C), if this is open, or the pressure in the manometer (M) is increased. The operator can readily use the apparatus single-handed and can exercise delicate control on the pressure in the manometer. There is no danger of flooding the animal with anticoagulant fluid, as has been known to occur through inadvertently omitting to close the clip to the pressure bottle. A rubber tube is also connected to the top of the manometer, which is normally kept closed by a screw clip. This enables bubbles of air when present in the manometer to be easily washed out.

The apparatus has been in use in these laboratories for some considerable time and has so far proved entirely satisfactory.

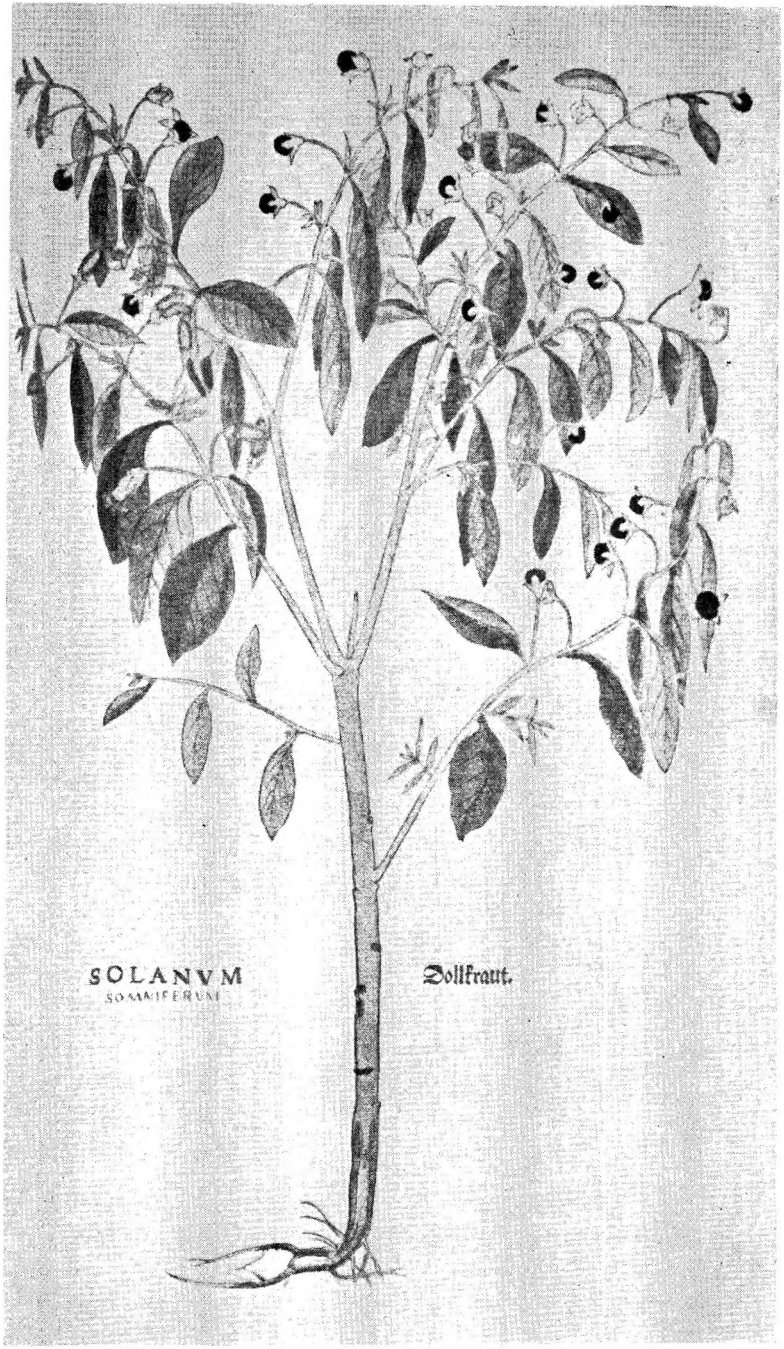


FIG. 1.—*Atropa belladonna* Linn., Reproduction of figure from Fuchs, "*De Historia Stirpium*." 1542.

[*To face page* 202, *Vol.* II, *No.* 4