REVIEW ARTICLE

THE PHARMACOGNOSY OF *ATROPA BELLADONNA* LINN.

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Introduction

Atropa belladonna Linn., family Solanaceae, is a perennial herb growing in calcareous soil to a height of about 2 metres: it is indigenous to Britain and most countries of Central and Southern Europe. A broad rootstock produces several aerial shoots which may branch and which bear numerous leaves; the leaves are broadly ovate, entire, petiolate, borne alternately in the lower part of the stem but occurring in pairs of unequal size in the upper part; flowers occur singly on short drooping pedicels in the axils of the pairs of leaves in the upper part of the stem, often associated with a few small leaves of a suppressed shoot. Eichler[1] has described and figured the upper part of each stem as a monochasial cymose inflorescence with adnation of the subtending leaf through one complete internode, the second leaf at each node and the dwarf leafy shoot representing a suppressed dichasial system. The flower has a campanulate purple corolla up to 3·5 cm. long, K(5) (C5) A5 G(2), stamens epipetalous; fruit a black fleshy berry up to 2 cm. in diameter with persistent calyx and containing numerous sub-reniform seeds. A number of tapering cylindrical roots up to 50 cms. long are attached to the rootstock.

It seems probable that belladonna, which is indigenous to Greece and Italy, was known in classic times and was used therapeutically. Unfortunately the botanical descriptions, recorded by Theophrastus, Dioscorides and Pliny for the materials *Mandragora, Strychnos* and *Solanum* respectively, are insufficient to enable them to be identified with belladonna, and some characters described by these authors are certainly not those of belladonna[2,3,4]. The first definite descriptions of belladonna date from the early 16th century; it was mentioned in the *Grand Herbier* printed in Paris about 1504, was described by Tragus[5] in 1532 as *Solanum hortense nigrum* and was both figured and described in 1542 by Fuchs[6] as *Solanum somniferum, S. somnificum* or *Dollkraut*. A photograph of the drawing by Fuchs is reproduced as Figure I.

Linnaeus[7], in 1753, named both the genus ATROPA and the species *belladonna,* but 4 years previously, in his *Materia Medica*[8], he had recorded the following monograph:—

89 ATROPA *Hort. Cliff* 57, *Hort. Upsal.* 45.
Solanum maniacum multis *Bauh. Hist.* 3. 611.
LOC: Pannonia, Austria, Anglia, Perennis, cicur.
PHARM: BELLADONNAE Baccae, Folia, ▽.
QUAL: venenata. *Insueta, praestans, caute.*
VIS: phantastica, paralytica, narcotica, anodyna.
USUS: Dysenteria, Rubor, Cachoëtes, Tumor mammarum, Fistula.
COMP:
(▽ is the Linnean symbol for water).

The leaves or flowering tops, also the root and rootstock, of belladonna are used for medicinal purposes. The leaf was introduced into the London Pharmacopœia of 1809 and the root was introduced into British medicine by Squire about 1860.

It is the purpose of this review to summarise the more recent work which has been carried out upon the pharmacognosy and physiology of belladonna. A well established corpus of knowledge of the morphology, anatomy, histology and chemistry of the drug, both leaf and root, may be found in the textbooks, to which the reader is referred.

Cultivation

A certain amount of belladonna herb and root is collected from wild plants, but the greater quantities are grown commercially. Gerrard[9] examined materials from wild species growing on limestone and from commercially grown samples and concluded that the wild plants were superior in alkaloidal content. This conclusion was challenged by Schmidt[10], by Carr and Reynolds[11] and by Carr [12], amongst others, who have found the tops and roots from cultivated plants to be richer in alkaloids than the material from the wild stand. Such a sub-division of all materials into " wild " or " cultivated " without further definition of the factors governing the growth of either group is too arbitrary to be of critical value. The amount of alkaloid present in the dried drug depends, amongst other things, upon the strain of plant from which it has been produced, the soil in which it has grown, including the addition of manure to the soil, the climatic conditions during growth, the age of the material when collected and the conditions under which drying has been carried out. When the plant is cultivated a number of these factors may be controlled and the optimum conditions for growth selected, hence we shall consider each of these factors in detail.

Germination. Many samples of belladonna seed are slow and erratic in germination. This is to some extent due to the occurrence in fully matured berries of a small proportion of light seeds which float on water and are non-viable[13], but the major cause of erratic behaviour is a physiological or mechanical dormancy which is not associated with the size or colour of the seed but is, to some extent, correlated with the separation of the seeds from the berries. Thus seeds separated from the ripe berries, washed free from pulp and dried, had a higher germination percentage than those which were allowed to dry within the fruit before separation[14]. Several chemical and physical methods have been proposed to break down this dormancy; Sievers[13] found that soaking the seeds in a 60 per cent. solution of commercial hydrogen peroxide for 18 to 24 hours gave the best results, freezing the seeds, also scarifying the testa, accelerated the germination, but soaking the seeds in strong sulphuric acid for different periods of time was of little value. The stimulus of initial refrigeration was supported by L'Vov and Iakovleva[15] (seeds stratified in moist sand in a cellar throughout the winter), by R. Melville and Metcalfe[16] (seeds refrigerated for 3, 7 and 14 days) and by Heit[17] (seeds pre-chilled at 3° to 5°C. for 25 days). Melville and Metcalfe found that

the germination of their controls increased greatly as the length of the experiment was increased, this they suggested was due to the diurnal fluctuation in temperature of the greenhouse in which the seeds were grown, Heit also found the use of fluctuating temperatures a suitable method of breaking dormancy when seeds were in the presence of a 0·2 per cent. solution of potassium nitrate. Scarification of the testa resulted in fungal attack, but a number of seeds were observed to germinate[16]. Stillings and Laurie[18] obtained a 50 per cent. germination by immersing seeds in 70 per cent. sulphuric acid for 1 minute, soaking in water and starting in an environment of high temperature and high humidity. A stimulus to germination and to growth using a mixture of aneurine hydrochloride and ascorbic acid has also been reported[19].

Plant Selection. A number of investigations have been made to select strains of plants which contain a high proportion of alkaloids and which retain this character in their progeny. Sievers[20] found that the individuality of the plant exerted a greater influence upon the ultimate yield of the drug and upon the amount of alkaloid therein than did variations in soil, manuring, weather and other factors. He crossed selected individuals, examined the F_1 and F_2 generations grown in different habitats, and concluded that the alkaloidal character of the maternal parent was inherited[21]. Miller and Reid[22] also Arny[23] claimed to have produced strains of plants of high alkaloidal content by selection and inbreeding. James[24,25,26,27,28,29] maintained some 40 strains in cultivation and produced hybrids between some of the races. A number of these were examined over a period of 5 seasons, but no one strain appeared to be consistently outstanding in plant vigour or in alkaloidal production: although 3 strains showed themselves capable of giving good growth and alkaloidal production in cultivation. James concluded that there was so much variation between different parts of the same plant and between the same parts at different ages as to render difficult the establishment of a factor for constant alkaloidal content in a particular strain of plant. No final conclusions may be drawn from these results as to the occurrence of distinct genetic races possessing a high alkaloidal content, it is certain that the environment exerts a marked influence upon alkaloidal yield of the plants and more detailed and carefully designed experiments will be necessary to elucidate the matter.

Manures and Soils. In analysing the results obtained by manurial treatments, three distinct objectives must be considered; they are (1) the weight of the plant material produced, (2) the assay, or alkaloidal content, of that material and (3) the absolute weight of alkaloid produced by the plant. To consider the assay value alone may be to obtain a false impression of the value of the treatment under investigation. Thus decrease in the dry weight of a leaf containing an unchanged weight of alkaloid will result in an increased assay figure, but to interpret such a result as an increase in alkaloid production is wrong. In consequence many modern results are expressed in terms of total weight of alkaloid rather than as a percentage of the dry weight.

(1) *Weight of Plant Material.* A number of workers have shown

that the application of manures, especially those containing nitrogen, resulted in increased growth and weight of belladonna plants. Thus Ransom and Henderson[30] obtained the following weights of green tops per acre: control (shade) 4 tons, control (sun) 7½ tons, sodium nitrate (1 cwt. per acre) 8¾ tons, mixed fertiliser (17 cwts. per acre) 17¾ tons. Stillings and Laurie[18] obtained an increase in crop yield by the use of high concentrations of balanced fertilisers containing nitrogen, potassium and phosphorus, whilst Brewer and Laurie[31] found that high levels of nitrogen manuring were necessary to produce heavy crops, but low levels of potasssium and phosphorus were satisfactory. Similar results have been obtained by Dafert[32] and by Dafert and Himmelbaur[33], and different organic or inorganic sources of nitrogen appear equally suitable. James[34] has shown that ammonium sulphate 1 cwt. per acre increased the weight of tops, but that heavier applications of the fertiliser decreased the yield. The following table by James indicates the effects of different nitrogenous fertilisers.

TABLE I

ATROPA BELLADONNA.—EFFECTS OF NITROGENOUS FERTILISERS ON DRY WEIGHT (JAMES)

Treatment	Dry weight per plant (g.)		
	Leaf	Stem	Root
Control	9·8	4·9	23·1
Nitro-chalk...	23·2	11·9	21·7
Ammonium sulphate	23·6	10·4	22·3
Sodium nitrate	20·9	11·9	35·1
Dried blood	23·2	10·7	25·0

By withdrawing essential elements from balanced sand cultures James has shown that potassium, calcium and phosphorus exert a slight positive influence on the crop yield, calcium withdrawal caused poor root formation and potassium withdrawal resulted in poor leaf development.

(2) *Alkaloidal Assay.* Contradictory evidence exists as to the ability of nitrogenous or other fertilisers to increase the percentage alkaloidal content of belladonna. Carr and Reynolds[11] found that all manurial treatments (kainit, basic slag, farmyard manure, sodium nitrate or superphosphates) produced lower assays than the controls. Ransom and Henderson[30] obtained similar results using different commercial fertilisers and Carr[12] found no change in assay of tops when farmyard manure or calcium cyanamide were used as sources of nitrogen, but nitrates (2 cwt. per acre) produced a lower assay. On the other hand Chevalier[35] produced leaves with 0·76 per cent. of alkaloid (control 0·32 per cent.) using farmyard manure and nitrates: Boshart[36] also Torricelli[37] have shown that nitrogenous fertilisers increase the amount of alkaloid present. Brewer and Laurie[31] applied nitrogenous fertilisers to the plants in the vegetative stage and noted an increase in alkaloidal content and De Conno[38] found that nitrogenous fertilisers, especially ammonium nitrate, increased the total alkaloidal content of the root. Cromwell[39] found an

increase in alkaloidal content in plants treated with ammonium sulphate but no increase when calcium or potassium nitrates were employed. Using pot cultures James[27,28,29] found increases in alkaloidal content of plants treated with different nitrogenous fertilisers; similar but less pronounced results were obtained in field experiments, these are shown by Tables II and III.

TABLE II

ATROPA BELLADONNA—EFFECTS OF NITROGENOUS FERTILISERS ON ALKALOIDAL CONTENTS

POT CULTURES (JAMES)

Treatment	Assay (per cent.)			Alkaloids per plant (mg.)			
	Leaf	Stem	Root	Leaf	Stem	Root	Whole plant
Control	0·21	0·21	0·31	20·8	10·3	71·6	102·7
Nitro-chalk	0·29	0·24	0·45	67·7	28·3	94·6	190·6
Ammonium sulphate	0·30	0·27	0·42	70·8	28·1	83·8	192·7
Sodium nitrate ...	0·40	0·28	0·39	83·6	33·3	136·9	253·8
Dried blood ...	0·42	0·24	0·53	97·5	25·6	132·4	255·5

TABLE III

ATROPA BELLADONNA—EFFECTS OF NITROGENOUS FERTILISERS ON ALKALOIDAL CONTENTS OF TOPS

POT CULTURES AND FIELD PLOTS (JAMES)

	Control	Ammonium sulphate	Sodium nitrate
Dry weight per plant (g.) :—			
Pot culture	9·8	23·6	20·9
Field plot	104·3	109·1	137·3
Assay (per cent.) :—			
Pot culture	0·21	0·30	0·40
Field plot	0·63	0·71	0·59
Alkaloids per plant (mg.) :—			
Pot culture	20·8	70·8	83·6
Field plot	650·8	773·1	811·4

Added potash salts produced a slight decrease in alkaloidal content or had no effect (Boshart[36], Carr[12], Chevalier[35], James[34]). Phosphatic manures produced a slight increase in alkaloids (James, Carr) or had no effect (Chevalier), and liming produced a slight increase in alkaloids (James).

(3) *Total Alkaloids per plant.* Tables I, II and III show that nitrogenous fertilisers produce considerable increases in dry weight per plant, some increase in assay and hence considerably increased weight of alkaloid per plant. James has also shown that a slight increase in total weight of alkaloid has resulted from liming or from phosphatic manuring but no change resulted from potassic manuring.

(4) *Soils.* A number of conflicting results described above may be due to differences in soils, whilst Table III shows a marked contrast between the results of pot cultures and field experiments. Carr and Reynolds[11] indicated that soil variations produce belladonna plants of different activities. Warin[42] found that the alkaloidal contents of extracts of belladonna

prepared from plants grown on chalky soil were greater than from plants on sandy soil or on argillaceous soil. Torricelli[37] reported that dried tops from plants grown in damp clay loam contained 0·3 per cent. of alkaloids, but when grown in light sandy soil they contained 1·0 per cent. Stillings and Laurie[18] have shown that a soil *p*H of 5·5 to 6·5 is optimum for alkaloid production whilst Sukhorukov[43] also stressed the influence of soil *p*H upon the accumulation of alkaloid in the plant.

We may thus conclude that a light calcareous soil with abundant balanced nitrogenous manuring during plant growth are essential for the production of a good belladonna crop possessing high alkaloidal activity.

Climate and Season. Marked variation occurs in the alkaloidal contents of belladonna crops grown under identical cultural conditions in different years[11,12,25,26,27]. Average temperature, sunlight and rainfall may contribute to these variations. A number of workers have shown that plants grown in full sunlight are richer in alkaloid and more luxuriant in growth than those grown in the shade, a total increase in yield of alkaloid per acre up to eightfold being recorded[30,44,45,46]. Carr[12] compared assay figures of commercial crops with meteorological records during the growing seasons for six years and found the lowest percentage assays occurred in years that were either very wet or very dull. Burmann[47] compared the assays of the harvested crop with the average diurnal temperature (4 observations per day) throughout growth and found a positive correlation between the values over a period of 5 years; he concluded that the cloudiness of rainy weather influenced the potency rather than the rainfall itself[48]. More recently Runge[49] has suggested that the alkaloidal content of a sample is dependent on climatic conditions throughout growth, being higher after warm sunny weather. This suggestion summarises the situation; insufficient statistics are available to determine the detailed individual significance of light, temperature and rainfall.

The two following experiments are of interest, but are not readily correlated with the foregoing observations. During three growing seasons Carr[12] shaded certain plants with white, green and red muslins and found the average assays of tops to be: control 0·51 per cent., under white muslin 0·51 per cent., red 0·68 per cent., green 0·77 per cent. Ripert[50] covered certain root crowns with cartons during March and allowed the shoots to grow in the dark for 84 days, the blanched leaves and stems were much richer in alkaloid (0·9 per cent.) than those of controls (0·5 per cent.), whilst the roots of blanched plants (0·21 per cent.) contained less alkaloid than the controls (0·24 per cent.). Exposure of the blanched tops to daylight for 9 days resulted in some decrease in alkaloidal content (0·75 per cent.).

Collection and Drying. Much evidence exists to show that the highest assay is found in tops at the time of flowering[31,36,24,25,26,27,28,51], although a number of workers have found little difference in the assay of leaves collected from May to September[12,42,49]. The analyses of Runge[49] for alkaloids in all aerial parts harvested at different times were: —April 30th 0·63 per cent., July 2nd 0·64 per cent., early August 0·71 per cent., September 26th 0·61 per cent. Commercial growers harvest three crops

annually (May, August and early October) from plants in their second and subsequent years of growth; thus three cuttings of actively growing leaves and young flowering tops are obtained. There appears to be little variation in alkaloidal content of roots throughout the year or over a period of several years[12,52]. Rapid drying at 50° to 60°C. in a free current of air or at 30°C. *in vacuo* prevents loss of alkaloid by enzyme activity, whilst Flück has shown that the enzymes responsible for alkaloid degeneration may be destroyed by heating at 100°C. for 15 minutes before drying is commenced with improved alkaloidal content of the product[53,54]. No differences in assay have been found between sun-dried or shade-dried leaves, but shade drying is preferred since such leaves retain their green colour[55,56]. Brewer and Laurie[31] dried entire plants and found that during the process there was a migration of alkaloids from the root to the foliage, and when only the aerial parts were dried the alkaloid initially present in the stem passed into the leaves.

Structural Characters

Aerial Parts. The anatomical and histological characters of the leaf, stem, flower, immature fruit and immature seed of *Atropa belladonna* are described briefly in the monograph of the British Pharmacopœia 1948 for Belladonna Herb. Formerly the leaves only were official and details of their macroscopical and microscopical characters are found in the textbooks of pharmacognosy or in the anatomical atlases[57,58,59,60,61,62,63]. The histological characters of the stem are described by Moll and Janssonius[64], but no detailed drawings are given; the striated cuticle, cruciferous stomata and protective trichomes of the epidermis, also the idioblasts of sandy crystals of calcium oxalate found in the cortex, phloem and pith are similar to those of the leaf; phloem, cambium and xylem form a continuous cylinder, the pitted vessels of the xylem being 50μ in diameter; perimedullary groups of phloem are present; small groups of fibres are present in the pericycle and also adjacent to the perimedullary groups of phloem. A description of the histology of belladonna flower is given by Moll and Janssonius and the structures, including the immature fruit and seed, are figured and described by Wallis and Butterfield[65]. Figure 2, taken from that paper, shows the characters of the powdered flowers, the chief diagnostic characters of which are the fibrous layer of the anther wall, subspherical tricolpate pollen grains about 40μ in diameter, papillose inner epidermal cells from the upper part of the corolla, thickened epidermal cells from the lower part of the corolla and pigment present in a number of the corolla cells.

Subterranean Organs. The morphology, anatomy and histology of belladonna root and rhizome have been described in pharmacognostical literature and the characters are summarised in the British Pharmacopœia 1948 for the root and rootstock which are official. C. Melville [66] has prepared detailed descriptions and sketches of the histological characters of the root, rootstock, stem bases and stolon. The root has a well-marked cork and phelloderm; wide, parenchymatous secondary phloem; large parenchymatous xylem with radially arranged scattered groups of

xylem vessels, tracheids and fibres and with scattered groups of interxylary phloem; the central primary xylem is diarch. The parenchymatous cells are filled with starch grains and idioblasts of sandy crystals of calcium oxalate occur; in older roots a concentric zonation of the xylem may occur. The rootstock may be up to 15 cm. in diameter; the broad xylem is pronouncedly thickened as a dense woody cylinder with lignified xylem parenchyma, groups of vessels, often with groups of interxylary phloem on the inner side, and an abundance of fibres forming the greater

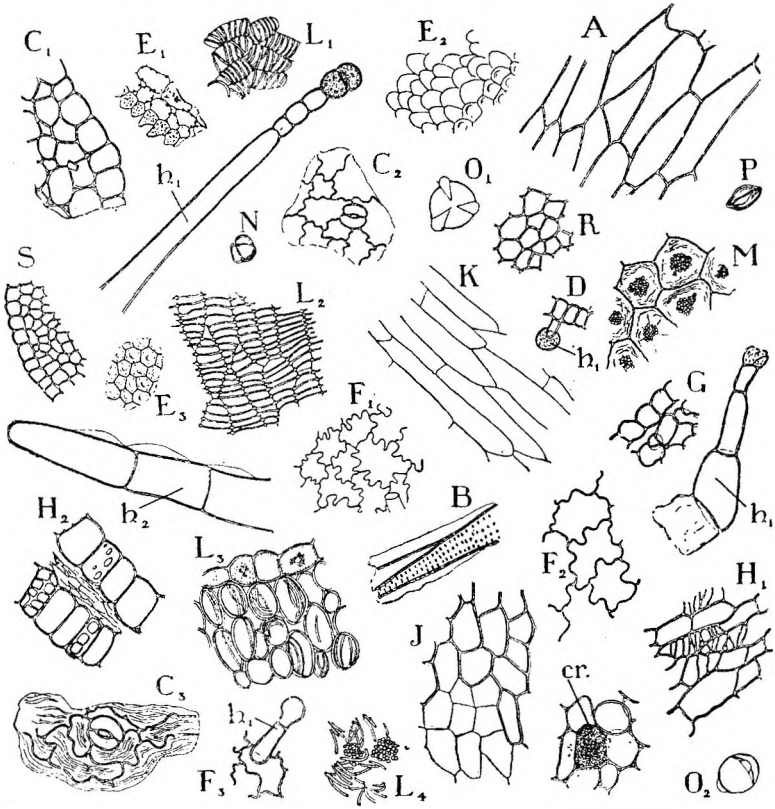

FIG. 2. *Atropa Belladonna* Linn., powdered flower. A, Epidermis of pedicel. B, Vessel from pedicel. C_1, Section of epidermis and mesophyll of calyx. C_2, Epidermis of calyx. C_3, Epidermis of calyx showing cuticular striations. D, Section of epidermis of calyx. E_1, Section of papillose epidermis of corolla. E_2 and E_3, Papillose epidermal cells of corolla. F_1, F_2 and F_3, Outer epidermis from upper parts of the corolla. G, Section of non-papillose epidermis of inner side of corolla. H_1, Thickened epidermal cells from base of corolla. H_2, Section of epidermis from base of corolla. J, Transitional region between unthickened and thickened cells near the base of the corolla. K, Epidermis from filament. L_1 and L_2, Fibrous layer of anther wall in surface view. L_3, Longitudinal section through wall of anther-lobe. L_4, Broken spirals from fibrous layer and chloroplasts from the epidermis of the anther. M, Epidermis of anther lobes. N, Young pollen grain. O_1, Pollen grain in polar view. O_2, Pollen grain from the side. P, Abortive pollen grain. R, Epidermis from ovary wall. S, Fragment of ovule. *cr.*, idioblast containing microsphenoidal crystals of calcium oxalate. h_1, glandular trichomes from calyx, pedicel and outer surface of corolla; h_2, covering trichome from inner surface of corolla or base of filament. All × 140. (Wallis and Butterfield.)

part of the tissue; concentric zones of unlignified xylem parenchyma are also found in the older rootstocks; a perimedullary phloem is present and also a central pith which may be lacunar; groups of pericyclic fibres are, at times, present. The vessel elements of the rootstock are shorter, and those of the stem bases are longer and narrower, than those

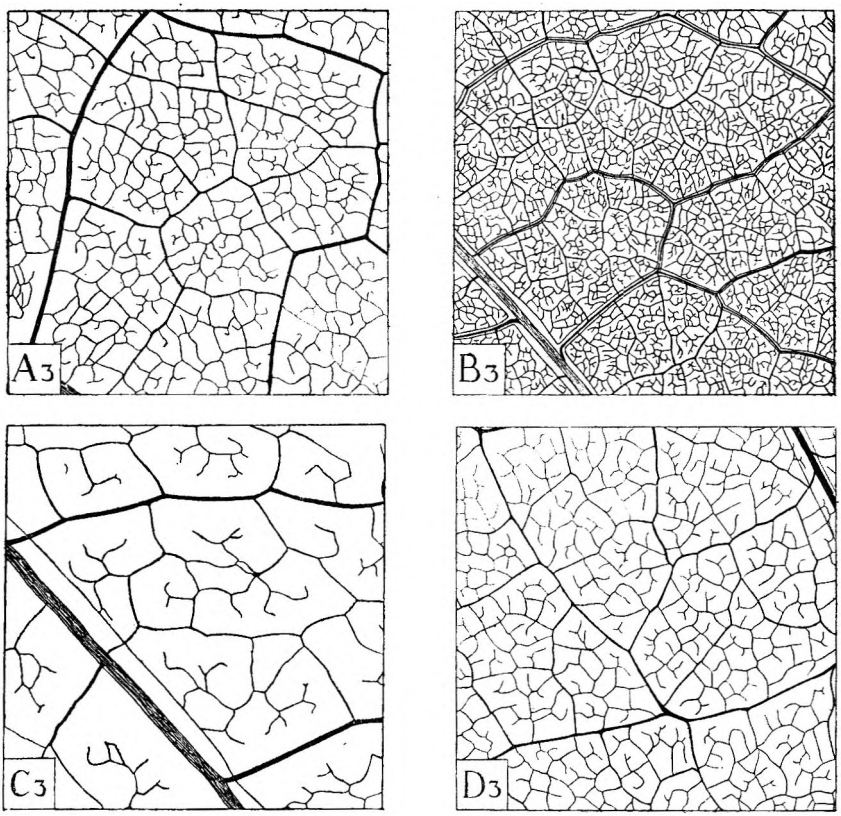

FIG. 3. Vein islets in cleared leaves; A3, *Atropa belladonna;* B3, *Ailanthus glandulosa;* C3, *Phytolacca decandra;* D3, *Solanum nigrum.* All × 8. (Forsdike.)

of the root; the vessels of the stolon are similar to those of the root. Xylem fibres are more abundant in both rootstock and stem bases than in the root.

Numerical Values. These methods have been developed in recent years for the characterisation of certain drugs and also, at times, to distinguish between a number of drugs possessing similar characters.

The following values have been recorded for belladonna: —

(1) *Palisade Ratio* of leaf: —

6 to 10 (Wallis and Forsdike[67]),
5·6 (Bogarosh[69]),
6 to 10 (Boswijk[71]),
6·5 to 7·25 (Feinstein and Slama[68]),
1·6 to **6·0** to 10·4 (George[70]),
4·5 to **6·9** to 9·2 (Youngken and Hassan[72]).

(2) *Stomatal Number* per square mm. of leaf epidermis: —

Upper Surface	*Lower Surface*	
60	150	(Moll and Janssonius[64])
7 to 18	77 to 177	(Rowson[73]),
0 to 50	75 to 140	(Boswijk[71]).

These values are very variable within the species and are of little diagnostic use.

(3) *Stomatal Index* for lower surface of leaf is 19 to 24 (Rowson[73,74]).

(4) *Vein Islet Number*. Forsdike[75] has used the appearance of these islets in cleared leaves to distinguish between belladonna, ailanthus, *Phytolacca decandra* and *Solanum nigrum*. Figure 3 shows the leaves of these four species at the same magnification.

(5) *Cork Cells* per square mm. of root surface (when in 22/44 powder) is 400 to 600 (C. Melville[76]).

(6) *Vessel Index* (the percentage of vessels wider than 135μ in the root when in 22/44 powder) is 0 to **0·5** to 2·35 (C. Melville[76]).

Boswijk[71] recorded the number of idioblasts of calcium oxalate in 1 square mm. of leaf as 9 to 40 but considered the figure too variable to be of diagnostic importance.

Belladonna Alkaloids and their Biogenesis

The Alkaloidal Mixture. Good samples of dried belladonna herb contain 0·25 to 0·9 per cent., the dried root 0·3 to 1·0 per cent., of total alkaloids, of which *l*-hyoscyamine is the main component, occurring along with small amounts of *l*-hyoscine and of optically inactive atropine (*dl*-hyoscyamine). Earlier workers [77,78,79] found that racemic atropine rather than *l*-hyoscyamine was the dominant alkaloid in some material and concluded that samples varied considerably in the proportional contents of these two alkaloids. More recent critical methods for the quantitative separation of *l*-hyoscyamine, atropine and *l*-hyoscine have been developed[80,81,82,83] and they have shown that the alkaloidal mixture from belladonna root may contain 0 to 26 per cent. of atropine, and from the tops 0 to 40 per cent. of atropine. There is strong evidence to suggest that in the actively growing plant atropine is absent but as the plant becomes less vigorous some of the *l*-hyoscyamine is racemised to atropine. Kuhn and Schäffer found two maxima of total alkaloid production and of hyoscyamine content in all parts of the plant, the first occurring at the time of flowering, the second occurring towards the end of the growing season. The roots were found to show the greatest dominance of hyoscyamine at these two maxima, but in winter the proportion of hyoscyamine in the total alkaloidal mixture from the roots fell to 33 per cent. Racemisation also occurs if the drying process is protracted and in order to ensure the presence of the more active *l*-hyoscyamine the actively growing tops should be carefully and quickly dried immediately after collection. The hyoscine content of the total alkaloidal mixture has been found to be remarkably constant between 5 and 11 per cent.[83]. Mesnard[84] has examined the racemisation of *l*-hyoscyamine in galenical preparations

and considered the rate to be much slower than would be anticipated from a knowledge of the *p*H of the solution; this retardation he attributed to the presence of resins in the extractive matter.

Genetical Control. Under the heading of Plant Selection we have already seen that the great variations in alkaloidal content due to climate, soil and age of collection of plants have precluded a critical analysis of the possible existence of a gene for high alkaloid production in belladonna. The remarkable constancy of the proportion of hyoscine to total alkaloid is of interest in this connection for it may indicate an inherent character of the plant which maintains such a balanced production.

A study of the influence of colchicine on belladonna seeds or stem apices has shown that tetraploid plants or branch chimeras may be produced. Such plants are characterised by having in each cell nucleus twice the number of chromosomes present in the normal diploid plant ($2n = 72$); in consequence of the increased nuclear volume the pollen grains, stomata and other cells are also larger in size. The physiological balance of such plants is changed and both Szomolanyi[85] and Rowson[86] have reported increased potential for alkaloid production. A maximum of 153 per cent. increase in alkaloidal content of a 2nd year tetraploid plant has been recorded with an average increase of 93 per cent.[86]. From these results it has been concluded that the ceiling of production of alkaloid is determined by the nuclear complement but that the factors of environment determine the achievement of this production[87].

Distribution. Histochemical examination of living material has shown that the alkaloids occur only in living cells and are most abundant in metabolically active tissues, e.g. young cortical and pith cells, endodermis, bundle parenchyma and phellogen. Alkaloids do not occur in vessels, fibres, sieve tubes, collenchyma or the pith of older stems but are found in the xylem storage parenchyma of mature roots: they appear to be stored in the vacuole of each cell[27,28,88]. In the berries alkaloids have been found in the fruit wall, placenta and seed coat. The removal of the testa does not prevent germination and James found alkaloids formed by the actively metabolising cells of the young roots and stem apices in seedlings when about 3 weeks old[89,90].

A typical analysis[25,37,49,91] of the percentage alkaloidal distribution in the dried aerial growth of belladonna is:—leaf 0·9, young stems 0·5, old woody stem 0·1 to 0·4, unripe fruit 0·8, ripe fruit 0·6, seed 0·4. The incorporation of reasonable quantities of stem in belladonna tops is thus permissible without adversely affecting the total alkaloidal content of the official drug.

Locus of Synthesis and Translocation. For many years it has been tacitly assumed that the leaf was the site of synthesis of alkaloids and that there was no large-scale transportation of alkaloid throughout the plant. Kuntz[92] showed that the percentage of alkaloid gradually increased in root and seed throughout the growing season, it steadily decreased in leaf and stem, but there was no marked downward migra-

tion of alkaloids towards the root at the end of the growing period. Sukharukov[43] found very little evidence of alkaloid diffusion throughout the growth of the plant.

Recent investigations by means of reciprocal grafts have shown that for a number of species of the Solanaceae, including *Atropa belladonna*, the region of alkaloid production in the plant is the root[27,28,40,87,93,94]. When belladonna stems are grafted onto tomato roots as stock the aerial parts, after growth, are free from mydriatic alkaloids, but in reciprocal grafts the aerial parts of tomato grown upon belladonna roots contain abundant mydriatic alkaloids. In grafts between belladonna and tobacco the aerial parts of belladonna contained nicotine, and only traces of atropine, when granted on tobacco roots. Grafts between belladonna and potato have also been produced, and in every instance the nature of the root has determined the presence or absence of alkaloids in the aerial parts, thus indicating that the alkaloids themselves or their immediate precursors are synthesised in the roots. Stem exudations from the cut xylem of actively growing belladonna plants have been shown to contain hyoscyamine in the ascending transpiration stream, and this indicates that the alkaloids themselves are synthesised in the root, the method of translocation to the leaves being in the transpiration stream of the xylem. The finding by Stillings and Laurie[18] of the migration of alkaloids from root and stems into the leaf during the drying of entire plants, to which reference has been made previously (p. 207), supports this fact, although the authors make no comment thereon. It also explains the observation of Rosenkranz[95], amongst others, that in the leaf the alkaloids appear to reside near the veins and midrib. The entire absence of alkaloids from the sieve tubes supports the conclusion that there is no translocation of alkaloids in the downwards direction from leaf to root.

Biogenesis. Histochemical investigation, sand culture or manurial experiments have endeavoured to show a parallelism between the production of proteins and of alkaloids in the living plant[27,28,29,39,40,96,97]. Soluble nitrogenous bodies such as the amines may to some extent be diverted from protein synthesis to alkaloid synthesis, alternatively these fractions used in alkaloid production may be derived from protein breakdown. Cromwell concluded that the alkaloids of belladonna were built up from the products of protein breakdown and carbohydrate intermediaries. Robinson[98,99] synthesised tropinone from succindialdehyde, methylamine and acetone-dicarboxylic acid; he considered that the succindialdehyde and the methylamine may arise in the living tissue from the interaction of formaldehyde and ornithine, and acetone-dicarboxylic acid from citric acid. Cromwell made stump injections of 18 different nitrogenous substances into belladonna plants and found significant increases in alkaloidal contents when using putrescine; he also isolated putrescine from fresh belladonna plants and concluded that this substance was an intermediate in the synthesis of hyoscyamine, being derived by deamination from arginine. An enzyme capable of oxidising putrescine, with the production of ammonia and an aldehyde, has been isolated from belladonna

root and Cromwell hypothesised the production of succindialdehyde, required for the Robinson synthesis, as:

Direct synthesis

arginine—ornithine→putrescine→succindialdehyde.

Protein breakdown

Cromwell also hypothesised a synthesis of scopine and hence of hyoscine and of the volatile base N-methyl-pyrroline.

James[97] fed isolated leaves with solutions of different amino acids and found small but significant increases in weight of alkaloid when *l*-arginine and *l*-ornithine were used, and concluded that some *in situ* synthesis of alkaloid occurred in the leaf. The enzyme arginase, capable of converting *l*-arginine into *l*-ornithine and urea, also free ornithine have been isolated from belladonna. Since *l*-arginine is a universal constituent of plant proteins, and since alkaloid production can occur simultaneously with protein breakdown, James concluded that *l*-arginine and *l*-ornithine can act as precursors of the tropane alkaloids.

Dawson[100] has discussed the work outlined above, and considered Robinson's "model" synthesis to be improbable in nature; James' evidence for synthesis in the leaf he rejected as too slender in comparison with that from graft experiments, and hence the conclusions drawn from isolated leaves are invalidated. The presence of the arginase enzyme system and the isolation of certain amino acids from belladonna were only considered as very remote evidence of their relationship to alkaloidal synthesis since they are found in the tops of belladonna where no synthesis occurs, moreover they are normally geared to protein synthesis and are found in many plant species which do not produce alkaloids.

Varieties

A number of strains of belladonna have been noted by James[25,26], differing in plant habit, in the development of the suppressed axillary shoots, colour of corolla, and in the size and shape of mature fruit, but full descriptions of these strains have not been published as yet. A yellow flowered and yellow berried variety of the species is also well known[26,86,101].

The claims made for Bulgarian belladonna root in the treatment of Parkinsonism resulted in a full investigation of the anatomy and chemistry of the root which was shown to be identical with that of *Atropa belladonna* grown in England[102,103,104,105].

Indian Belladonna. The taxonomy of this drug has been a problem for many years; *Atropa belladonna* is known to grow in India and to be used commercially[106,107,108]. In 1917 and 1918 Holmes drew attention to the occurrence of a distinct commercial drug derived from wild plants of *Atropa lutescens*, formerly regarded as a variety of *A. belladonna*[109,110]. Wartime shortages of European belladonna during the last decade led to renewed interest in this drug[111,112] and monographs in the 5th and 7th Addenda to the British Pharmacopœia 1932, and in the British Pharmacopœia 1948 defined Belladonna Herb and Belladonna Root as derived from either the European or the Indian plants.

The " Index Kewensis " equates *A. acuminata* Royle and *A. lutescens* Jacquemont to *A. belladonna*. R. Melville[113] has shown the species of Royle and Jacquemont to be identical, the name *A. acuminata* taking precedence. Melville has indicated the differences between that species and *A. belladonna* and considers these sufficient to regard the Indian material as a separate species. A detailed investigation of the anatomy of the subterranean organs of *A. acuminata* has been carried out by C. Melville[114]. The numerical values in Table IV indicate the differences between the Indian and European drugs:—

TABLE IV

Value	*A. belladonna*	*A. acuminata*	Author
Vessel Index (Root No. 22/44 Powder)	0 to 2·35	11·2 to 14·3	Melville[76]
Cork Cells Number (Root No. 22/44 Powder) ...	400 to 600	265 to 371	,,
Palisade Ratio (Leaf)	1·6 to 10·4	2·2 to 13·5	George[70]
Stomatal Index (Leaf, lower surface)	19 to 24	16 to 18	Rowson[73,74]

Conclusions

A discussion of recent advances in our knowledge of the pharmacognosy of belladonna has been presented under the four main headings of Cultivation, Structural characters, Alkaloids and their biogenesis, Varieties. The conclusions which may be drawn from the evidence available have been recorded along with the discussions in each section of the work and the following results arising from modern research work have been presented:—

(1) The breaking of dormancy in seeds by chilling, fluctuations in temperature or acid treatment.

(2) The slight effect of calcium, potassium and phosphorus upon yield of plant matter and of alkaloid in comparison with the marked effect of nitrogenous manuring of the growing plant.

(3) The importance of collecting actively growing aerial parts and the rapid drying of leaf, stems and roots in order to obtain maximum content of *l*-hyoscyamine in the crude drugs.

(4) Anatomical characters of the flower, immature fruit, stem, rootstock, root and stolon and the use of numerical values to characterise belladonna leaf and root.

(5) The influence of chromosome number of the nucleus upon the potentiality of the plant for alkaloid production.

(6) Alkaloids are produced by the plant in the root only and move to the aerial parts in the transpiration stream. There is no downward translocation of alkaloids in the phloem.

(7) The use of putrescine and arginine by the plant as intermediates in the biosynthesis of hyoscyamine has been suggested but the evidence is not conclusive.

(8) The taxonomy, numerical characters of leaf or root, and the anatomy of the subterranean organs of Indian Belladonna have been discussed.

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RESEARCH PAPERS

STUDIES OF RICINOLEIC ACID AND A TURBIDIMETRIC METHOD OF EVALUATING THE BACTERICIDAL ACTION OF SOLUTIONS OF PHENOLS IN POTASSIUM RICINOLEATE

PART I. THE EFFECTS OF HEAT AND AGEING UPON RICINOLEIC ACID

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THE object of these studies was threefold: (1) To trace the effects of heat on the acid value of samples of the fatty acids, obtained by hydrolysis of castor oil, and known as Ricinoleic Acid B.P. (2) To ascertain the value of a nephelometric method of evaluating bactericidal values, from the point of view of the reproducibility of results. (3) The application of this method to investigate the effects of using ricinoleic acids of different acid values to solubilise insoluble phenols in potassium ricinoleate solutions.

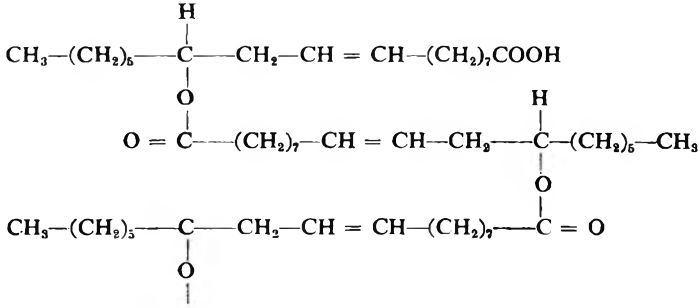
INTRODUCTION.

The objects of the work described in Part I were to prepare a ricinoleic acid of high acid value, to trace the effects of heats and ageing on this acid, to investigate the relationship between refractive index and acid value, and to prepare a series of potassium soaps of ricinoleic acid for further investigations. Work by Maquenne[1], Goldsobel[2], and Vernon and Ross[3] has established the formula of ricinoleic acid as

$$CH_3(CH_2)_5CH(OH).CH_2CH:CH(CH_2)_7.COOH.$$

Several workers, Koszowski[4], Rider[5], and Valette and Salvanet[6], and Brown and Green[7] have described attempts to obtain a pure sample of the acid. The latter two workers concluded that even by using a pure methyl ester it was difficult to obtain an acid of more than 95 per cent. purity. They attributed this difficulty to the ease with which ricinoleic acid forms polyricinoleic acids.

This formation of polyricinoleic acid has been described by Chowdhury *et al.*[8], Jakes and Hökl[9], and Esafor and Shpadi[10] and the explanation is that the COOH group of one molecule reacts with the OH group of another molecule to give a chain-like formation of from 2 to 6 molecules of ricinoleic acid, i.e.



As a result of the formation of these poly-acids or estolides the acid value of ricinoleic acid will be lower than that of a pure ricinoleic acid. This estolide formation would also explain the variation of acid value in commercial samples of ricinoleic acid and the drop in acid value which occurs when samples of ricinoleic acid are stored. Esafor and Shpadi[10] reported that these estolides were formed when ricinoleic acid was heated at under 200°C., but that prolonged or more vigorous heating resulted in the breakdown of the estolides and the formation of other polymerisation and thermal degradation products.

Experimental

1. Preparation of a Ricinoleic Acid of High Acid Value

Since heat promotes the formation of estolides of ricinoleic acid a method of preparing the acid in the cold was attempted. The method adopted was the saponification of castor oil to give potassium ricinoleate and the conversion of this soap to ricinoleic acid with hydrochloric acid. A supply of castor oil was stored in a refrigerator and from time to time samples of this oil were taken and filtered free of any solid particles and used in the experiments.

A refrigeration temperature of 4°C., however, introduced a complication in that at such a temperature the acid formed a semi-solid from which the last traces of moisture were difficult to remove. The solubilising power of the soap (Berry and Bean[11]) also presented a difficulty in that, if alcoholic potassium hydroxide was used in the preparation, some of the alcohol would be solubilised by the soap and find its way into the final acid from which it would be difficult to remove except by the use of heat. This solubilising effect also hindered the removal of the unsaponifiable matter since any solvent used in its extraction would also be solubilised by the soap.

The acidification of the soap with hydrochloric acid in the cold resulted in a small portion of the soap remaining unchanged, with the result that the process of washing the acid free of chloride produced extremely stable emulsions from which the acid was difficult to separate.

The scheme evolved was to prepare acids in the cold (a) making no effort to remove the unsaponifiable matter, using aqueous potassium hydroxide (b) in a similar manner using aqueous and alcoholic potassium hydroxide and removing the unsaponifiable matter, and then to repeat the experiments using a minimum amount of heat.

Acid values were determined by the method of the British Pharmacopœia 1932.

(a) *Cold Aqueous Potassium Hydroxide; Unsaponifiable Matter not Removed.* 6 g. of potassium of hydroxide was dissolved in 75 ml. of distilled water and mixed with 25 g. of castor oil; the mixture was stored at 4°C. and shaken frequently. Even after 6 months' storage at 4°C. a considerable portion of the oil remained unsaponified in this mixture. A portion of the mixture, after 3 weeks' storage, was filtered through a filter paper wetted with distilled water, which served to remove the majority of the unsaponified oil and gave a filtrate with only slight opalescence. The filtrate was acidified with hydrochloric acid, thoroughly

shaken and left for 48 hours for the acid to separate. This mixture separated into 3 distinct layers, a clear amber layer of acid, a layer of thick white emulsion and a lower aqueous layer. The acid layer was separated off and washed with distilled water until the washings were free from chloride. An emulsion layer formed at each washing, and considerable amounts of acid were lost in discarding this layer each time. The acid was dried by gentle heat under reduced pressure. Acid value of product, 168·6.

(b) *Cold Aqueous Potassium Hydroxide; Unsaponifiable Matter Removed.* A portion (25 ml.) of the soap solution from experiment (a) was placed in a separator, 25 ml. of ether were added and the majority of this was solubilised by the soap so a further 25 ml. were added, after shaking and allowing to stand the mixture separated into 3 layers and the emulsion layer was discarded with the ethereal extract, further portions of ether were added to complete the extraction of the unsaponifiable matter. The ether solubilised by the soap solution was removed by gentle warming under reduced pressure. Addition of hydrochloric acid converted the soap to acid which was washed with water and dried with anhydrous sodium sulphate. Acid value of product, 166·5.

(c) *Cold Alcoholic Potassium Hydroxide; Unsaponifiable Matter Removed.* 25 g. of castor oil was mixed with 75 ml. of 95 per cent. alcohol in which 6 g. of potassium hydroxide was dissolved, and the mixture was left at 4°C., and shaken frequently, for 7 days. The unsaponifiable portion was extracted with light petroleum (this being less soluble in water than ether, separation of the layers was easier), 6 quantities, each of 25 ml., being used. The resulting soap solution was divided into 2 portions. The first was treated with hydrochloric acid and left at room temperature for several days, then the liberated ricinoleic acid was washed free of chloride and dried with anhydrous sodium sulphate. Acid value of product, 180·8.

The second portion was refluxed for 30 minutes with hydrochloric acid. The liberated ricinoleic acid was washed and dried at 100°C. under reduced pressure. Acid value of product, 174·7.

(d) *Hot Aqueous Potash; Unsaponifiable Matter not Removed.* 50 g. of castor oil was refluxed for 2 hours with 150 ml. of aqueous potassium hydroxide when saponification was shown to be complete by the absence of an oily layer. The resulting soap solution was divided into 2 equal portions. The first was acidified with hydrochloric acid and refluxed for 30 minutes. The resulting acid was separated, washed free of chloride and dried at 100°C. Acid value of product, 165·7.

The remainder of the soap solution was acidified with hydrochloric acid at 0°C. and kept at 4°C. for 7 days. The ricinoleic acid so formed was washed free of chloride and dried with anhydrous sodium sulphate. Acid value of product, 164·8.

(e) *Hot Alcoholic Potassium Hydroxide; Unsaponifiable Matter not Removed.* When alcoholic potassium hydroxide was used, refluxing for 30 minutes was sufficient to complete the saponification. As in experiment (d) the soap solution was divided into two portions, both

of which were converted to acid, one by 30 minutes' refluxing and the other in the cold. Acid value of acid obtained, by refluxing 181·6; in the cold 164·8.

An attempt was made to repeat these results. Acid value of acid obtained, by refluxing 157·9; in the cold 179·5.

(f) *Hot Alcoholic Potassium Hydroxide; Unsaponifiable Matter Removed.* A soap solution was prepared as above (e) and the unsaponifiable matter extracted with 6 lots of light petroleum. As in the previous experiments, half the soap solution was acidified with hydrochloric acid, refluxed for 30 minutes and then the liberated acid separated off, washed free of chloride and dried at 100°C.; the second half of the soap solution after acidification was left at room temperature for 7 days before the liberated acid was washed and dried with anhydrous sodium sulphate. Acid value of acid prepared, by refluxing 178·4; in the cold 177·8.

(g) *Summary of Acid Values.*

(i) Cold Saponification.

Aqueous potassium hydroxide, unsaponifiable matter not removed	168·6
Aqueous potassium hydroxide, unsaponifiable matter removed	166·6
Alcoholic potassium hydroxide, unsaponifiable matter removed:	
(a) cold acidification	180·8
(b) hot acidification	174·7

(ii) Hot Saponification.

Aqueous potassium hydroxide, unsaponifiable matter removed:	
(a) cold acidification	164·8
(b) hot acidification	165·7
Alcoholic potassium hydroxide, unsaponifiable matter not removed:	
(a) cold acidification	179·5
(b) hot acidification	157·9
Alcoholic potassium hydroxide, unsaponifiable matter removed:	
(a) cold acidification	177·8
(b) hot acidification	178·4

(h) *Preparation of Stock Potassium Ricinoleate Solution.* 250 g. of potassium hydroxide was dissolved in 3 l. of alcohol (90 per cent.) and 1000 g. of castor oil was added. This mixture was refluxed on a water-bath for 30 minutes. The unsaponifiable matter was removed from the soap solution with light petroleum (50° to 60°C.). The alcohol and solubilised light petroleum were removed from the soap solution by diluting it with an equal quantity of water and evaporating the resulting solution down to half its volume under reduced pressure. The resulting solution was made up to 5 l. to give an approximate 1 in 5 solution of potassium ricinoleate.

Over a period of 18 months, samples of this soap solution were taken, from time to time, and converted to ricinoleic acid by acidification with hydrochloric acid. The acid values of the acids so prepared were (a) 174·5, (b) 165·8, (c) 179·0, (d) 181·2, (e) 183·4, (f) 186·4. This latter sample was prepared by mixing the soap solution and hydrochloric acid at 0°C. and allowing the reaction to proceed at 4°C. for 14 days, with frequent shaking; after washing free from chloride it was dried by two separate treatments with anhydrous sodium sulphate.

2. Effects on Acid Value of Heating Samples of Ricinoleic Acid at 150°C. and 160°C.

Samples of some of the acids prepared above were placed in open or closed containers and subjected to a temperature of 150°C. in a hot-air oven for varying lengths of time, cooled in a desiccator and the acid values determined immediately the samples were cool. One sample in an open container was heated in an autoclave and after cooling the condensed moisture was removed, the acid was dried and then its acid value determined.

Table I is a summary of results obtained:—

TABLE I

Effect of Heat Treatment on the Acid Value of Ricinoleic Acid

Initial Acid Value	Heat Treatment			Final Acid Value
	Time (hr.)	Temperature °C.	Container	
174·5	1	150	Sealed	164·1
	3	150	Sealed	140·5
	1	150	Sealed in presence of anhydrous sodium sulphate	171·8
	3	150	Sealed in presence of anhydrous sodium sulphate	144·4
	7½	150	Sealed	133·4
	3	150	Open, in autoclave	172·7
	7½	150	Open	121·7
	8½	150	Open	93·5
	2	160	Open	137·5
165·8	1	150	Open	156·8
	2	150	Open	155·2
	3	150	Open	153·3
	4	150	Open	141·6
	5	150	Open	128·7
	6	150	Open	111·7
	7	150	Open	94·9
186·4	4½	150	Open	157·4
	4⅔	150	Open	152·1
	5⅞	150	Open	142·0
183·4	½	150	Open	184·5
	1	150	Open	182·6
	1½	150	Open	178·7
	2	150	Open	175·7
	2½	150	Open	170·2
	3	150	Open	168·0
	3½	150	Open	161·5
	4	150	Open	154·6
	4½	150	Open	150·3
	5	150	Open	144·3
	5½	160	Open	108·1

It was noted that a gradual darkening in colour accompanied the decrease in acid value of any one sample of original acid, although the acid of acid value 164·1 obtained by heating the acid of acid value 174·5

for 1 hour was considerably lighter in colour than the original acid of acid value 165·8.

3. Effects of Ageing on Acid Value

Samples of some of the acids prepared above were stored in closed containers for 12 months or more at room temperature and then their acid values were determined. Table II is a summary of the results obtained.

TABLE II

Decrease of Acid Value on Storage

Initial Acid Value	Time Months	Final Acid Value	Decrease
174·4	19	158·0	16·4
164·2	18	147·0	17·2
165·8	12	152·4	13·4
156·8	12	144·9	11·9
155·2	12	143·7	11·5
153·3	12	149·4	3·9
141·6	12	132·1	9·5
128·5	12	120·1	8·4
111·7	12	104·8	6·9
94·9	12	92·8	2·0

The logarithms of the reductions in acid value occurring after 12 months' storage were plotted against the initial acid values and a graph showing close approximation to a straight line was obtained. The long

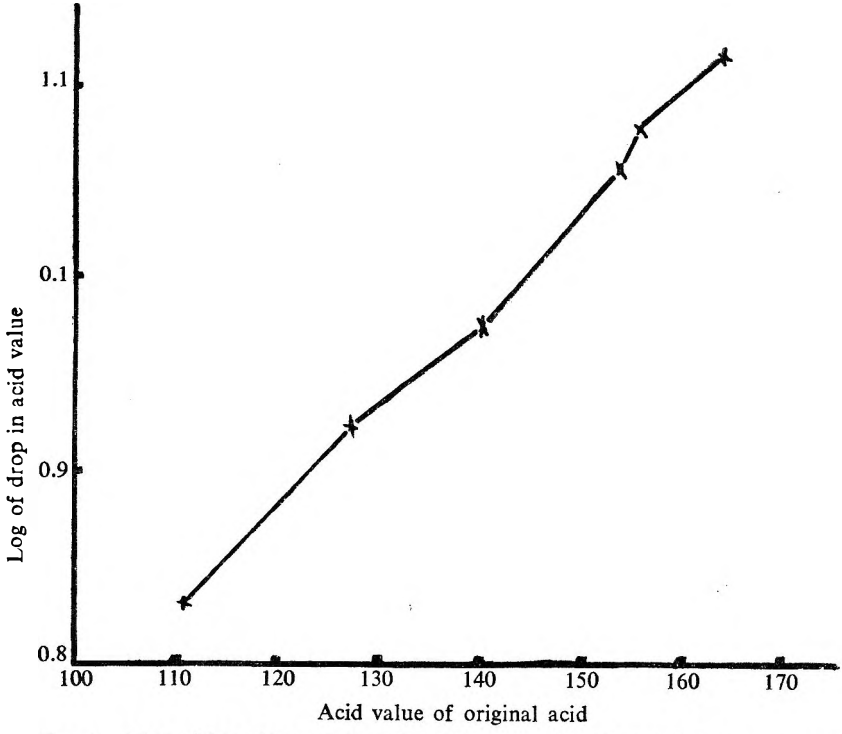


Fig. 1. Plot of logarithm of drop in acid value after 12 months' storage, against original acid value of ricinoleic acid.

period of storage, 12 months, made it difficult to obtain any confirmation of these results.

4. Refractive Index and Acid Value

The British Pharmacopœia 1932 stipulates 40°C. as the temperature for the determination of the refractive index of ricinoleic acid; this temperature was therefore maintained for these measurements, which were made with an Abbé Refractometer. The refractive indices of three series of acids were measured. The acids of each series were obtained from the same original acid, the reduction of acid value had been brought about by:—Series I—heat treatment and 18 months' ageing; Series II—heat treatment and 12 months' ageing; Series III—heat treatment only. The refractive index and acid value for each acid were determined on the same day or within three days of each other. Table III summarises the results.

TABLE III

The Results of Acid Value and Refractive Index Determinations

Series I		Series II		Series III	
Acid Value	Refractive Index	Acid Value	Refractive Index	Acid Value	Refractive Index
160·9	1·4620	152·4	1·4623	183·4	1·4611
157·9	1·4611	149·4	1·4624	184·5	1·4614
147·0	1·4613	144·9	1·4621	182·6	1·4615
		143·7	1·4630	178·7	1·4619
		132·1	1·4650	175·1	1·4621
		120·1	1·4640	170·2	1·4624
		104·8	1·4649	168·0	1·4627
		92·9	1·4650	161·5	1·4630
				154·6	1·4630
				150·3	1·4634
				144·3	1·4639
				108·1	1·4656

Using the last two figures of the refractive indices in Series I and II as abscissæ and acid values as ordinates these results were plotted in Figure II.

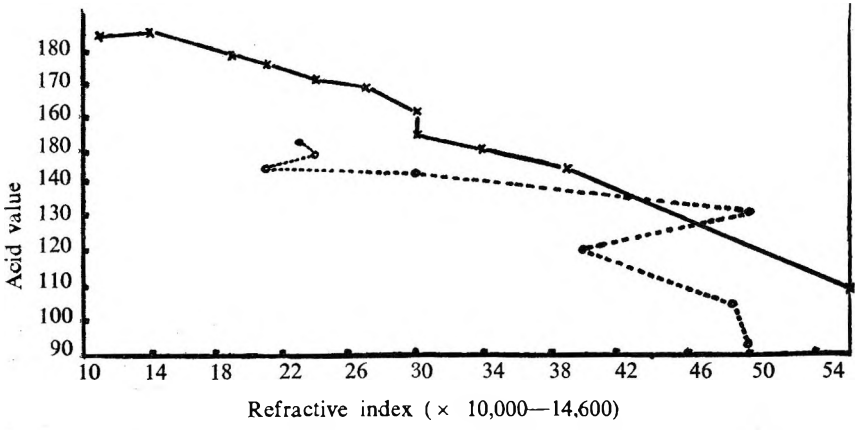


Fig. 2. Showing relationship between acid value and refractive index for 2 series of acids.

Discussion

Results showed that the preparation of a ricinoleic acid of high acid value from castor oil is possible. The complete saponification of the glycerides in the oil is best accomplished by the use of alcoholic potassium hydroxide, and there is no indication that moderate heating at this stage in any way affects the final acid value. The removal of unsaponifiable matter, difficult on account of the solubilising effects of the soap solution is recommended to ensure the production of an acid of high acid value, but it is not always essential. The soap was converted to ricinoleic acid by addition of hydrochloric acid; the acids of highest acid value (170 to 185) were obtained when this reaction was done in the cold and allowed at least 7 days at 4°C. for complete conversion. Emulsion difficulties were experienced and slight loss of acid resulted when the acid was washed free of chloride. It was possible to convert samples of potassium ricinoleate into ricinoleic acids of similar acid values to that of the original acid used to make the soap, even after the soap solution had been stored for over 12 months. The acid value appeared to depend upon the amount of heat used in converting the soap to acid.

The heating of ricinoleic acid at 150°C. in an open container resulted in a regular drop in acid value of something less than 10 units of acid value per hour, although slight difficulty was experienced in stopping this reduction at a level between acid values of 140 and 125. Heating at 160°C. produced a more rapid fall in acid value.

Twelve months' storage of several ricinoleic acids resulted in falls in acid value, the higher values suffering a greater drop than lower values. A straight line relationship was indicated when the logarithm of the decrease in acid value over 12 months was plotted against the acid value of the original acid. If this drop in acid value was due to the formation of estolides, then such a proportionate drop in acid value is to be expected as the concentration of individual molecules of ricinoleic acid available for estolide formation would be greater in the acids of higher acid values. The long time interval (12 months) did not permit of confirmation of the relationship found for one series of acids.

Results of refractive index measurement showed that the drop in acid value resulting from heat treatment was proportional to the increase in refractive index. A similar proportionality was not as clearly defined in the case of acids whose acid values had dropped through ageing. No similarity was found between the refractive indices of acids of the same acid value but prepared by different means.

Summary

1. A method of preparing a ricinoleic acid of high acid value from castor oil has been described and the desirability of not using heat in the conversion of the potassium ricinoleate to ricinoleic acid has been shown.

2. It has been shown that the storage of ricinoleate soap solutions does not result in any drop in acid value of the acid prepared from the soap.

3. The drop in acid value of ricinoleic acid when heated at 150°C. has been traced.

4. The drop in acid value of ricinoleic acid when stored for 12 months has been shown to be proportional to the original acid value in the case of one series of acids.

5. It has been shown that the use of refractive index as a measurement of the acid value of ricinoleic acid has very restricted application.

This work forms part of a thesis submitted for the degree of Doctor of Philosophy in the University of London.

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A NOTE ON THE USE OF FREE-LIVING PROTOZOA AS TEST ORGANISMS FOR DRUGS

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This method was developed in order that the toxicities of known anti-protozoal drugs could be determined on certain free-living organisms and compared with the activities of these drugs against pathogenic organisms. The results of this investigation are published elsewhere (James[1]).

The principle of the method employed was to determine the weakest concentration of drug that killed all the organisms in a standard amount of culture in a given time. Strains of *Paramoecium caudatum* and of *Colpidium colpoda* were used; the former was derived from a single individual isolated from soaked hay, and the latter from a pure bacteria-free culture kindly supplied by Prof. R. A. Peters. Cultures of each organism were grown on a medium of diluted hay infusion to which 0·04 per cent. of acetone-dried yeast had been added. The hay infusion had been boiled on two consecutive days before dilution to ensure freedom from other infusoria. Subcultures were prepared by adding to the fresh medium one-twentieth of its volume of a culture of the organism, and leaving the freshly prepared subcultures exposed to the atmosphere for a period of 48 hours to ensure adequate bacterial contamination. Several attempts to propagate *P. caudatum* on a medium devoid of bacteria failed, and the production of rich cultures of both organisms on the medium described was dependent on the bacterial contamination of the culture medium. Thus arose the necessity of a further procedure, namely, testing at the end of the experiment for surviving bacteria and so making sure that the activity of the drug was not an indirect one through the destruction of the bacterial flora of the culture.

Earlier work in this field had shown that tests carried out with the same organisms on different occasions would often give rise to variable results. It was desirable, therefore, to establish what factors were responsible for these variations, and so to standardise a procedure that would involve the minimum of errors. The considerable variation in the susceptibility of *C. colpoda* to atropine was shown by Prowazek[2] to be due to the age of the culture. Groupe[3] showed that the ciliate *Tetrahymena geleii* became less resistant to mepacrine as the culture aged and this he attributed mainly to an increase in *p*H, but partly to the presence of metabolic products.

At the time of inoculation the cultures showed *p*H values of 6·0 to 6·2, which became steadily less acid as growth proceeded, reaching a value of 6·8 to 6·9 on the seventh or eighth day. At this stage the

cultures showed the greatest number of organisms (*P. caudatum*, 450 to 600 per ml.: *C. colpoda*, 5,000 to 7,000 per ml.) and their motility was maximal. A period of stability ensued for 6 days, after which the culture became alkaline (reaching a final *p*H value of 7·5), the organisms became sluggish and collected at the bottom of the culture. Eight to 14 day-old cultures were selected and used throughout this work for three reasons:— (a) they gave a constant *p*H, (b) the organisms showed a maximum degree of activity, and (c) the number of organisms in successive subcultures did not vary by more than 40 per cent.

Preliminary experiments indicated that an increase from 20° to 25°C. in the temperature at which the experiment was conducted decreased the resistance of the organisms to most of the drugs tested to such an extent that their activity appeared doubled. Experiments were therefore conducted at a constant temperature.

Drugs strongly acid or alkaline in solution should be neutralised beforehand, particularly when high concentrations have to be used. This factor was not involved in the work on antiprotozoal drugs, partly on account of the fact that the solutions used were sufficiently dilute and partly on account of the slight buffering effect of the culture medium.

Procedure

The method of preparing drug dilutions was that described by Gunn and Simonart[4], whereby twelve 0·5 ml. serial dilutions of a 1 per cent. stock solution of 5 drugs were prepared and transferred to small tubes of 1 to 2 ml. capacity set up in suitable racks. 0·5 ml. of a 8 to 14 day-old culture of *Colpidium* or of *Paramœcium* was added to each tube, the contents well mixed and the tubes placed in an incubator at 20°C. The time was taken when the mixing was complete and the tubes were examined for survivors after 20 minutes, 1, 3, 6 and 24 hours' exposure to the drugs, and again each day until there was no further change. The tube containing the weakest dilution in which there were no survivors was noted at the end of each period of time and the final concentration of drug (half the initial concentration) in the tube recorded. This is referred to as the minimum lethal concentration.

This procedure using 12 tubes is convenient for preliminary use as it will almost certainly cover the range of activity of any drug. Afterwards it can be shortened as a rule by using only 6 dilutions of the drug.

Each experiment was repeated on 4 further occasions at different times and using different cultures. The agreement was found to be good, for in practically all cases at least 4 of the 5 results tallied.

The results were represented on a graph where the logarithm of the reciprocal of the minimum lethal concentration was plotted against time.

Colpidium was found to be more resistant to the antiprotozoal drugs than was *Paramœcium*, particularly in the case of the more active drugs. Their effects against the two organisms, however, showed a parallelism so that these drugs fell into the same order of activity when either organism was used in the test.

The test for aerobic bacteria in the drug-culture mixture consisted in

inoculating tubes of broth infusion with a few drops of the contents of the tubes from the above test after its completion and incubating for 48 hours. A control was carried out using a 1 in 2 dilution of untreated culture. The rate of appearance of turbidity in the tests was compared with that in the control. This test, when applied to the antiprotozoal drugs, showed that the bacteria were unaffected by concentrations which destroyed the protozoa.

Summary

1. A simple method of testing the activity of drugs on certain infusoria is described, whereby cultures of the organisms are exposed to varying dilutions of the drugs and the lethal concentrations recorded after different periods of exposure.

2. The smaller organism, *Colpidium*, was found to be more resistant to the action of the drugs than was *Paramœcium*, but the results obtained with both species gave the same relative order of activity.

I am indebted to Prof. R. St. A. Heathcote for his helpful criticisms and also for suggesting this work.

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THE PREPARATION OF ESTERS

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In the course of investigations carried out in these laboratories, a considerable number of different esters has been required. A simple method, resulting in very satisfactory yields, has been described by Clinton and Laskowski[1] involving the refluxing of a mixture of the acid, commercial methyl alcohol, ethylene dichloride and sulphuric acid for from 6 to 15 hours. This method has the advantage of not requiring the separation of water formed during the reaction.

We have employed this method with success in the preparation of several methyl esters not described by Clinton and Laskowski, and have investigated the extension of the method to include other alcohols. Ethyl esters have been prepared from the following acids:—9-undecylenic, sebacic, oxalic, tartaric and citric. Equally successful preparations of *n*-butyl esters have been carried out from sebacic and citric acids. Citrates have been obtained in satisfactory yield from both β-chloroethyl and β-bromoethyl alcohols, and a good yield of cinnamic ester has been obtained from tetrahydrofuryl alcohol.

TABLE 1

Ester	B.pt. or M.pt. °C.	Yield Per Cent.
Methyl sebacate	B.pt. 164° to 168°/12 mm.	89
Ethyl sebacate	B.pt. 192° to 197°/25 mm.	80 to 84
*iso*Propyl sebacate	B.pt. 181° to 183°/10 mm.	55
*n*Butyl sebacate	B.pt. 216° to 220°/14 mm.	92
*tert*Butyl sebacate	——Olefine evolved——	
Methyl oxalate	B.pt. 163° ; m.pt. 51°	44 to 47
Ethyl oxalate	B.pt. 182° to 192°	48 to 51
Methyl citrate	M.pt. 79°	56
Ethyl citrate (a)	B.pt. 136° to 138°/2 mm.	55
Ethyl citrate (b)	B.pt. 179° to 180°/12 mm.	40
Ethyl citrate (c)	B.pt. 176° to 178°/10 mm.	35
βChloroethyl citrate	B.pt. 215° to 216°/0·5 mm.	32
βBromoethyl citrate	B.pt. 226° to 228°/0·4 mm.	30
*n*Butyl citrate	B.pt. 170° to 172°/0·5 mm.	65
Ethyl tartrate	B.pt. 158° to 160°/12 mm.	39 to 41
Ethyl 9-undecylenate	B.pt. 137° to 139°/13 mm.	88
Methyl (p-methoxyphenyl)-propionate	B.pt. 154° to 156°/16 mm. ; m.pt. 38°	86
Methyl (m-methoxyphenyl)-propionate	B.pt. 154° to 156°/16 mm. M.pt. 29° to 30°	76
Methyl p-hydroxybenzoate*	M.pt. 128°	85
Tetrahydrofuryl cinnamate	B.pt. 207° to 209°/11 mm.	67
Methyl piperonylate*	B.pt. 146°/11 mm. ; m.pt. 52°	71

Standard conditions : 1 mol. of aliphatic carboxyl group, 3 mol. of the alcohol, 300 ml. of ethylene dichloride and 3 ml. of concentrated sulphuric acid heated under a reflux condenser for 15 hours.

* —aromatic acid, as above but with 15 ml. of concentrated sulphuric acid per mol. of carboxyl group.
(a) anhydrous ethyl alcohol.
(b) ethyl alcohol (95 per cent.)
(c) industrial methylated spirit used.

Primary alcohols appear to react with readily esterifiable acids in a satisfactory manner by this method. An attempted esterification of benzoic acid by benzyl alcohol gave a product containing so high a

proportion of dibenzyl ether as to render the method unsuitable for the preparation of benzyl esters: it is possible that the use of other catalysts than sulphuric acid might eliminate this difficulty.

In the case of the one secondary alcohol examined there are indications that esterification proceeds rather less readily, since, under the standard conditions, a mixture of products was obtained from sebacic acid with *iso*propyl alcohol. The yield of di-*iso*propyl sebacate was 55 per cent., and a considerable quantity of mono-*iso*propyl sebacate was also formed. Attempted esterification of readily dehydrated alcohols by this method, in the presence of sulphuric acid, leads to the formation of the olefine. While dibasic acids, in which the carboxyl groups are widely separated, appear to esterify very readily, in the special case of oxalic acid, with two vicinal carboxyl groups, it does not appear possible appreciably to exceed a yield of 50 per cent. The general yield of 50 per cent. of ethyl oxalate is unaltered whether hydrated or anhydrous acid be employed, and the yield is not reduced by halving the amount of alcohol in the reaction mixture. Whereas we were able to obtain consistent yields of 55 per cent. of methyl citrate, using the standard method, and a similar yield of ethyl citrate when anhydrous ethyl alcohol was used, the yield of ester from industrial methylated spirit was of the order of 35 per cent. Using ethyl alcohol (95 per cent.) a yield of 40 per cent. was obtained.

It would appear that, in general, this method of esterification is of considerable value, and is of much wider application than is at present indicated in the literature.

We wish to thank Mr. D. E. Waller and Mr. W. C. Austin for technical assistance, and the Directors of Messrs. Allen and Hanburys Ltd. for permission to publish these results.

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THE STERILISATION OF SULPHANILAMIDE POWDERS

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Introduction

Although sulphanilamide has been largely superseded by other substances and preparations, we feel that the work described below should be placed on record as illustrative of pharmaceutical problems wherein seemingly trivial impurities can exert a marked effect upon the quality of a final preparation, and that the results obtained may be useful in dealing with such problems.

The use of sterilised sulphanilamide powder for topical application to wounds during the last war led us to examine the problems involved in the sterilisation of this substance in unit containers. Some of the difficulties have been indicated in the many methods of preparation described[1-6, 8-10] for the production of a powder which is free from discolouration and is free-flowing to facilitate sprinkling the powder evenly over a wound.

The free-flowing property may be improved by controlling the particle size of the powder[2] or by admixture with certain substances[1,3], although caking and discolouration are not necessarily prevented by these methods. The admixture with insoluble substances, such as zinc oxide, kaolin, etc., is undesirable when the powder is to be used in the peritoneal cavity or in deep wounds, since foreign body reactions may arise.

In order to avoid discolouration Buckland[5] sterilised the powder in plugged tubes by heating in a dressings autoclave. McCartney and Cruikshank[6] used tightly closed screw-capped bottles and, providing the powder contained not less than 0·2 per cent. of moisture, sterilisation could be effected by heating in an autoclave. Caking and discolouration of the sulphanilamide was later reported by these authors[7].

Long[4] described a process for sterilising paper envelopes containing 5 g. of sulphanilamide. Discolouration of both the sulphanilamide and the paper in contact with it occurred when samples of sulphanilamide available in this country were used.

A package containing 5 g. of sulphanilamide in a paper envelope and enclosed in a second envelope was most suitable for use in the field since it was compact, light in weight, and easily and cleanly opened for use.

In our experimental work sterilisation was controlled by deliberately adding hay dust to a number of the packages in each batch so that the sample was grossly infected with spores of *Bacillus subtilis*, and tests

for sterility were carried out using aerobic and anaerobic media containing 0·1 per cent. of *p*-aminobenzoic acid. This was necessary, as the method referred to below as " heating at 150°C. for 1 hour " was carried out in a thermostatically controlled electric oven, the shelf temperature of which was found to vary between 145° and 155°C. and the time of heating at this temperature range was about 1½ hours to effect sterility.

Experimental

Certain factors, found to be important in the preparation of a suitable powder, were examined separately.

1. *The Effect of Electrification of the Powder During Grinding.* The particles appeared to become " electrified " when sulphanilamide was ground to a fine powder. It was then not free-flowing and could not be sprinkled evenly from a container. Sulphanilamide crystals were carefully broken and the resulting powder graded by means of silk sieves. Particles which passed through an 80-mesh, or more coarse, sieve, but were retained by a 100-mesh sieve formed free-flowing powders, but particles which were electrified were those that passed through a 100-mesh sieve.

McDougall and Shotton[1] had found that if certain substances were mixed with finely ground sulphanilamide a mobile powder was immediately formed, the mobility being retained after the powder was heated at 150°C. for 1 hour. Zinc oxide and kaolin were outstanding in this respect, calcium phosphate and the carbonates of calcium, magnesium and zinc were slightly less effective, whilst anhydrous sodium sulphate, anhydrous sodium citrate and potassium hydrogen tartrate gave a very weak effect in producing a free-flowing powder. Davis[3] suggested the added substance probably acted as an absorbent for the moisture present in the sulphanilamide. We found that a sulphanilamide in fine powder containing less than 0·1 per cent. of moisture was not free-flowing unless mixed with zinc oxide or kaolin. Anhydrous sodium sulphate, which could be expected to absorb moisture, was much less effective than zinc oxide, and it would seem, therefore, that the main effect of the added substance is concerned in some way with the removal of the charge on the particles.

In the above experiments the sulphanilamide was heated at 150°C. for 1 hour in screw-capped tubes of approximately ½-inch diameter and some discolouration of the powders occurred even in the presence of 5 per cent. of zinc oxide.

2. *The Effect of the Solvent used for the Crystallisation of Sulphanilamide.* The sulphanilamide used in the preliminary experiments had been crystallised from alcohol, thus the loss of weight on drying would represent this solvent. Alcohol was detectable by smell when the powder was warmed. The effect of the presence of a small amount of alcohol was examined. A quantity of sulphanilamide in 40/100 powder was divided into three portions, " A," " B " and " C." " A " was kept as a control " B " was crystallised from boiling alcohol

(95 per cent.) after charcoal treatment and filtration of the hot solution. "C" was crystallised similarly, but using distilled water as the solvent.

The crystals obtained from "B" and "C" above were dried at a low temperature and reduced to a 40/100 powder. The loss of weight on drying was determined. 15 g. of each of the three powders was heated at 150°C. for 1 hour in square, screw-capped bottles of 1 fl. oz. capacity. The results obtained are summarised in Table I.

TABLE I

EFFECT OF THE RESIDUAL SOLVENT ON THE COLOUR OF SULPHANILAMIDE POWDER HEATED AT 150°C. FOR 1 HOUR

Sulphanilamide Powder (40/100)	'A'	'B'	'C'
Loss of weight on drying	0·2 per cent.	0·32 per cent.	0·06 per cent.
Heated immediately after re-crystallisation ...	Brownish discolouration	Discolouration slight ; less than 'A'	White
Heated after 3 months storage at laboratory temperature ...	As above	Discolouration greater than 'A'	White

From these results it appears that the presence of a small quantity of alcohol is a cause of the discolouration which occurs when sulphanilamide is heated.

A little acetaldehyde was added to a portion of the above powder "C" both alone and also with 5 per cent. of zinc oxide present. Both powders developed a brownish colour and were caked after heating at 150°C. This suggests that on storage a change takes place in the powder crystallised from alcohol with the formation of a compound which is decomposed by heat. It seems probable that the alcohol undergoes atmospheric oxidation to acetaldehyde and this subsequently reacts with the sulphanilamide.

3. *The Effect of the Moisture Content of Sulphanilamide Powder.* Sulphanilamide was recrystallised from distilled water as above and divided into portions. These were dried slowly at about 40°C. in order to obtain powders with differing moisture content.

The moisture content of each portion was determined and the powders heated at 150°C. alone and with 5 per cent. of zinc oxide both in screw-capped bottles and in paper envelopes. When heated in screw-capped bottles, from which the moisture could not readily escape, caking occurred when the moisture content was above approximately 0·25 per cent., a small hard central core being formed when the moisture content approached 0·25 per cent. This result was obtained also when 5 per cent. zinc oxide was mixed with the sulphanilamide. Water vapour was lost quite readily from the paper envelopes containing sulphanilamide and a sample of powder containing 0·94 per cent. of moisture gave a satisfactory powder after heating in such a package. It was noticed, too, that

powders containing more than 0·5 per cent. of moisture were less readily electrified during grinding.

Paper Envelopes. Envelopes were made from many types and qualities of paper, but after heating some became too brittle and others were discoloured by, or caused discolouration of, the sulphanilamide in contact with them. Somerville[11] has described a colour reaction between sulphanilamide and an aldehyde accompanying lignin and this may afford an explanation of the mutual discolouration of the envelope and the sulphanilamide which occurred. A good quality vegetable parchment was found to be suitable, but as some variation occurred each delivery was tested for suitability.

Sulphathiazole. We found that if sulphathiazole was recrystallised from distilled water, as previously described for sulphanilamide, this too could be heated at 150°C. for 1 hour without becoming noticeably discoloured, whereas if recrystallised from alcohol it was discoloured.

The charcoal used in these experiments was a commercial sample purified by boiling with strong hydrochloric acid and washing with distilled water until free from acid. It was then substantially free from arsenic and lead.

Summary

1. A sterile free-flowing sulphanilamide powder may be prepared providing the powder is not finer than an 80/100 mesh.

2. Powders finer than 100 mesh appear to become electrified during grinding and are then not free-flowing. Powders containing more than 0.5 per cent. of moisture are less readily electrified by grinding.

3. The addition of certain substances, e.g., zinc oxide or kaolin, to fine powders resulted in free-flowing powders. This appears to be mainly connected with the loss of the electric charge.

4. Sulphanilamide recrystallised from alcohol became discoloured when sterilised by heating at 150°C. The discolouration was increased after the sample was stored for some time at laboratory temperature before sterilisation.

5. Sulphanilamide recrystallised from water, as described, was not discoloured when heated at 150°C. and remained satisfactory after storage at laboratory temperature.

6. Sulphanilamide powder heated in screw-capped bottles at 150°C. became caked, to some degree, if more than about 0·25 per cent. of water was present.

7. Sulphanilamide powder packed in thin layers in paper envelopes did not cake after heating at 150°C. even when the moisture content was nearly 1·0 per cent. since water vapour was readily lost through the paper.

8. A good quality vegetable parchment was found to be a suitable paper for the envelope in contact with the sulphanilamide.

9. Sulphathiazole recrystallised from distilled water was not discoloured when heated at 150°C. but if recrystallised from alcohol it was discoloured by heat in a similar manner to sulphanilamide.

We wish to express our thanks to Mr. A. C. McDougall for much encouragement and advice throughout this work.

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SOME STUDIES OF THE CHEMISTRY AND PHARMACOLOGY OF ADRENOCORTICOTROPHIC HORMONE

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The final establishment of certain biochemical actions of the adrenocorticotrophic hormone (ACTH) has aroused the interest of clinicians, physiologists, pharmacologists and chemists alike, and has made it desirable that larger quantities of the hormone should be obtainable. It is unfortunate, however, that various reports have been apt to mislead in the matter of potential supplies of this hormone. We believe that the opinion that only minute amounts of the hormone can be produced at high cost needs rectifying in the light of our own experience. Sufficient hormone for therapeutic use should be available if the maximum yield attainable is achieved and the hormone is used in the optimal way from the clinical and pharmacological point of view.

Yields

It has been known for many years that the adrenocorticotrophic hormone concentration of pig pituitaries is far higher than that of cattle or sheep pituitaries. Although 1 kg. of frozen pig pituitaries contains only 20 times as much adrenocorticotrophic hormone as 1 kg. of cattle pituitaries, in practice one can prepare at least 100 times more, adequately purified for clinical use, from the pig pituitaries, than is obtained from the cattle pituitaries. This is due to the fact that the same extraction methods which are used to prepare the crude preparation yield, from pig pituitaries, a preparation which is 20 to 100 times purer than the preparation from the cattle pituitaries. In order to get from cattle pituitaries adrenocorticotrophic hormone of purity similar to that of the preparation from pig pituitaries, additional purification steps have to be introduced which are sometimes connected with very considerable losses.

The only yield calculation we can find published so far comes from Fishman[1], who has worked in connection with the White-Sayers group. He prepares from 1 kg. of pig pituitary 6·5 g. of crude extract, from which about 1 g. of purified preparation can be obtained. According to this conception only forty 25 mg. ampoules for clinical use could be prepared from 1 kg. of pig pituitary. Most of the purification methods described so far are only of academic interest. Their adoption as routine methods for the production of the pharmaco-therapeutic preparation would make the clinical use of adrenocorticotrophic hormone prohibitive.

The procedures to be described below give the following calculated yield. From 1 kg. of frozen pig pituitary 35 to 40 g. of a crude preparation which is already free from gonadotrophic, thyrotrophic or growth hormones is obtained. Its potency is at least one-third as high as that of

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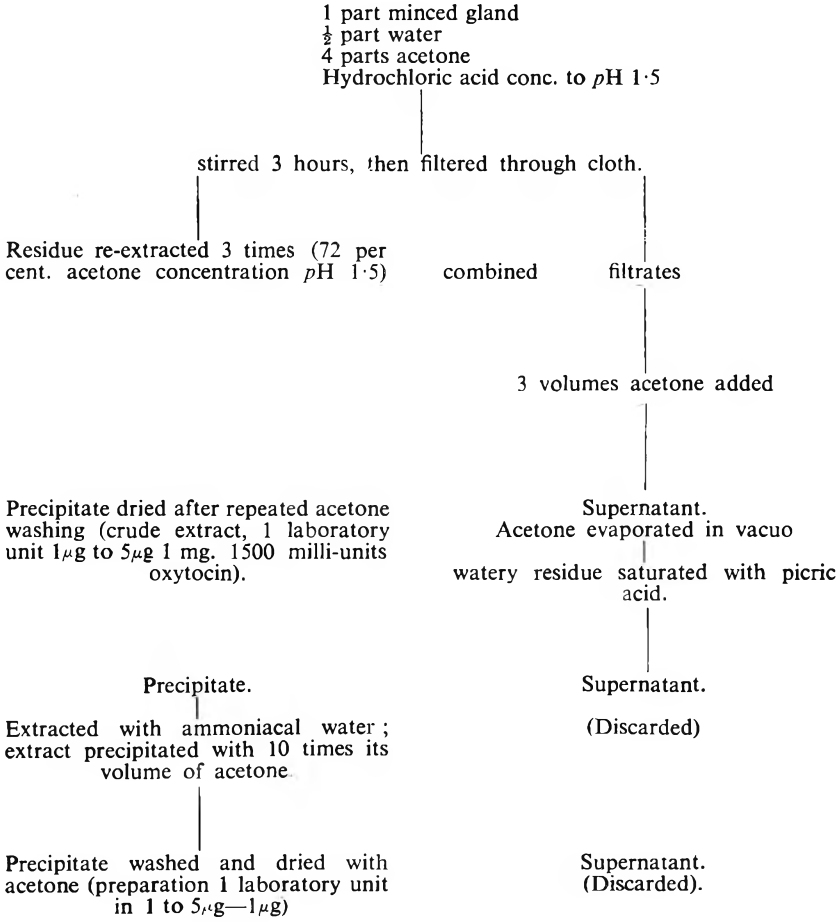
some of the pure preparations described, such as the Armour standard preparation La-I-A. Only the very low (nearly negligible) content of lactogenetic hormone and the posterior lobe hormone present in the preparation must be removed before it can be used therapeutically. These procedures are connected with the loss of about 25 per cent. of the active material. It should be possible to prepare 600 to 800 ampoules of an activity equivalent to that of 25 mg. of standard hormone from 1 kg. of pig pituitaries.

Chemical Preparation

The crude hormone preparation has been made by a process similar to that of Lyons[2]. The glands were extracted with strong acid acetone for 3 hours. This was carried out in accordance with the process shown

TABLE I



in Table I. The high acetone concentration used does not extract the gonadotrophic and thyrotrophic hormones. The extraction is repeated

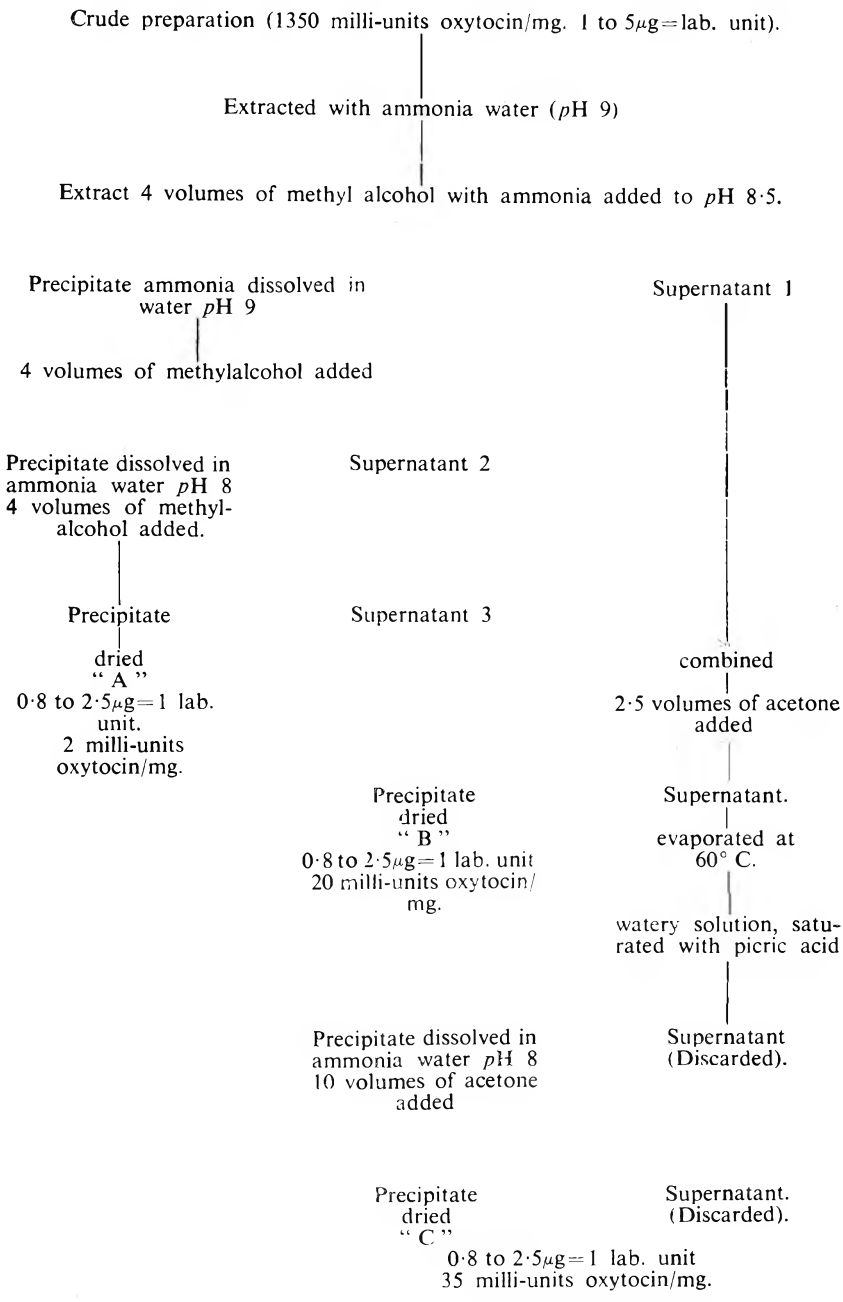
3 or 4 times. After precipitation of the bulk of the adrenocorticotrophic hormone with 95 per cent. acetone some activity still remains in solution and the hormone can be precipitated by picric acid, after evaporation of the acetone. The biological activity of this picrate is similar to that of the bulk of the preparation and constitutes about 5 per cent. of the total yield. It consists apparently of an adrenocorticotrophic hormone of smaller molecular size than is present in the 95 per cent. acetone precipitate.

Removal of the lactogenic hormone from the crude extract is best carried out in accordance with the various American methods by precipitation with sodium chloride at *p*H 3·0 (Li, Simpson and Evans[3]).

The main difficulty of the whole purification procedure, however, is the removal of the posterior lobe hormone contamination. This is done in all published methods by destruction of the posterior lobe hormone by adding large amounts of concentrated ammonia, but it is difficult to emphasise sufficiently how wasteful this method is, as not only posterior lobe hormones, but also very considerable amounts of adrenocorticotrophic hormone are destroyed by this procedure. A purification method (shown in Table II), based on the fact, found in our investigations, that the posterior lobe hormones are more soluble in alkaline methyl alcoholic solution than the adrenocorticotrophic hormone, allows not only the removal of the posterior lobe hormones, but also the separation of the total adrenocorticotrophic activity into three different hormone fractions. The crude powder is extracted with ammoniacal water and by adding methylalcohol to 80 per cent. concentration a precipitate deposits which is twice more re-precipitated and finally dried with acetone. This preparation (" A") is highly active in the ascorbic acid test and nearly completely free from posterior lobe hormones. It comprises about 25 per cent. of the total yield of the purified preparation. The combined supernatants are mixed with two and a half times their volume of acetone, whereupon the main fraction precipitates. This preparation (" B ") too exhibits only very little oxytocic activity (about 20 milliunits per mg.) and is equal in potency to the preparation " A " as shown by the adrenal ascorbic acid test. The supernatant liquid is evaporated until nearly all the acetone and methyl alcohol are removed. The watery solution is saturated with picric acid, the resulting picrate dissolved in ammoniacal water at *p*H 8 and precipitated with 10 volumes of acetone. The precipitate is then washed and dried with acetone. It has the same activity as shown by the ascorbic acid test as the " A " and " B " fractions and only contains small amounts of oxytocic activity. This last fraction constitutes at least 3 per cent. of the total yield.

It is not intended to deal here with further chemical characteristics of these apparently distinct adrenocorticotrophic hormone fractions, nor has the investigation as to the possibility of different biological actions of these fractions been completed. It is only conceivable that different molecular sizes or different sizes of protein carriers of the same active prosthetic hormone group may have been separated in the three different fractions.

TABLE II



Certain parallelisms with the relations between, for instance, thyroglobulin and thyroxine or dialysable and undialysable adrenocorticotrophic hormone are obvious. We have reported several years ago (Reiss

and Golla[4]) on the high alcohol solubility of an adrenocorticotrophic hormone fraction prepared from cattle pituitaries.

The preparation "B" has been used on our patients clinically for adrenal response tests and for treatment. Concentrated solutions can be Seitz-filtered or irradiated by 30 watt ultra-violet germicidal lamp λ 2537, without any loss of activity. The resulting sterile* solution is filled into ampoules and freeze-dried.

One of the shortcomings of clinical treatment with adrenocorticotrophic hormone is its speedy disappearance from the circulation. We have found that the heating of the powder to 180° to 200°C. does not reduce its biological activity if it is kept under a high vacuum during this procedure, but its solubility is considerably diminished, and it remains to be seen whether such powders used as implants will not make the clinical use of adrenocorticotrophic hormone more economical.

Biological Assay

The biological assay of adrenocorticotrophic hormone has been greatly simplified since the adrenal ascorbic acid depletion method of Sayers and Sayers[5] was introduced.

For the purpose of comparison in our purification attempts the dose that reduced the ascorbic acid content of the adrenal of hypophysectomised rats by 20 per cent. was taken as the laboratory unit. This laboratory unit is approximately equivalent to a 1 μg. dose of the Armour standard La-I-A and is also equivalent to about 1/30th to 1/75th of the sudanophobic repair unit (Reiss, Balint, Oestreicher and Aronson[6]: Simpson, Evans and Li[7]). The animal unit is only used for following the course of the preparation of the hormone. A final estimation of the potency of a preparation is always made by a simultaneous test of the preparation against the one which has been adopted as a temporary standard of reference (La-I-A Armour standard).

Sayers, Sayers and Woodbury's[8] arrangement of the ascorbic acid depletion method appeared to us to be the most satisfactory. We quote, however, in Table III the results of an assay which show that one is not likely to achieve the degree of precision required using the small group of animals recommended by the authors.

TABLE III

La-I-A				Unknown Preparation B			
No. of rats	Dose	Mean Resp.	S.D.	No. of rats	Dose	Mean Resp.	S.D.
14	1·0	106	27·4	14	1·0	75	22·0
11	0·25	67	20·2	11	0·25	48	24·2

* The sterility test was carried out in our Pathological Laboratory by Mr. G. Pope. The contents of ampoules chosen from each batch at random were dropped into suitable media such as peptone water or broth and examined after 24 to 72 hours.

	La-I-A	Preparation B	Combined
b	64·78	44·85	54·81
s	24·5	23·0	23·8
$\lambda = \frac{s}{b}$	0·3782	0·5128	0·4342

M = 1·53075; ratio of potency Prep. B/La-I-A = 0·3447 with Standard (La-I-A) at 100 per cent. Preparation B = 34·5 per cent.

Sm = ± 0·1559; limits of error at p = 0·95 are 49·5 to 202·8 per cent. Range of potency Pre. B/La-I-A in 95 cases out of 100 is 16·78 to 68·75 per cent.

In Table III are given: the number of rats used for each dose, the dose expressed in μg./100 g. of bodyweight, the mean response to each dose (i.e., the average of the differences between right and left adrenal ascorbic acid in mg/100 g. of fresh tissue) and the standard deviation of a single observation of the response to each dose; b is the slope of the log dose response curve for each preparation and for the two combined, S is the standard deviation for each preparation (derived from both doses) and the standard deviation for both preparations. λ = an index of precision, M = the log. of the ratio of the potency of the preparation B to La-I-A, Sm = the standard error of M. The statistical analysis of the results was carried out according to the method of Irwin as given by Pugsley[9].

The slope of the log dose-response curve obtained in the standardisation is less steep than that mentioned in the example published by Sayers, Sayers and Woodbury[8] although twice the number of animals were used in the experiment described in the table. The slope described here is typical of a series of our standardisations and it remains to be seen whether an improvement of the experimental arrangement of the method or (what appears to us at the moment more probable) a considerable increase in the number of experimental animals used will improve the exactitude of the method.

Clinical Diagnostic and Therapeutic Technique

The introduction of the clinical adrenal cortex response test, as worked out by Thorn[10], Pincus[11] and others, is of great importance. Clinical treatment with adrenocorticotrophic hormone will only prove successful if it is applied in suitable selected cases. Treatment with this hormone can only be considered in patients with an adrenal cortex which responds to it. There again, it will be necessary to distinguish between patients who show a positive glucose tolerance or cold test and those who do not. Or in other words, between patients in which the pituitary is able to produce endogenous hormone and patients in whom it cannot do so. Only in those cases where the adrenal cortex can react to this hormone; but who are unable to produce enough of it themselves, is adrenocorticotrophic hormone therapy indicated.

Doses and duration of treatment must also be considered very carefully by the clinician in co-operation with the pharmacologist. Prolonged treatment with high doses of adrenocorticotrophic hormone might not only be wasteful but contra-indicated. The hormone given over a long

period may produce considerable enlargement of the suprarenal cortex, and finally an exhaustion of the hormone-producing capability of the gland. Unpleasant side effects too, have been reported which were due to the increased mobilisation of some adreno-cortical compounds, the physiological excretory balance of which was disturbed by the dose of adrenocorticotrophic hormone administered.

It still remains to be seen whether there do not exist different fractions which are responsible for the mobilisation of the different adrenocortical compounds.

In our experience the 17-ketosteroid excretion of patients can sometimes, after a few days' treatment with the high 25 mg. doses, rise to figures which have been seen by us so far only as a sequel to severe adrenal cortex hyperfunction and hyperplasia, or even adenoma. We have further seen a mental patient who improved considerably during the first seven days of treatment with the high dose, and deteriorated again when the injections were continued for a further 10 days. On the other hand, one injection of the high dose as given sometimes for the purpose of the response test can initiate an improvement which occasionally lasts for many weeks.

Future experience might still show whether prolonged treatment with small 1 or 2 mg. doses, as we carried out some years ago (Hemphill and Reiss[12]) will not prove the method of choice.

Considering in how many physiological processes the hormones of the adrenal cortex participate, it can scarcely be foreseen how manifold the indications for the use of adrenocorticotrophic hormone may be, and it is therefore imperative to start to define its pharmaco-dynamic qualities.

Our thanks are due to Mr. S. H. Grover of Messrs. C. and T. Harris, Ltd., Calne, for collection of the glands, and Mr. R. H. Orpen of this Department for making the necessary arrangements; and to The General Electric Co, Ltd., for very kindly putting the Germicidal Lamps at our disposal. We are very indebted to Mr. F. E. Badrick, Miss Gwen Stephens and Mr. Donald Dickinson for very valuable help in the preparation and standardisation of the hormone, and to Mr. G. Pope for carrying out the sterility tests.

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THE EVALUATION OF THE BACTERICIDAL ACTIVITY OF ETHYLENE GLYCOL AND SOME OF ITS MONOALKYL ETHERS AGAINST *BACTERIUM COLI*

PART X

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IN the preface to the previous communication[1] it was pointed out that the statement of a time taken to reach a certain mortality level of an inoculum, was not by itself a sufficient criterion of the bactericidal efficiency of a disinfectant substance; other considerations should be taken into account in the assessment, namely, the effects of dilution and of temperature. The dilution factor was dealt with in the last paper; in this paper the temperature factor is expounded.

THE TEMPERATURE COEFFICIENT AND ITS METHOD OF DETERMINATION

The influence of temperature on the disinfection rate. Early observers were not slow to notice that the rate of disinfection was faster at higher temperatures. Koch[2] recorded that anthrax spores were killed more quickly by phenol at elevated temperatures; Madsen and Nyman[3] found the same to be true with mercuric chloride. Experiments at different temperatures were also conducted by Henle[4], Behring[5] and Abbot[6], and the same conclusions were reached.

Chick[7], from her studies of disinfection reactions, deduced that there was a close analogy between the manner in which the reaction velocity of disinfection and that of chemical reactions, increased with temperature. She calculated that a rectilinear relationship existed between the logarithm of the disinfection rate and the temperature of the reaction; this was confirmed by her (*loc. cit.*) when studying the disinfection of bacteria by hot water. Since it was believed that the disinfection rate (k) was constant throughout its course, k was taken as being indirectly proportional to the time for disinfection; the plot of the disinfection times against the respective temperatures (for the same strength disinfectant solution) should therefore yield a straight line.

Phelps' equation. Phelps[8] likened the increase of disinfection rate with temperature to a relationship common in physical chemistry in which the velocity of a reaction increased in geometrical progression as the temperature increased in arithmetical progression. Temperature coefficients, θ *and* Q_{10}, were postulated, these being figures indicating the proportionate increase in the rate of the process (or decrease in time of completion) for a 1°C. and 10°C. increase in temperature respectively. Mathematically, the relationship may be expressed as $k_2/k_1 = Q_{10}^{(T_2-T_1)}$ where k_2 and k_1 are the velocity constants of the disinfection processes at temperatures T_2 and T_1 respectively, differing by 10°C. (When $T_2 - T_1 = 1$, $k_2/k_1 = \theta$). When, for example, $Q_{10} = 2$, it means that

the death rate is doubled by a 10°C. rise; a further increase of 10°C. will double it again, so that a total increase of 20°C. will quadruple the death rate. From the list complied by Chick[9] it is clear that θ and Q_{10} vary with the nature of the disinfectant; for example, for phenol $Q_{10}=7$ to 8, whereas for metallic salts it is 2 to 4.

The significance of the temperature coefficient. The significance of θ (or Q_{10}) does not make itself apparent in a phenol coefficient. The comparison between the standard and the unknown substance is performed at a constant temperature, and hence the coefficient so obtained is dependent upon the temperature selected for the test. The correct relative relationship between the standard and the unknown can be calculated at other temperatures only when θ is the same for both substances. If the temperature coefficients are not the same, then the increase in rates of disinfection with temperature will be disproportionate, and then the phenol coefficient will vary when the test is performed at different temperatures.

The use of extinction times as the basis of comparison for the determination of temperature coefficients. For end point methods of investigation, Phelps' equation is interpreted as $t_1/t_2=\theta^{(T_2-T_1)}$ where t_1 and t_2 θ, it is necessary to determine the extinction times for the same strength disinfectant solutions at two temperatures.

The use of LT50 as the basis of comparison for the determination of temperature coefficients. A summary of the evidence that has accumulated to indicate that the disinfection rate varies along its course has been presented in Part II[10] of this series of communications; it has also been emphasised in Part IX that the use of disinfection rates for the comparison of bactericidal activity is not always reliable.

Throughout this work the LT50 has been used to compare activities of disinfectant solutions and it is suggested that it be substituted for t in the above expression to calculate θ for the compounds. In order to achieve this it is necessary to calculate the LT50's for the same concentration of disinfectant solution at the two temperatures employed, viz., 20°C. and 30°C.

Calculation of the Temperature Coefficients of Ethylene Glycol and its Monoalkyl Ethers

LT50's for the same concentration of disinfectant were calculated for the disinfection reactions at 20°C. and at 30°C. Suitable concentrations were selected for each compound, this being readily achieved by inspection of the data in Table I, Part IX[1]. The ratio of the two LT50's at each temperature gives Q_{10}, from which θ is calculated.

Example of a calculation

In the equation $y=\bar{y}+b(x-\bar{x})$, $y=\log$ LT50 at x, the logarithm of the selected concentration; $\bar{y}=$mean log LT50 of the data; b=the slope of the regression $(=n)$; $\bar{x}=$mean log concentration of the data.

Calculation of LT50 for 70 per cent. ethylene glycol at 20°C.

$$y = 1{\cdot}550 - 15{\cdot}87(1{\cdot}845 - 1{\cdot}904) = 305 \text{ minutes.}$$

Calculation of LT50 for 70 per cent. ethylene glycol at 30°C.

$$y = 1{\cdot}678 - 18{\cdot}46(1{\cdot}845 - 1.821) = 17 \text{ minutes.}$$

Calculation of the temperature coefficient

$$Q_{10} = 305/17 = 18$$

$$\theta = \sqrt[10]{18} = 1{\cdot}334$$

Results. Table I sets out the values of Q_{10} between 20°C. and 30°C. and θ at the two concentrations for each compound. It is seen that θ falls within the range 1·0 to 1·9, the generally accepted range for biological processes (Bělehrádek[11]). Except for the monoethyl ether, Q_{10} is seen to vary between 7 and 45; the monoethyl ether appears to be anomalous and exceedingly high values have been obtained. In all the compounds except ethylene glycol the values of θ and Q_{10} are greater at the lower concentration of disinfectant used for the calculation. As Q_{10} is based on two observations only in each case, a large error of estimation is involved; there are no means of knowing whether the differences with concentration are really significant.

Discussion

Variation of the temperature coefficient with the concentration of disinfectant. In this work, temperature coefficients have been recorded at two temperatures, viz., 20°C. and 30°C. Broad generalisations only, therefore, on the nature of the coefficients, can be drawn from the results; experiments at intermediate stages involving smaller temperature ranges, should be performed before detailed inferences are made. Nevertheless, it is clear from the results in Table I that the value of θ and Q_{10} varies considerably with the concentration of the disinfectant. The variation in the values of Q_{10} is very noticeable and although θ (the tenth root of Q_{10}) appears to alter but little, this is deceptive as small differences in its value will manifest themselves when the temperature range is protracted.

Influence of the concentration exponent on the variation of the temperature coefficient with concentration of disinfectant. The variation of the temperature coefficient at different concentrations appears to be dependent on the behaviour of the concentration exponent of the disinfectant. This general phenomenon was first observed by Chick[6]. The results of Jordan and Jacobs[12] suggest a possible explanation of this effect. These authors detected that the relationship between log *v.s.t.* and log concentration of phenol was not rectilinear over a wide range of concentrations. When two regressions of this nature set up at two different temperatures, are compared in order to determine the temperature coefficient, the value of the latter will vary with the concentration selected and the trend of the variation will depend on the actual shapes of the curves.

The results obtained in this work can be used to illustrate the variation of the temperature coefficient with concentration by comparing the slopes of the log LT50-log concentration of disinfectant regressions. Figure 1 gives an example; it shows the slopes of the log LT50-log per cent. con-

centration regressions (i.e., the concentration exponents) of ethylene glycol monopropyl ether at 20°C. and at 30°C. It can be seen that the slopes are not parallel and hence the ratio between the values of the two LT50's at the same concentration (i.e., Q_{10}) will vary with the concentration chosen. The level of mortality selected for the calculations does not enter into the argument; the deciding factor is the concentration exponent and if this is not the same at the two temperatures then different values of Q_{10} must be obtained at different concentrations. When n does not alter, the magnitude of the slopes at different temperatures will be identical and at whatever concentration the comparison is made, the value of Q_{10} will be constant.

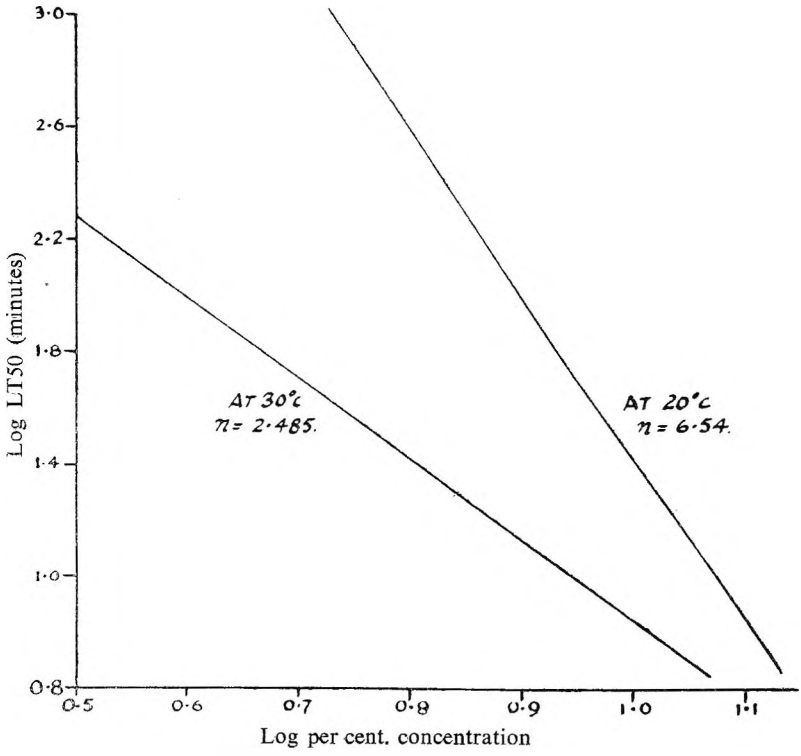

FIG. 1. Comparison of the concentration exponents (n) of ethylene glycol monopropyl ether at 20°C. and at 30°C.

Comparison of the temperature coefficients of chemical reactions and disinfection processes. The pioneer workers carried out investigations on disinfection with metallic salts and obtained Q_{10} values of between 2 and 3. Chemical reactions have a Q_{10} of the same order and the similarity of the range for the two processes gave added evidence to Chick's[6] hypothesis that a stoichiometric relationship existed between disinfectant and bacterial protein. The researches of Paul, Birstein and Reuss[13] on hydrochloric acid enhanced this conception; they obtained Q_{10} values between

1 and 3, whereas the temperature coefficient of chlorine fell between 2 and 4 (Weber and Levine[14]; Ames and Smith[15]). However, Charlton and Levine[16] found that the coefficient of this latter substance was affected by *p*H and that at *p*H 6 the temperature coefficient was between 5 and 6.

Phenol and fundamentally similar disinfectants possess higher values of Q_{10}. For phenol against *Bact. paratyphosum*, Chick[6] obtained values between 7 and 15, and against anthrax spores, between 7 and 8·6. Tilley[17] confirmed this range for phenol and also found that cresols and resorcinol were similar. Tilley's results also showed that the values of Q_{10} depended on the position on the temperature scale chosen for the determination; although a variation in the value was noticeable with different positions, no definite trend could be detected. Against *Bact. typhosum* the same author obtained extremely high values for ethyl alcohol and normal butyl alcohol; between 30°C. and 40°C., Q_{10}'s of 54 and 40 respectively were obtained, and between 20°C. and 30°C. the corresponding values were 43 and 31. The values for the compounds in the present investigation are of the same order.

High values of Q_{10} are not in line with the view that the process of disinfection is analogous to a chemical reaction; if the two processes were analogous then values of 2 to 3 for Q_{10} should be obtained consistently. Extremely high coefficients have also been recorded for the coagulation of proteins by hot water; Chick and Martin[18], for example, returned a figure of 650 for egg albumin. Henderson Smith[19] investigated the disinfection action of hot water on Botrytis spores and found that Q_{10} increased at lower temperatures. The highest value recorded by him was 690. None of these can be considered as chemical reactions.

A classification of disinfection temperature coefficients. Cooper and Haines[20] classified disinfectants into three groups according to the values of the temperature coefficient. In the first group there was no increase in the disinfection rate with increased temperature; in the second group the increase was approximately the same as that for chemical reactions, while in the third group the temperature coefficient was extremely high. Rahn[21] cast doubt on the validity of this classification because the authors had calculated the temperature coefficients from the ratio of the minimum concentrations of phenol (the standard) and the test substance required to kill the inoculum in a fixed arbitrary time; had other times been used, different temperature coefficients might have been obtained.

Limitations of the accepted formula for determination of temperature coefficients. Jordan and Jacobs[22] found that the temperature coefficient of phenol (as calculated from the usual formula) increased with temperature, especially at the higher concentrations of disinfectant. At the lower concentrations there was a tendency for the coefficients to increase again when the temperature fell below a certain level. Henderson Smith[19] had pointed out that the accepted relationship between temperature and reaction velocity was purely empirical and was devised to meet the case of chemical reaction in which the velocity of the process changed slowly over a wide temperature range; in disinfection reactions the velocity was often changing rapidly over a short range. By adaption of Arrhenius'[23]

equation Henderson Smith was able to procure a rectilinear relationship between mortality times and temperature.

Proposal of a more satisfactory temperature coefficient for disinfection processes. Jordan and Jacobs[22] employed some of the established formulae commonly used in the calculation of the rate of increase of biological processes with temperature listed by Běláerhdek[11] in the hope of deriving a constant temperature coefficient for the disinfection process. They suggested that the variation in Q_{10} at different temperature levels could be predicted on theoretical grounds, since a minimum (or " threshold ") temperature existed for any given phenol concentration and at which the disinfection reaction became infinitely small: Q_{10} must therefore rise as the temperature approaches the threshold value. Experimental evidence was secured in support of this hypothesis. From further studies[24] it was observed that the distal portion of the *v.s.t.*-temperature curve appeared to be asymptotic and it was not possible to estimate accurately the temperature at which the *v.s.t.* was zero. However, they assigned an arbitrary time of 10 minutes to the *v.s.t.* at the " maximum " temperature for each phenol concentration, i.e., the temperature at which the *v.s.t.* may be taken as equal to 10 minutes. The curves of (*v.s.t.* – 10) when plotted against temperature fell from infinity to zero as the temperature rose from the minimum to the maximum value. When log (*v.s.t.* – 10) for given concentrations was plotted against temperature, sigmoid curves were obtained which could be regarded as asymptotic to the ordinates at the minimum and maximum temperatures. The authors found that the equation known as the " Pearl-Verhulst logistic equation " (Pearl[25]), adequately described the shape of the curve. One of the constants in the formula was of the nature of a temperature coefficient and hence it was possible to derive a truly constant temperature coefficient for each phenol concentration. The values of the new temperature coefficient did not vary greatly with phenol concentrations within the range studied.

Summary

1. It has been proposed that LT 50 be used in place of the extinction time in the adoption of Phelps' equation[8] for the determination of temperature coefficients.

2. The temperature coefficients of ethylene glycol and its monoalkyl ethers have been found somewhat large, the monoethyl ether exceptionally so. These high values have been used as evidence against the thesis that disinfection and chemical processes are analogous.

3. The temperature coefficients have been shown to vary with the concentrations of disinfectant used for their calculation; the variation of the concentration exponent with temperature has been suggested as an explanation of this phenomenon.

4. Reference has been made to the proposal of Jordan and Jacobs[24] of a more satisfactory temperature coefficient which does not vary with the temperature of the disinfection concentration.

TABLE I

VALUES OF THE TEMPERATURE COEFFICIENTS BETWEEN 20°C. AND 30°C. FOR CONCENTRATIONS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS

Compound	Concentration	Q_{10}	θ
	per cent.		
Ethylene glycol	70·0	18·00	1·334
	80·0	24·17	1·375
Monomethyl ether	40·0	45·37	1·464
	50·0	35·83	1·430
Monoethyl ether	20·0	291·00	1·764
	25·0	113·10	1·604
Monopropyl ether	7·0	16·09	1·320
	8·0	7·24	1·222
Monobutyl ether	3·0	32·15	1·515
	3·5	27·10	1·391
Monohexyl ether	0·30	12·26	1·284
	0·45	10·25	1·262

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Ascaridol, in Chenopodium Oil, Determination of. H. Lepetit. (*Trav. Lab. Mat. Med.*, 1943-5, **32**, Part 2.) The method of the French Codex for the determination of ascaridol in oil of chenopodium gives very consistent results, but the results are inaccurate, as the factor used was determined on pure ascaridol, whereas actually the factor varies according to the concentration of ascaridol. The method recommended is as follows. Dissolve a weighed quantity of the oil, between 2·50 and 2·60 g., in sufficient acetic acid to make a total volume of 50 ml. Place a flask, containing 3 ml. of 83 per cent. solution of potassium iodide with 5 ml. of concentrated hydrochloric acid and 10 ml. of acetic acid, in an ice bath. When this has cooled (after about 6 to 8 minutes), add, with shaking, 5 ml. of the chenopodium solution, close the flask, and allow to stand in ice for 5 min. Add 5 ml. of carbon tetrachloride, and titrate the iodine liberated with 0·1N sodium thiosulphate until the aqueous layer is decolorised. Carry out a blank experiment without the oil, adding finally 20 ml. of water before titration. The cooling conditions for this test should be the same as for that with the oil. If N and n are the volumes of thiosulphate solution used for the test and the blank respectively, and a is the weight of sample taken, then the percentage of ascaridol in the oil is given by the formula $0{\cdot}605\ (N-n)/a$. The limits suggested for the official oil are from 60 to 80 per cent. of ascaridol. G. M.

Aspirin, Phenacetin and Caffeine, Infra-Red Determination of. W. H. Washburn and E. O. Krueger. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 623.) A method suitable for the determination of aspirin, phenacetin and caffeine in commercial tablets, etc., depends upon the presence of absorption peaks in the spectra of these substances at 9·27μ, 8·99μ, and 10·26μ respectively. In each case, the absorption due to the remaining 2 substances is a minimum. A quantity of powder, chosen so as to bring the absorption at each of the 3 wave-lengths within the working-range of the spectrometer, is suspended in water and extracted with chloroform. The optical density is determined at each of the 3 given wave-lengths. Reference is made to working curves showing the absorption of aspirin, phenacetin and caffeine at each of these wave-lengths and the proportion of each ingredient is determined approximately, by ignoring the absorption due to the other 2 ingredients whose absorption is not maximal. These approximate figures are used to make a more accurate computation in which allowance is made for absorption due to all 3 ingredients at each wave-length, the exact answer being arrived at by successive approximation. The determination is reproducible within ± 2 per cent., and can be completed within 4 hours. G. B.

Calcium and Magnesium, Separation by Oxalate Method. T. Holth. (*Anal. Chem.*, 1949, **21**, 1221.) For the determination of calcium as oxalate in solutions containing calcium ions the concentration of the solution should be about 0·01M and the precipitates should stand for 4 hours at room temperature before filtration. To obtain quantitative precipitation of calcium oxalate the solution must not contain even relatively small amounts of a strong acid (calcium oxalate cannot be precipitated quantitatively from

a solution containing free oxalic acid) although to obtain precipitates with a particle size convenient for filtration the precipitation should be carried out in neutral or in an acetic acid medium. After precipitation the solution is filtered and the precipitate is washed with hot ammonium oxalate solution 3·0 g./l.), dried at 105°C. for half an hour, and then heated in an electric furnace at 480° to 500°C. to constant weight (40+20 minutes). When calcium is precipitated as oxalate from solutions containing calcium chloride and magnesium chloride, some magnesium oxalate is always occluded. The precipitate should therefore be dissolved in hydrochloric acid and reprecipitated with ammonia; a large excess of ammonium oxalate prevents the precipitation of magnesium from supersaturated magnesium oxalate solutions. In samples containing less than 25 per cent. of magnesium the precipitation can be carried out by neutralising a hydrochloric acid solution of the calcium ion and an excess of oxalate ion with ammonia; ammonium chloride should not be added. For samples containing from 25 to 90 per cent. of magnesium an amount of ammonium oxalate (ten times the amount of magnesium present but not less than 3 g.) is dissolved in 200 ml. of water, the solution is cooled to room temperature, and enough acetic acid is added to make the solution 0·05M. The solution under test containing 100 to 150 mg. of calcium plus magnesium is added, the beaker is then placed on a steam bath for 15 minutes, with stirring, and is then allowed to stand for 4 hours at room temperature after which the filtration is carried out as described previously. R. E. S.

Cinchona and Nux Vomica, Assay of, by Microsublimation. J. A. Zapotocky and L. E. Harris. (*J. Amer. pharm. Ass.*, 1949, **38**, 557.) Heating plant material containing alkaloidal salts produces only minute amounts of crystalline sublimate, and treatment with an alkaline agent such as sodium carbonate is first necessary. A number of alkaloidal bases and the corresponding salts were wetted with sodium carbonate solution to form a paste, dried, powdered, and approximately 25 mg. subjected to microsublimation in a tube (17 cm. long, 7 mm. in diameter) at 5 mm. pressure. The sublimates obtained from the alkaloids were compared microscopically with the sublimates obtained from the respective alkaloids and alkaloidal salts treated with sodium carbonate and in each instance the crystals were found to be identical in shape. Using this procedure the sublimation temperatures of a number of alkaloids were determined and also the temperatures at which alkaloids sublimed from cinchona and nux vomica. In the quantitative determination of the total alkaloids of cinchona, a known weight of dried sample, in No. 60 powder was mixed with anhydrous sodium carbonate in the ratio of 3 to 1. Sufficient distilled water was added to wet the mixture which was then triturated in a mortar and dried in a warm desiccator. The dried mixture was again powdered and approximately 50 mg. was placed in the closed end of a sublimation tube. A piece of cotton wool was inserted over the sample and sublimation was carried out at 5 mm. pressure and through a temperature range of 140° to 175°C. Heat was applied until crystals no longer appeared. The percentage of total alkaloids obtained by this method was 3·25 per cent. against an average of 3·88 by the N.F.VIII assay. If the N.F.VIII assay residue of total alkaloids (including insoluble portions and colouring matter) was purified by treatment with dilute sulphuric acid and activated charcoal followed by extraction with chloroform, an average result of 3·28 per cent. was obtained which was close to the result of 3·25 per cent. obtained by microsublimation. A similar microsublimation assay of nux vomica gave results of 1·26 and 1·22 per cent. alkaloids whereas the N.F.VIII

assay gave results of 1·33, 1·28 and 1·26. It is considered that the micro-sublimation procedure gave results which were more nearly a true determination of the alkaloids present. R. E. S.

Codeine and Diamorphine, Determination of, in Admixture. C. Stainier and J. Bosley. (*J. Pharm. Belg.*, 1947, **2**, 218.) The method is devised specially for the determination of codeine and diamorphine in pilules, containing also terpin hydrate. A quantity of powdered material corresponding to 10 pilules, is treated with 8 ml. of water and 2 ml. of 20 per cent. solution of sodium hydroxide. The mixture is heated, under a reflux condenser for 30 minutes on the water-bath, and transferred to a separating funnel, the solution being rinsed in with 12 ml. of water. It is then extracted in succession with 30, 30, 20, 10 and 10 ml. of chloroform. The chloroformic solution is dehydrated with sodium sulphate, filtered and evaporated to dryness. The residue of codeine is taken up in a mixture of 15 ml. of neutral alcohol and 15 ml. of water, and the solution is titrated with 0·1N hydrochloric acid, using methyl red as indicator. The titration is checked by adding 5 ml. of the acid in excess, and titrating back with alkali. One ml. of 0·1N acid is equivalent to 0·0317 g. of codeine (1 H_2O), or 0·0433 g. of codeine phosphate (2 H_2O). The alkaline solution which has been extracted with chloroform is treated with 2 g. of ammonium chloride, and extracted with 40, 30, 20, 10 and 10 ml. of a mixture of 3 volumes of chloroform and 1 volume of isopropyl alcohol. After dehydration with sodium sulphate, the solution is evaporated to dryness and the residue is dissolved in N hydrochloric acid so that 1 ml. of the solution contains about 1 mg. of diamorphine. To 1 ml. 0·2 ml. of 10 per cent. solution of sodium nitrite, 0·2 ml. of 25 per cent. hydrochloric acid, and water to 5 ml. are added. After 20 min., 1 ml. of 30 per cent. sodium hydroxide solution is added and the colour is determined photometrically in a 10 mm. cell, using filter S47. The amount of diamorphine is then determined from a standardisation curve prepared with morphine hydrochloride, the factor 1·127 being used to convert morphine hydrochloride into diamorphine hydrochloride. G. M.

Ethyl Alcohol in Ethyl Ether, Determination of. J. Lamond. (*Analyst*, 1949, **74**, 560.) The method recommended is as follows. Extract 50 ml. of the ether in a separating funnel with three 15 ml. quantities of water and transfer the combined extract to a 50 ml. Nessler cylinder. Into a second cylinder measure 40 ml. of distilled water and 5 ml. of the reagent (667 g. of ceric ammonium nitrate, $(NH_4)_2Ce(NO_3)_6$, dissolved in the minimum quantity of water, made up to 1 litre and filtered). Add 5 ml. of the reagent to the cylinder containing the aqueous extract and rapidly titrate the contents of the second cylinder with a 3 per cent. w/v solution of alcohol in water. When the colour of the blank almost matches that in the original tube, adjust the volume of liquid in the two tubes to the same level before completing the titration. When the colour is matched the alcohol content of the ether, g./100 ml., equals (ml. of 3 per cent. alcohol required × 0·06). Extraction of alcohol was complete under the conditions of the method and estimation of quantities between 0·10 and 0·20 per cent. was satisfactory. The end-point could be judged to within 0·1 ml., corresponding to an error of 3 per cent. on 0·10 per cent. of alcohol; 0·01 per cent. of alcohol could be detected. Alcohol-free ether could be prepared by washing with a 10 per cent. solution of sodium chloride. The colour is given by all the lower alcohols and interference is caused by aldehydes and amines. R. E. S.

Gallic Acid and Pyrogallol, Paper Partition Chromatography of. S. Rydel and M. Macheboeuf. (*Bull. Soc. Chim. biol.*, 1949, **31**, 1265.) It has been shown that ions resulting from the dissociation of alkaloids cause difficulties when the method of paper partition chromatography is applied to their solutions, but that this difficulty can be overcome by adding sufficient ammonia to repress the ionisation. It is now shown that with acids also, the ions are absorbed on the paper and produce tracks which confuse the chromatograms. The trouble may be avoided by using solvent phases which contain an acid which represses the ionisation. The tests were carried out on gallic acid and pyrogallol, and the spots were developed with ferric chloride or ammoniacal silver nitrate. In *n*-butyl alcohol saturated with water, pyrogallol, which has no acid function, gave well-defined spots, while with gallic acid there were elongated tracks. Both compounds gave satisfactory results in an acid solvent prepared by shaking 4 volumes of butyl alcohol with 5 of water and 1 of acetic acid, and removing the aqueous layer. In alkaline medium (collidine) the phenolic function is ionised and unsatisfactory results are obtained with both compounds. G. M.

Hydrastis, Use of Selective Adsorption in the Analysis of. J. Mendez, E. R. Kirch and R. F. Voigt. (*J. Amer. pharm. Ass.*, 1949, **38**, 538.) The proposed assay is based on the separation of hydrastine from berberine by adsorption on florisil (synthetic magnesium silicate) columns, with selective elution with ammoniacal alcohol followed by spectrophotometric estimation using the colour produced in hydrastine solutions by oxidation with potassium dichromate and sulphuric acid. An extraction of the crude drug is carried out according to the N.F.VIII method to the point where a measured quantity of ethereal extract is taken for analysis. An aliquot of this extract is warmed on a water-bath with 10 ml. of 0·5N sulphuric acid. The resulting acid solution of the alkaloids is quantitatively transferred to a glass wool filter and passed through the prepared adsorption column. The column is washed with 300 ml. of distilled water and then with 50 ml. of ether followed by elution with 100 ml. of ammoniacal alcohol, the ether fraction and the eluate being combined and evaporated to dryness. The residue is dissolved in 0·1N sulphuric acid, aliquot portions of this solution are taken in a 25 ml. flask and 4 ml. of 1 per cent. potassium dichromate solution is added, followed by 5 ml. of concentrated sulphuric acid. This mixture is thoroughly shaken and allowed to stand for 5 minutes in order to develop the maximum colour. Distilled water is added to the final volume, and the light absorption is measured at 600 mμ against a reagent blank. Any berberine present in the eluate aliquot is estimated directly by means of spectrophotometric readings at 430 mμ. Standard curves for hydrastine and berberine are given. It is claimed that the procedure simplifies the purification of the hydrastine; results obtained are lower than those from an N.F.VIII assay on the same sample of hydrastis, the difference representing the other ether-soluble extractives included in the official assay. The average amount of hydrastine in the sample of hydrastis was found to be 2·51 per cent. as compared to 2·67 per cent. when determined by the official N.F. method. R. E. S.

Pentavalent Antimony in the Presence of Pentavalent Arsenic, Polarographic Determination of. I. M. Kolthoff and R. L. Probst. (*Anal. Chem.*, 1949, **21**, 753.) Polarographic measurements on the reduction of pentavalent antimony showed two waves at hydrochloric acid concentrations equal to or greater than 6N, the first wave corresponding to a reduction of Sb^{V} to Sb^{III} and the second to that of Sb^{III} to Sb amalgam. With

concentrations lower than 4N only one wave was observed, the height decreasing with decreasing acidity. No reduction wave was observed in an alkaline medium. The reduction of Sb^{V} to Sb^{O} was complete in a medium of N hydrochloric acid and 4·0 M potassium bromide; one wave only was observed, the anodic bromide wave occurring at a more negative potential than that of the first reduction wave of Sb^{V} to Sb^{III}. The half-wave potential in the bromide-containing medium did not correspond to the true half-wave potential of Sb^{V} to Sb^{O} because this potential is also shifted to a more negative value by the anodic bromide current when the bromide concentration was greater than 2·0 M. The diffusion current constant in the presence of 4·0 M bromide was found to be 7·48 while in 6N hydrochloric acid it was 7·50, indicating that the reduction was complete in 4·0 M bromide. Reduction waves of As^{V} were not observed in solutions containing less than 4N hydrochloric acid or in solutions of 4N potassium bromide and N/1 hydrochloric acid. Waves of small height were found in 6 to 8N hydrochloric acid and this medium is not suitable for the determination of Sb^{V} in the presence of large amounts of As^{V}. The polarographic behaviour of trivalent arsenic and antimony was examined and the characteristics of the waves were discussed; both gave typical anodic waves in 0·1 to 0·5N potassium hydroxide. R. E. S.

Procaine, Photometric Determination of. C. Lapiére. (*Anal. chim. Acta,* 1947, **1**, 337.) The solution of procaine, containing 0·25 to 1·25 mg. of procaine in 0·5 per cent. hydrochloric acid, is cooled in ice and treated with 0·1 ml. of 10 per cent. sodium nitrite solution. After 2 minutes 2 ml. of a 1 per cent. solution of βnaphthol in 10 per cent. sodium hydroxide solution is added. After 30 minutes, 2 ml. of alcohol is added and the volume is made up to 10 ml. with water. The colour is determined, using filter S47. When applied to tablets, the procaine is extracted with peroxide-free ether in presence of ammonia; from ointments, it is extracted by 0·5 per cent. hydrochloric acid from a light petroleum solution. G. M.

Quinine, Spectrophotometric Determination of. C. V. St. John. (*Bull. nat. Form. Comm.*, 1949, **17**, 208.) The proportion of quinine in aqueous solutions of quinine salts may be determined by measurement of the optical density of a solution at 346 mμ. The quantity of quinine is calculated by reference to a calibration curve prepared by using solutions of a pure quinine salt. Using a Beckman quartz spectrophotometer with a band-width of 1 to 5 mμ and a 1 cm. quartz cell, suitable concentrations are 0·005 to 0·3 mg. of anhydrous quinine dihydrochloride per ml. The solutions should be acidified with a little hydrochloric acid, and the reference cell should contain distilled water. Provided that the *p*H is kept below 2·5, the absorption spectrum is unaffected by the concentration of acid present, and identical curves are obtained with sulphate, hydrochloride and dihydrochloride. Interfering substances can be detected by comparing the absorption spectrum with that of a solution of a pure quinine salt. If the logarithm of the optical density is plotted against wave-length in each case, the curves can be superimposed unless interfering substances are present, in which case special correcting systems have to be developed. Provided the cells are carefully cleaned with chromic-sulphuric acid mixture and care is exercised in making the test dilutions accurately, the results obtained spectrophotometrically agree within 1 per cent. with those obtained by the method of chloroform extraction and weighing. G. B.

Strychnine, Colorimetric Determination of. S. Rolland-Leclercq. (*J. Pharm. Belg.*, 1947, **2**, 283.) The method is based on the colour given by

tetrahydrostrychnine with oxidising agents. The solution, containing 0·1 to 0·5 mg. of strychnine, is placed in a large test-tube, the volume being made up to 5 ml. with water. It is then treated with 5 ml. of concentrated hydrochloric acid and 3 g. of amalgamated zinc, and heated on the water-bath for 20 minutes. After cooling, the mixture is made up to 10 ml. and treated with 2 drops of 0·1 per cent. solution of sodium nitrite. After 5 minutes the red colour is determined, using a 10 mm. cell and 520 or 440 filter. The colour given by similar quantities of brucine under these conditions is yellow and much weaker; when using the 520 filter it is negligible. On the other hand, the presence of brucine interferes with the determination of strychnine and produces an irregular increase of the colour due to the strychnine. Thus, in applying the method to mixtures of the two alkaloids, it is necessary first to destroy the brucine by treatment with nitric acid. G. M.

INORGANIC CHEMISTRY

Sodium Citrate, Action of, on Sparingly Soluble Compounds of Calcium and Barium. O. Gengou, P. E. Grégoire, G. Lagrange and J. Thomas. (*Arch. int. Pharmacod.*, 1950, **81**, 61.) Sparingly soluble compounds, such as barium carbonate and sulphate, and calcium oxalate, are more crystalline when produced from less concentrated solutions. Most of these can be dispersed in water by sodium citrate, but generally the more crystalline forms are much less easily dispersed. Sodium citrate is also able to prevent the precipitation of compounds of this type, or, in lower concentration, it modifies fundamentally the microscopic appearance of such precipitates. Independently of the dispersing action, trisodium citrate dissolves a certain quantity of these salts, the amount depending on their nature, the concentration of citrate, and the temperature. Dispersion and dissolution appear to be different phenomena, in which one part of the citrate is used to dissolve the precipitate, while the remainder assists the dissolution of the residue. The resulting solutions have the character of true solutions, following an interchange of ions betwen the trisodium citrate and the barium or calcium compound. In some cases the compound formed crystallises.

G. M.

ORGANIC CHEMISTRY

Oxophenarsine Hydrochloride (Mapharsen), Decomposition Products of. C. K. Banks. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 503.) Arsenicals with the 3-amino-4-hydroxyphenyl structure being known to decompose under unfavourable conditions, investigations were made into the types of decomposition undergone by oxophenarsine as the hydrochloride and as the free base in solid form, as well as solutions of these compounds at various *p*H levels. The oxidative and hydrolytic decompositions under a variety of conditions were studied and the products resulting were determined quantitatively and examined for possible toxicity. Various solid forms of the drug were also investigated. Results showed solid oxophenarsine hydrochloride to be extremely stable when anhydrous, showing no appreciable decomposition in 5 years, but with increasing water content stability decreased. Oxygen did not react with the hydrochloride but was liable to act on hydrolytic products giving coloured oxidation products. Solid hydrated oxophenarsine hydrochloride and oxophenarsine were decomposed to *o*-aminophenol and inorganic arsenic compounds in the absence of oxygen. With oxygen, part of the *o*-aminophenol was converted to iminoquinone intermediate which reacts with more of the aminophenol to produce non-arsenated

*iso*phenoxazones. Solutions of oxophenarsine without oxygen decomposed slowly in the cold, more rapidly with heat, to *o*-aminophenol and inorganic arsenite. In the presence of oxygen, *o*-aminophenol was oxidised and below *p*H 7 condensed with unoxidised o-aminophenol giving non-arsenated *iso*phenoxazones. Above *p*H 7 the *o*-aminophenol was oxidised to give, as final products, arseno*iso*phenoxazones. The decomposition products of oxophenarsine hydrochloride were found to be less toxic than the drug itself, when injected intravenously into rats. G. R. K.

Oxophenarsine Hydrochloride (Mapharsen), Decomposition Rate of. C. K. Banks. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 509.) The rate of decomposition of solid oxophenarsine hydrochloride was found to depend on the moisture content and on temperature, the extent to which it occurred being limited by, and in proportion to, the amount of water available. When anhydrous, the substance decomposed only slightly over a 5-year period at temperatures ranging from 20° to 70°C. Oxophenarsine hydrochloride containing moisture to the extent allowed by the U.S.P. had a decomposition rate approaching a limit of the equivalent of arsenical decomposed for each equivalent of water present. Since the amount of water permissible is small, the maximum decomposition is within the tolerance for the product and is too small to be detected by pharmacological methods. At higher temperatures than the product would encounter the amount of arsenical decomposed for a given moisture content is doubled. Commercial ampoules were stable for 4 years. Solutions of the hydrochloride decomposed by a unimolecular mechanism in a first-order reaction. At a *p*H of about 3·25 the rate was at a minimum. Solutions of *p*H 6 were stable enough for clinical purposes but not for use over a long period of time. When maintained at room temperature or below, solutions made from multiple dose packages were adequately stable for 3 days. Diffused light did no harm but sunlight was deleterious. Such solutions should be kept under a vapour proof stopper to prevent evaporation and subsequent overdosage. G. R. K.

TOXICOLOGY

Morphine, Codeine and Diamorphine from Viscera, Isolation and Determination of. A. Stolman and C. P. Stewart. (*Analyst.*, 1949, **74**, 536.) The use of florisil as an adsorbent in the isolation of morphine, codeine, and diamorphine is described. In experiments on pure alkaloids 1 mg. of the alkaloid was dissolved in 100 ml. of distilled water and the solution was run through a column of untreated florisil. The adsorbed alkaloid was eluted by refluxing with methyl alcohol for 1 hour. Quantitative determination of the eluted alkaloid showed an apparent recovery of 105 to 120 per cent. with morphine or codeine, and 94 per cent. with diamorphine. In order to avoid large quantities of solvent, elution of the alkaloids was carried out from a glass tube containing the florisil in a modified Soxhlet type apparatus. Solid sodium carbonate was placed on top of the adsorption column before elution and oxalic acid was added to the solvent flask to prevent decomposition of the alkaloids in an alkaline solution. Recovery of alkaloids was then substantially complete from *p*H 6·0 to 8·0. Above *p*H 8·0 the morphine was not completely adsorbed; below *p*H 6·0 adsorption of morphine and diamorphine was incomplete, but codeine was still adsorbed at *p*H 4·0, though not at *p*H 2·0. A description is given of initial trial work which indicated that the method could be adapted for the isolation of the alkaloids from dilute alcoholic (Stas-Otto), acidified aqueous (Dragendorff) or from trichloracetic acid (Stewart) extracts of biological tissues. For tissues 100 g.

of liver were macerated in a Waring blender with 200 ml. of alcohol (95 per cent.) acidified with tartaric acid; the mass was mixed with 300 ml. of alcohol and then filtered. Each volume of filtrate was mixed with 4 volumes of 5 per cent. w/v aqueous trichloracetic acid solution and filtered, the filtrate being used for adsorption experiments after the adjustment of the *p*H and the addition of specified amounts of alkaloid. Trichloracetic acid extracts were made by macerating 100 g. of liver with 200 ml. of 10 per cent. w/v trichloracetic acid solution; each volume of filtrate was mixed with an equal volume of water to reduce the concentration of trichloracetic acid to 5 per cent.; each 100 ml. of the 5 per cent. trichloracetic acid solution was mixed with 25 ml. of alcohol (95 per cent.) and then used for adsorption. For blood analyses, 1 volume of blood was mixed with 1 volume of a 10 per cent. w/v aqueous solution of trichloracetic acid and one-half volume of alcohol (95 per cent.); the coagulated proteins were removed by filtration and the filtrate then used for adsorption experiments after the adjustment of the *p*H. Urine was mixed with an equal volume of water and half of its volume of alcohol (95 per cent.), the solution being filtered if necessary and the *p*H adjusted. In the recovery of alkaloids added to tissue extracts it was found that the optimum *p*H found previously for pure aqueous alkaloidal solution gave low results and adjustment of the *p*H to 8·0 to 9·0 was necessary. With this method morphine, codeine, or diamorphine, in amounts ranging from 0·5 to 5·0 mg., contained in 100 to 250 ml. of alcohol or trichloracetic acid extract of tissue, blood or urine, could be quantitatively adsorbed and quantitatively eluted by methyl alcohol. The eluted alkaloids were readily identifiable by the purity of their colour reactions. The determination of the alkaloids was effected by modifications of standard colorimetric methods. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Alvein. K. Gilliver, A. M. Holmes and E. P. Abraham. (*Brit. J. exp. Path.*, 1949, **30,** 209.) Alvein is a new antibiotic formed by *Bacillus alvei* in certain liquid media. It inhibits the growth of a number of Gram-positive and Gram-negative bacteria. Production was obtained by deep fermentation in a medium containing glucose, corn steep liquor and inorganic salts. Chemically the preparation behaved as if it consisted mainly of a strongly basic polypeptide. It is hæmolytic. H. T. B.

Ergothioneine: Preparation from Blood and Ergot. G. Hunter, G. D. Molnar and N. J. Wight. (*Canad. J. Res.* (*E*), 1949, **27,** 226.) The yields from pig blood were very much lower than those reported earlier, 30 gallons giving less than 2 g. Although this variation may be due to dietary factors, the lack of a reliable method of determining ergothioneine in blood precludes an adequate explanation. Ergothioneine was prepared from ergot in 0·26 per cent. yield by the following method. 500 g. of ergot in fine powder was extracted by boiling successively with 2000 ml. of water containing 1 ml. of glacial acetic acid, and two quantities, each of 1000 ml. of water, straining and pressing between each boiling. The filtrate was treated with a slight excess of a saturated aqueous solution of uranium acetate, allowed to stand and the supernatant liquid removed. The residue was washed twice with water and the combined liquid and washings made acid (0·5N) with sulphuric acid and heated to 60°C. A slight excess of an aqueous suspension of cuprous oxide was added, the mixture shaken, allowed to

stand, and the supernatant liquid removed. The copper precipitate was washed three times at 60°C. with its own volume of 0·5N sulphuric acid containing a little copper. The washed copper precipitate was suspended in hot water, treated with hydrogen sulphide and filtered. The residue was washed and the combined filtrate and washings freed from hydrogen sulphide and treated with warm barium hydroxide solution until the solution was neutral on litmus paper. The solution was evaporated *in vacuo* to 15 ml., boiled with charcoal, filtered, and the filtrate evaporated *in vacuo* to 5 ml. when crystals appeared. After further purification, 1·30 g. of anhydrous ergothioneine was obtained. The twice recrystallised product melts with expansion and decomposition at 280°C. It was found that ergothioneine from blood was frequently contaminated with hypoxanthine. Since free ergothioneine is soluble in water and free hypoxanthine is insoluble they may be readily separated. If, however, an acid radical is present, both are soluble and the purine also forms crystals similar to those of ergothioneine. Although copper oxide precipitates purines as well as ergothioneine, it is an eminently suitable precipitant for obtaining the alkaloid from ergot because the latter contains little or no purine, and nitroprusside reacting SH- groups are absent. It has, however, a tendency to gel formation. G. R. K.

Local Anæsthetics and the Potassium Ion. O. Peczenik and G. B. West. (*Nature*, 1949, **164**, 354.) The action of 5 local anæsthetics (procaine-amylocaine, cocaine, amethocaine and cinchocaine) on the isolated rat phrenici nerve diaphragm was studied, with particular reference to the potassium on., All the preparations were left for 2 hours before use in a bath of 70 ml. of Tyrode solution containing 0·2 per cent. of glucose. From the results of 3-minute contacts it was found first that large doses of the anæsthetics produced neuro-muscular block *per se*, and secondly that whereas the addition of potassium chloride potentiated the inhibition produced by amylocaine and amethocaine it had little or no action on procaine, suggesting a difference of action for this compound. In a further experiment in which the potassium chloride was added to the bath for 3 minutes in the recovery phase, after washing out the anæsthetic, it was found that in the case of amethocaine or cinchocaine this always produced block ; without cocaine or amylocaine it had either no action or produced block, while in the case of procaine it was found that the addition of potassium chloride sometimes aided but never prevented recovery. On the other hand the increase of the refractory period of the rabbit auricles produced by cocaine is potentiated by potassium chloride. S. L. W.

BIOCHEMICAL ANALYSIS

Amidopyrin in Body Fluids, Colorimetric Determination of. R. Pulver. (*Arch. int. Pharmacod.*, 1950, **81**, 47.) The reagent is prepared by treating 2 ml. of a 1 per cent. solution of *p*-nitraniline in N hydrochloric acid with 2 ml. of 1 per cent. sodium nitrite solution. After 2 minutes 2 ml. of 1 per cent. sulphamic acid solution is added, and, after a further 20 minutes, 14 ml. of water. The reagent may be kept for 12 hours. This solution gives, with amidopyrin, an intense yellow colour, which does not appear to be due to coupling. For the examination of body fluids, 2 ml. of plasma, serum or liquor is treated with 1 ml. of acetate buffer solution (56·5 ml. of glacial acetic acid and 118 g. of sodium acetate in water to 1 l.) diluted with 10 ml. of water, and heated for 5 minutes on the water bath. The solution is then filtered into a separating funnel, the filter being washed twice with 2ml. of hot water. After cooling, 1 ml. of 2N sodium hydroxide is added, and the liquid is shaken out with two 20 ml. quantities of ether. The ethereal extract

is evaporated to dryness, and the residue is treated with 2 ml. of 10 per cent. sodium acetate solution, and, after cooling, 0·5 ml. of reagent. After 10 minutes, the mixture is transferred to a separating funnel, being washed in with 2 ml. of 2N hydrochloric acid and then with 2 ml. of water. The colour is shaken out into 10 ml. of chloroform, the chloroformic solution being dried with sodium sulphate prior to photometric determination in a 2 cm. cell using filter S43. G. M.

***p*-Aminosalicylic Acid in Biological Media, Determination of.** M. Pesez. (*Bull. Soc. Chim. biol.*, 1949, **31**, 1369.) The author has shown previously that diazotised *p*-aminosalicylic acid, unlike the corresponding compound of *m*-aminophenol, is very unstable, and passes into *p*-hydroxysalicylic acid. *p*-Aminosalicylic acid itself is easily converted into *m*-aminophenol by warming in acid solution. Thus the method of diazotisation and coupling with *p*-hydroxysalicylic acid (derived from *p*-aminosalicylic acid and nitrite) may be used for the determination of both *p*-aminosalicylic acid and of *m*-aminophenol. The sample containing 10 to 50 μg. of *p*-aminosalicylic acid, is diluted to 2 ml. with water, treated with 1 ml. of 10 per cent. solution of trichloracetic acid, and heated for 30 min. in a water-bath. A similar sample is kept in ice during the same period, then treated with 1 ml. of trichloracetic acid. Each tube, cooled in ice, is then treated with 3 ml. of water, 0·5 ml. of 0·1 per cent. solution of sodium *p*-aminosalicylate ($2H_2O$) and 0·5 ml. of 1 per cent. sodium nitrite solution. After exactly 3 minutes at 0°C. 2 ml. of 10 per cent. solution of anhydrous sodium carbonate is added, then, after 15 minutes at ordinary temperature, the volume is made up to 10 ml. with water. The difference in colour between the two tubes is determined photometrically using filter 43. In the case of blood, serum or plasma, 2 ml. of water and 2 ml. of trichloracetic acid solution are added to 1 ml. and, after shaking, the mixture is diluted with 5 ml. of water, filtered and centrifuged. The test is then carried out on 2 ml. of the filtrate. Urine is diluted to 100 volumes with water, and 1 or 2 ml. taken for the test. G. M.

Ergothioneine: Determination in Simple Solution and in Blood. G Hunter. *Canad. J. Res.E.*), 1949, **27**, 230.) *Simple Solution.* If x ml. is the volume of test solution to be used, 2x ml. of water, 1 ml. of diazo reagent, 2 ml. of alkaline buffer solution and x ml. of test solution are put, in that order, into a properly matched Evelyn photo-electric colorimeter tube, cooled in ice-water and mixed. In the presence of ergothioneine a lemon-yellow colour develops. After 45 seconds the tube is removed from the ice-water, 5 ml. of 10N sodium hydroxide is added and the contents mixed. After 3 to 4 minutes the final purplish pink colour has fully developed and the tube is read in the colorimeter A blank test with 2 ml. of water is used to set the colorimeter at 100 per cent. transmittance. The Evelyn filter No. 520, transmitting in the range 495 to 550 mμ, was found to be suitable. The diazo reagent is prepared as follows. To 1·5 ml. of a solution containing 9 g. of sulphanilic acid and 90 ml. of 37 per cent. hydrochloric acid per l., cooled in ice-water, add 1·5 ml. of 5 per cent. sodium nitrite solution, allow to stand for 5 minutes, add a further 6 ml. of nitrite solution and after a further 5 minutes adjust the volume to 50 ml. with ice-cold water. The alkaline buffer consists of an aqueous solution containing 1 per cent. of anhydrous sodium carbonate and 10 per cent. of anhydrous sodium acetate. *Blood.* To 0·45 ml. of whole blood in a centrifuge tube, add 4·5 ml. of 0·0045 N acetic acid containing 160 mg. of sodium oxalate per l., mix, immerse in water which is just boiling, and stir. Centrifuge, separate the supernatant liquid and to it add 0·05 ml. of lead

subacetate solution. Mix, centrifuge, separate the supernatant liquid and add 1 drop of a 10 per cent. aqueous solution of sodium dihydrogen phosphate. Mix, centrifuge and again separate. The supernatant liquid is the test solution and is assayed by the method described above. The strength of the acetic acid-oxalate solution used depends upon whether whole blood, corpuscles or plasma is being assayed and upon the extent of the dilution of the specimen. Ergothioneine added to whole blood was recovered quantitatively by this method. All plasmas were found to give a significant yellow colour, which was attributed to histidine or tyrosine or both and not to ergothioneine. G. R. K.

Penicillins, Total Determination of, by the Iodimetric Method. Report of the Analysts' Sub-committee of the Ministry of Health Conference on the Differential Assay of Penicillin. (*Analyst,* 1949, **74,** 550.) To express the total penicillins in a crystalline penicillin G sample the basic iodimetric method of Alicino (*Ind. Engng. Chem., Anal, Ed.,* 1946, **18,** 619) as modified by Mundell, Fischbach, and Eble (*J. Amer. pharm. Ass., Sci. Ed.,* 1946, **35,** 373) was used. The method was examined by collaborative work and the following modifications were tried (a) buffered reactants to reduce the effect of non-penicillin impurities to a minimum; (b) different strengths of alkali for inactivation of the penicillin; (c) longer or shorter periods of inactivation and iodination; (d) temperatures of inactivation and iodination ranging from room temperature to 30° C.; (e) higher concentrations of potassium iodide in the standard iodinating reagent. The final process recommended was as follows. Weigh accurately about 60 mg. of the sample, dissolve in distilled water and dilute to 50 ml. Transfer a 10 ml. aliquot to a stoppered flask, add 5 ml. of N sodium hydroxide and allow to stand for 30 minutes in a water-bath at 30° C. Acidify with 5 ml. of 1·1 N hydrochloric acid, add 30 ml. of 0·02 N iodine solution, close the flask with a wet stopper and place it in a water-bath at 30° C. for 15 minutes. Titrate the excess of iodine with 0·01 N sodium thiosulphate solution, adding 1 ml. of starch solution near the end-point. A blank determination is completed by transferring a 10 ml. aliquot of the penicillin solution to a stoppered flask, adding 30 ml. of 0·02 N iodine solution and titrating immediately with 0·01 N sodium thiosulphate. The difference between the two titrations (each should be duplicated) represents the amount of iodine that has reacted with the penicillin, each ml. of 0·01 N iodine being equivalent to 0·382 mg. of sodium benzylpenicillin. R. E. S.

Streptomycin B (Mannosido-streptomycin), Colorimetric Determination of. W. B. Emery and A. D. Walker (*Analyst,* 1949, **74,** 455.) The method developed deals with the assay of streptomycin B in the presence of streptomycin itself; it depends on the determination of streptomycin B from the mannose residue in the molecule using a 0·2 per cent w/v solution of anthrone in 95 per cent. v/v sulphuric acid as used for the quantitative determination of carbohydrates by Morris (*Science,* 1948, **107,** 254). Under the conditions described the glucosamine and streptose moieties in the molecules of both streptomycins did not react with the reagent. A calibration curve was prepared using various solutions of pure mannose (10 to 100 μg. per ml.) which were added to the reagent, allowed to stand for 20 minutes and examined photoelectrically using Chance glass O.R.2 red filters (peak transmission 640 mμ) and 2 cm cells. Experimental details are given for the measurement of the solution under test and at the same time the total streptomycin (in units /ml.) present in the test solution is determined by chemical assay based on the maltol-ferric ion reaction. The molecular proportion of

streptomycin B (i.e., the molecular percentage of the total streptomycin present) in the sample may be calculated from the expression:

$$\text{Molecular proportion of streptomycin B} = \frac{321\,z}{x} \text{ per cent.}$$

where a solution of streptomycin at x chemical units per ml. contains z μg. of mannose per ml. The method described has been applied to a number of samples of streptomycin hydrochloride and streptomycin calcium chloride complex containing from 3 to 95 per cent. of streptomycin B. A table is given of results obtained together with the biological/chemical assay ratios, and the proportions of streptomycin B calculated from these ratios; ratios of 0·2 and 1·0 for pure streptomycin B and streptomycin A respectively were assumed. The results obtained by the method given agreed with those calculated from chemical and biological assays assuming the relative biological activities. R. E. S.

PHARMACY

GALENICAL PHARMACY

Bentonite, Dried, as a Disintegrating Agent in Compressed Tablets of Thyroid. C. B. Granberg and B. E. Benton. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 648.) Tablets of thyroid, 65 mg. were prepared according to 12 different formulæ in order to compare the properties of dried starch and dried bentonite as disintegrating agents. The tablets were examined for appearance, for hardness expressed as kg. of pressure required to fracture the dry tablet in a special hardness tester, and for disintegration, expressed as the time taken for a weight of 20 g. to force a blade to cut through a tablet immersed in acid pepsin solution. A working plan of the apparatus for disintegration tests is given. The use of bentonite instead of starch as disintegrant gives tablets of greater hardness and longer disintegrating time, but tablets having a suitably short disintegrating time are obtained when some bentonite is used as a filler. A suitable formula is:—lactose, 35 mg. and bentonite, 10 mg. as filler; bentonite, 12 mg., as disintegrant; magnesium stearate, 1 mg. as lubricant; 15 per cent. starch paste, q.s. as binder. The presence of bentonite does not interfere with the U.S.P. XIII assay for iodine in thyroid. G. B.

NOTES AND FORMULÆ

Choline Chloride. (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **141**, 391.) Choline chloride, trimethylhydroxyethylammonium chloride, $CH_2OH.CH_2.N(CH_3)_3.Cl$, is white, crystalline and deliquescent, with an amine-like odour. It is soluble in water and alcohol, and insoluble in benzene, chloroform and ether; a 10 per cent. aqueous solution has a *p*H of about 4·65. It gives a pale yellow precipitate with mercuric potassium iodide, and an emerald-green colour with cobaltous chloride and potassium ferrocyanide. If free from neurine it gives a white curdy precipitate with phosphotungstic acid and no precipitate with tannic acid; heavy metals are limited to 30 p.p.m., ash to 0·05 per cent. and moisture to 0·5 per cent. The content of choline chloride is 97 to 101 per cent., calculated with reference to the dried substance, and is determined by titration with silver nitrate and spectrophotometrically. Choline chloride is given by mouth in daily doses of 1·5 to 3 g., but the precise dosage is not yet established. It has the lipotropic action and uses of other choline salts and is also recognised for clinical trial in the treatment of fatty infiltration of the liver. G. R. K.

Furtrethonium Iodide (Furmethide Iodide). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **141,** 264.) Furtrethonium iodide, furfuryltrimethylammonium iodide, $(C_4H_3O.CH_2.N(CH_3)_3.I)$ is a white- to cream-coloured crystalline powder, soluble in water and alcohol but insoluble in benzene; m.pt. 115° to 119°C. A 1 per cent. aqueous solution has *p*H5·3 to 6·0. With an acidified saturated solution of trinitrophenol it gives a yellow precipitate which melts at 167° to 170°C. It is assayed for nitrogen and iodide and contains 97 to 101 per cent. of furtrethonium iodide. When dried *in vacuo* over phosphorus pentoxide for 4 hours, the loss in weight is not more than 0·5 per cent. Standards are also given for tablets and ampoules. Furtrethonium idodide is similar in action to acetyl-β-methyl choline but is more effective when given by mouth and less active, when given by injection, on the cardiovascular, respiratory and gastrointestinal systems. It is given by mouth or by subcutaneous injection to stimulate micturition; it should not be given intravenously. For normal adults an initial dose of 3 mg. is usually effective within 15 minutes.

G. R. K.

Mephobarbital (Mebarol). (*New and Nonofficial Remedies: J. Amer. med. Ass.*, 1949, **141,** 920). Mephobarbital, *N*-methyl-5-ethyl-5-phenylbarbituric acid is methylphenobarbitone. It occurs as white, tasteless, odourless crystals, m.pt. 177° to 181°C. and soluble in chloroform and solutions of alkali hydroxides and carbonates, but only slightly soluble in water, alcohol and ether. The precipitate obtained on adding silver nitrate or mercuric chloride to a solution in sodium hydroxide is soluble in ammonia. It may be distinguished from barbitone by heating in a water-bath for 30 minutes with potassium nitrate and sulphuric acid, cooling, diluting with water, adding sufficient ammonia to make alkaline, heating to expel gas, cooling, filtering and adding to the filtrate ammonium sulphide, when a dark brown-red ring forms; the precipitate obtained when the filtrate obtained in the same manner is treated with dilute sulphuric acid melts at about 220°C. (distinction from phenobarbitone). When assayed by dissolving in alcohol and titrating electrometrically against sodium hydroxide, it contains 99 to 101 per cent. of mephobarbital; the nitrogen content determined by the Kjeldahl methods is 11·15 to 11·50 per cent. Mephobarbital is a long-acting barbiturate with a comparatively mild hypnotic action and is therefore more suitable as a sedative than as a sporific. It is chiefly used as an anticonvulsant in the treatment of epilepsy of the grand mal and petit mal types. G. R. K.

Naprylate Caprylic Compound. (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **141,** 992.) Caprylic compound is a mixture of sodium caprylate and zinc caprylate used for the treatment of superficial fungus infections of the skin and accessible mucous membranes. It is effective against infection due to trichophytons, microsporons and *Monilia albicans.* In moderate concentrations, it does not irritate the skin and is not absorbed from the skin or mucous membranes. Sodium caprylate occurs as cream-coloured granules soluble in water and sparingly soluble in alcohol. It contains 98 to 102 per cent. of sodium caprylate when assayed by the method for sodium propionate N.F.; when dried at 105°C. for 2 hours it loses not more than 2·5 per cent. of its weight. Zinc caprylate occurs as a fine white powder, insoluble in water and alcohol. When dissolved in dilute sulphuric acid and assayed by the method for zinc sulphate U.S.P., it contains 95 to 100 per cent. of zinc caprylate, calculated with reference to the dried substance; the loss on drying at 105°C. for 2 hours is not more than 3 per cent.

Naprylate is supplied as an ointment and powder containing 10 per cent. of sodium caprylate and 5 per cent. of zinc caprylate in a water miscible base and a mixture of starch and talc respectively, and as suppositories. G. R. K.

Phenylpropylmethylamine Hydrochloride (Vonedrine Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **141,** 133.) Phenylpropylmethylamine hydrochloride, *dl*-1-methylamino-2-phenylpropane hydrochloride, $C_6H_5.CH(CH_3).CH_2.NH.CH_3,HCl$, is not available in the dry state. It is supplied as a clear, colourless, nearly odourless, aqueous solution with *p*H 5·5 to 6·5, from which the hydrochloride may be prepared as fine white crystals, by the following method. Make the solution alkaline with sodium hydroxide, extract with ether, thoroughly dry with anhydrous sodium sulphate, remove the ether, dissolve the residue in benzene, dry the solution with anhydrous sodium sulphate, pass in dry hydrogen chloride and allow to crystallise. After filtration, washing with dry benzene and dry ether, and drying by air suction, the crystals melt at 147° to 150°C.; they are soluble in water, alcohol, ether and hot benzene. Phenylpropylmethylamine hydrochloride is a local vasoconstrictor with little or no stimulating action on the cardiovascular or central nervous system. It is usually applied as a 2·8 per cent. solution as nasal drops, spray, or tampons. G. R. K.

PHARMACOGNOSY

Belladonna and Datura Species, Post-Harvest Alkaloidal Movement in. W. R. Brewer and L. D. Hiner. (*J. Amer. pharm. Ass.*, 1949, **38,** 541.) An investigation has been made into the differences observed in the alkaloidal content of leaves cured after separation, or on intact belladonna plants; and also to assess the value of the normal drying method of hanging whole herbs. Studies were carried out from 1942 to 1947 on *Atropa belladonna*, L., the plants after harvesting providing leaf and stem parts which were separated and cured on drying trays, and also parts which were cured intact by impaling the whole plant on racks and separating leaf from stem after drying. The possibility that alkaloids could be transferred from the roots to other parts of the plant was investigated by dividing a harvest into two parts. The whole plants from one part were cured by hanging the entire plant, including roots, stems and leaves, tops downward from drying racks. Those from the other part of the same harvest were cured by drying their roots, stems and leaves in three different drying trays. Plants were also cured while remaining in an upright position, to investigate the action of gravity on any alkaloidal transference. All the results obtained were statistically analysed and it was found that the drying of intact leaves on the plant increased their alkaloidal content as compared with leaves which were dried separately; this effect was not due to the position of the plant during the drying procedure nor to gravity. The increase in alkaloidal content of the leaf was accompanied by a decrease in the alkaloids of the root or stem, suggesting relocation. The effect was found in at least 4 species of Solanaceous plants, *Atropa belladonna, Datura stramonium, Datura inermis,* and *Datura tatula.* R. E. S.

PHARMACOLOGY AND THERAPEUTICS

Aconite Tincture, Biological Assay of. M. Barr and J. W. Nelson. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 518.) A method is described for the quantitative assay of aconite tincture, using intravenous injections in mice, in which the comparison of LD50 doses of the Reference Standard

Aconitine solution and of diluted tinctures was used as a basis. The standard error of each series of assays was found to be less than 2 per cent. Aconitine is one of the most potent alkaloids known. Hence, any assay procedure giving a product of variable toxicity is dangerous. This is largely responsible for the fact that aconite has fallen into disuse. The tincture was chosen to be studied since it is the most used aconite preparation in medicine. Objections to the official guinea-pig method, though recommended for its constancy, were its lack of sensitivity and the time factor and expense involved. With mice, a simple and economical biological assay is possible, and the use of a 90-minute observation period reduces the time required to perform a complete assay as compared to the present N.F.VIII method. It is more accurate than the present guinea-pig method, and should also be applicable to other preparations of aconite. G. R. K.

9-Aminoacridinepenicillin, Toxicity of. D. C. Brodie and Elizabeth Lowenhaupt. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 498.) 9-Aminoacridinepenicillin is a bright yellow, crystalline powder, almost insoluble in water and soluble in alcohol, glycerin and propylene glycol. One mg, contains about 1,000 units of penicillin and 0·3 mg. of 9-aminoacridine. In general it resembles 9-aminoacridine in its toxic effects, although there is some modification. Thus, its LD50, when given to mice subcutaneously, is 250 mg./kg. expressed as 9-aminoacridine hydrochloride compared with 70 to 80 mg./kg. for 9-aminoacridine. Similarly, when given orally, the LD50 is equivalent to 100 mg. of 9-aminoacridine hydrochloride per kg. as compared with 78 mg./kg. for 9-aminoacridine. This may be due to the lower water-solubility of 9-aminoacridinepenicillin, to 9-aminoacridine being less readily available to the tissues, to the existence of the drug in solution as a colloidal electrolyte, or to a modification in the inherent toxicity of 9-aminoacridine. 9-aminoacridinepenicillin produces local tissue necrosis with œdema and inflammation when administered intramuscularly, subcutaneously or to scarified skin. G. R. K.

Ammonium Nitrate for Premenstrual Intoxication. E. J. Stieglitz and S. T. Kimble. (*Amer. J. med. Sci.*, 1949, **218**, 616.) Many authors have published suggestions as to the ætiology of a premenstrual syndrome characterised by one or more of the following symptoms: depression, irritability, abdominal fullness, headache, nausea, backache or mammary hyperæsthesia. While disagreement exists as to the specific physiological mechanism involved, excessive production of ovarian steroids or increased sensitivity to them is considered to be the main factor and on this basis the authors administered as a diuretic enteric coated capsules of ammonium nitrate, each containing 1 g., 1 capsule being given 3 times a day with a liberal fluid intake. The nitrate was given because of its greater diuretic efficiency than the chloride and to avoid increasing the chloride content of the tissues. Treatment was given for the 10 days preceding each menstrual period. Dramatic improvement occurred in 61 out of 67 patients treated, the maximum benefit frequently being observed after 2 or 3 months. H. T. B.

Artane: A New Antispasmodic. M. Canelis, F. J. Farnell and T. H. McGavack. (*Amer. J. med. Sci.*, 1949, **218**, 655.) Artane is 3-(1-piperidyl)-1-phenyl-1-cyclohexyl-1-propanol hydrochloride and belongs to a new series of antispasmodic compounds. Its pharmacological properties in animals suggested trial in parkinsonism. On isolated intestinal loops its relaxant effect is 20 times that of trasentin and half that of atropine, but its excitant action on the central nervous system is much less than that of atropine. It has a feeble antihistamine activity. Twenty-three patients with

parkinsonism were treated with the new compound for periods varying from 10 to 46 weeks, their age varying from 30 to 78 years. All but two received 2 mg. of artane three times daily; the two received respectively 2·5 mg. and 3 mg. Within 3 days 21 of the 23 were much improved, and those previously taking atropine or hyoscine were as well as on maximum tolerated doses of the alkaloids. Within 2 weeks there was improvement over all previous treatments but thereafter no increased benefit resulted even with increased dosage, although the improvement was maintained indefinitely, or at least for the 10 months of the trial without signs of tolerance appearing. Accompanying the objective improvement was an increase in the sense of well-being in 19 patients. Three were not improved in emotional status and one became much confused. Replacement of the drug by a placebo caused dramatic relapse within 3 days but the former condition was restored on resuming the drug. The effective safe daily dose is between 6 and 15 mg.; larger doses tend to produce blurring of vision. H. T. B.

***l*-Ephedrine Salt of Penicillin G (Tersavin).** G. Gunberg, L. O. Randall and R. J. Schnitzer. (*J. Pharmacol.*, 1949, **95,** 336.) Tersavin, which has the empirical formula, $C_{16}H_{18}O_4N_2S$, $C_{10}H_{15}ON$, is a white crystalline powder with a melting-range of 135° to 137°C. (decomp.). The specific optical rotation $[\alpha]_D^{26°C}$ in a 2·4 per cent. aqueous solution is +190°. It is highly soluble in water (60 per cent. at room temperature) and 1·5 per cent. aqueous solution has a *p*H of 6·2 The calculated unitage per mg. is 1187 units (this was confirmed by assay), and the potency of 1·4 mg. therefore corresponds to that of 1 mg. of crystalline sodium penicillin G. Tersavin is only slightly more toxic than ephedrine hydrochloride in mice and rats but about equally toxic in rabbits; its toxicity may be ascribed therefore to its ephedrine content. The vasoconstrictor potencies of tersavin and ephedrine hydrochloride are identical on a molecular basis and each compound produces tachyplaxis to the other; the bronchodilator effects of the two compounds were indistinguishable. Tersavin exhibited the characteristic antibacterial properties of penicillin G. There does not seem to be either an antagonism or a synergism between the two components. Two modifications might however be attributed to the presence of the vasoconstrictor. One is an influence on the blood level which is prolonged and higher with tersavin than with penicillin G; the other is an experimental local streptococcal infection. The authors conclude that tersavin offers the advantages of both the antibacterial properties of penicillin G and the vasoconstrictor effect of ephedrine by the administration of a single chemically defined compound. S. L. W.

Heparin Preparations, Sheep Plasma Method for the Bioassay of. O. F. Swoap and M. H. Kuizenga. (*J. Amer. pharm. Ass.*, 1949, **38,** 563.) The potency of an unknown heparin is determined by a simultaneous comparison of the minimum amounts of standard and unknown heparin which when contained in a volume of 0·3 ml. will keep 1 ml. of recalcified sheep plasma more than 50 per cent. fluid for 1 hour at room temperature. The sheep plasma is obtained by drawing blood from the animal into a sterile vessel containing 50 ml. of a 4 per cent. sodium citrate solution per 500 ml. of blood. The blood and sodium citrate solution is mixed by gentle agitation and by centrifuging. Such plasma can be stored at −20° C. for as long as one year without deterioration. The amount of calcium chloride necessary for recalcification need be determined only once for each lot of frozen plasma. One ml. samples of the strained plasma in each of 4 test tubes are added to 0·05, 0·10, 0·15, and 0·20 ml. quantities of a 2 per cent. solution of calcium chloride. The optimum amount of calcium chloride

solution is that which, in the shortest time, produces a firm clot which cannot be removed from the tube by inverting it. The coagulation tubes for the test are prepared by measuring varying amounts of the heparin dilution (in 0·9 per cent. sodium chloride solution) and the standard into a series of test tubes, the final volume being adjusted in each case to 0·3 ml. To each of these tubes is added 1·0 ml. of the strained sheep plasma and then, noting the time, the optimum amount of calcium chloride solution as determined above. Each tube is immediately stoppered and the contents are mixed by inverting the tube three times. The tubes thus prepared are kept at room temperature, and exactly 1 hour after recalcification are examined for coagulation. Any tube showing partial clotting is shaken sufficiently to estimate whether the clot is more, or less, than 50 per cent. of the total contents of the tube. If the clot is greater than 50 per cent. of the total volume, the tube is regarded as clotted, the end-point being taken as the minimum amount of heparin necessary to maintain the tube in a condition of less than 50 per cent. clot. It is considered that 20 to 25 samples may be assayed daily by this method; calculation of results is rapid and simple and the dosage response curve is steeper than that found with other methods or with other plasmas, while the end-point is easy to determine.

R. E. S.

Hetrazan (Banocide) in Treatment of Loiasis. F. Murgatroyd and A. W. Woodruff. (*Lancet*, 1949, **257**, 147.) Seventeen European patients were treated with hetrazan (1-diethylcarbamyl-4-methylpiperazine hydrogen dicitrate). At first 2 mg./kg. of bodyweight per day was given, but later this was increased to 4 mg./kg. for some patients and then to 6 mg./kg. This daily dose was given orally in three equal separate portions after breakfast, luncheon and supper. The total amounts of drug given varied from 1·2 to 10·5 g. After the first day's treatment 6 patients complained of itching and 3 of these had rashes. The rashes were transitory, lasting 48 hours, but the irritation persisted for 3 to 4 days and in one case 16 days. It was alleviated by giving 50 mg./kg. of benadryl or 100 mg./kg. of anthisan twice daily. In three patents cutaneous thickenings or nodules appeared which disappeared in 2 or 3 days. Three others showed cutaneous serpiginous linear swellings, the appearance of which suggested reactions around adult worms and in two patients specimens of dead adult *L. loa* were extracted. The embryos of *L. loa* rapidly disappeared from the peripheral blood. Embryos of *Acanthocheilonema perstans* in the blood of one patient were insensitive to the drug. During treatment some of the patients complained of nausea and headache. With one exception all patients remained entirely free from symptoms, 9 of them for 6 months or more after treatment. One showed a recurrence of calobar swelling possibly due to reinfection. Two healthy persons who received 6 mg./kg. of bodyweight of the drug daily for 14 days, showed none of the side-reactions indicating that they were probably due to the action of the drug on the parasites. These two people also showed no alterations in the total and differential leucocyte counts.

A. D. O.

Hetrazan, Action on Filariasis and Onchocerciasis. F. Hawking and W. Laurie. (*Lancet*, 1949, **257**, 146.) Hetrazan (1-diethylcarbanyl-4-methylpiperazine hydrogen dicitrate) apparently acts like an opsonin by modifying the micro-filariæ so that they are destroyed in the reticulo-endothelial system. A short and simple dose scheme was used, usually consisting of one dose per day for 7 days. The maximum tolerated dose was about 20 mg./kg. of body-weight per day. Doses as low as 1 mg./kg. twice daily or 0·5 mg./kg. 3 times daily, removed almost all the micro-filariæ from

the blood but doses of 0·2 mg./kg. twice daily did not completely clear the bloodstream. With larger doses, microfilariæ disappeared from the blood in 3 days or less, but in a few cases persisted for 3 months. Single doses of 20 mg./kg. removed all or most of the micro-filariæ from the blood in most of the patients treated; the possibility of mass treatment by such short high-dosage courses is being investigated. It was found that if the micro-filariæ were not in contact with large phagocytes, e.g. in a serious cavity, they are apparently not destroyed by hetrazan. Follow-up of the patients after treatment showed that hetrazan has a marked action on the microfilariæ of *Onchocerca volvulus* but this is less rapid and less permanent than the action on the micro-filariæ of *Wuchereria bancrofti*. The effect on adult worms, however, was less sure. Hetrazan was well tolerated by patients with filariasis due to *W. bancrofti* but caused violent allergic reactions in patients infected with *O. volvulus*. A. D. O.

Mercury, Absorption of, Through the Skin. A. Bass and E. D. Robinson. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 659.) The penetration of mercury through the skin of rabbits was compared by determination of the concentration of mercury in the kidney tissue. 10 mg. of ointment was inuncted over an area of 30 sq. inches of closely clipped skin, the area covered with a rubber sheet and left for 24 hours after which the animals were killed. The kidneys were removed, weighed and analysed for mercury, using the dithizone method with a photoelectric colorimeter. The following ointments were compared:—(a) boric acid, 2; anhydrous lanoline, 20; bees-wax, 10; resorcinol, 4; red mercuric oxide, 5; water, 8; soft paraffin to 100, and (b) the same, but without the resorcinol. The concentration of mercury in the kidney tissue was 6 times higher in the case of the ointment containing no resorcinol. It appears that resorcinol interferes with either the penetration of the mercury compound through the skin, or its storage in the kidney. When the ointments were compared for bacteriostatic power by the Food and Drug Administration agar cup-plate method, the zone of inhibition was more than twice as wide when the ointment contained resorcinol. This may have been due to the diffusion of resorcinol into the aqueous agor medium, in which it is soluble. G. B.

Octin (Methyl-*iso*-octenylamine) in the Treatment of Vasodilating Headaches. G. A. Peters and W. W. Zeller. (*Proc. Mayo Clin.*, 1949, **24,** 565.) Octin relieves the pain of vasodilating headaches by acting as a vasoconstricting agent. It may be administered by either the intramuscular or the oral route, but not intravenously. The best results may be expected in the treatment of the typical migraine attack and more variable results may be obtained in the treatment of headache due to nervous tension. Of 59 headaches in 26 patients, 48 were partially or completely relieved by intra-muscular injection of 0·5 to 1 ml. of octin hydrochloride in a concentration of 100 mg./ml. of solution (repeated if necessary after 30 minutes). With oral administration, using octin mucate in a dose of 2 gr. every 30 minutes until relief was obtained, only 15 of 26 headaches in 16 patients were relieved. None of the patients in this study experienced serious side-reactions from the use of the drug. It is a valuable adjunct of particular value in those patients who may have abused the use of the ergotamine preparations and in those for whom the use of ergotamine may be contraindicated. S. L. W.

Orthoxine. Evaluation for Bronchospasm. E. Bresnick, J. F. Beakey, L. Levinson and M. S. Segal. (*J. clin. Invest*, 1949, **28,** 1182.) Orthoxine is *o*-methoxy-β-phenyl*iso*propylmethylamine hydrochloride and differs from ephedrine in the presence of an *o*-methoxy group and in the

absence of a β-hydroxy group. Clinical trial, using doses of 100 to 200 mg. 3 or 4 times daily, showed that it is similar to ephedrine as an anti-asthmatic. Side effects were numerous and included anorexia, nausea, light-headedness and drowsiness. Administered as a syrup the drug gave clinical relief from troublesome cough in some cases. H. T. B.

Propylthiouracil for Exophthalmic Goitre in Children. A. S. Jackson and H. B. Haley. (*Amer. J. med. Sci.*, 1949, **218**, 493.) Exophthalmic goitre in children is accompanied by various signs and symptoms additional to those occurring with the same disease in adults, including gastro-intestinal symptoms, high susceptibility to upper respiratory infections, sleep disturbances, epistaxis and marked personality changes. In 8 cases ranging in age from 8 to 16 years it was found that propylthiouracil in doses of 0·025 or 0·05 mg. twice or three times a day was most useful for pre-operative preparation. The individual response was uncertain but in general the toxic manifestations of the disease were relieved. In only one case was the progress of the disease apparently permanently arrested so that operation is still the method of choice in young patients. H. T. B.

Protamine Zinc Insulin, Precision of the U.S.P. Assay for. C. I. Bliss (*J. Amer. pharm. Ass.*, 1949, **38**, 560.) The U.S.P. XIII assay (in which each rabbit is injected twice; once with the standard preparation and then with the "unknown" after an interval of about one week) is used in accordance with the Food, Drug, and Cosmetic Act, to assay each new batch of protamine zinc insulin against a specially prepared standard preparation in two or more rabbit cross-over tests in two different laboratories; it must pass both tests before it can be released. At the request of the U.S.P. Insulin Advisory Committee, a statistical analysis of the results obtained in parallel assays in different laboratories of 24 preparations of protamine zinc insulin has been made to test the precision of the U.S.P. XIII procedure. Both the mean difference over 6 periodic bleedings and the trend of these differences with time proved significantly greater than the residual or random sampling error in the two series of tests. The mean differences from two independent assays of the same preparation were not correlated. Within the official tolerance limits therefore the mean difference could be attributed to minor variations in laboratory procedure and not to actual differences in potency. The trend with time, however, was correlated significantly in parallel assays from different laboratories and therefore partly represents a real characteristic of a given preparation of protamine zinc insulin. It is considered that the assay for protamine zinc insulin is capable of distinguishing finer differences than it is called upon to make in actual practice. R. E. S.

Rutin, Effect of on the Biological Potency of Vitamin C. E. W Crampton and L. E. Lloyd. (*Science*, 1949, **110**, 18.) This note is a preliminary summary of an experiment to ascertain the biological value of vitamin C in two natural sources of the vitamin, and to test the effect of added rutin on the apparent vitamin C potency of these substances, as well as on synthetic ascorbic acid. Of three equal groups of guinea-pigs the first received crystalline ascorbic acid, the second canned orange-grape fruit juice, and the third dehydrated potato. Each assay material was given at four different levels of vitamin C content, namely 0·5, 0·79, 1·26 and 2·00 mg. of ascorbic acid daily. The amounts of orange-grape fruit juice and dehydrated potato to be fed were based on chemical analyses of samples of the two materials. Half the animals on each assay material were given orally 100 mg. per day of crystalline rutin. Using the odontoblast method of assay it was found that the rutin treatment gave significantly higher figures on the response-

dose curve at the 0·5, 0·79, and 1·26 mg. levels of vitamin C intake for both the synthetic ascorbic acid and orange-grape fruit juice assays. At the lower levels of vitamin C intake it appears that rutin either makes more available or delays *in vivo* destruction of ascorbic acid in the original source. A further possibility is that rutin forms the base for synthesis by the animals of additional ascorbic acid. The authors tentatively conclude that rutin present in some natural vitamin C sources may be the factor responsible for enhancing the apparent biological potency of vitamin C. S. L. W.

Suramin and Antrycide, Action on Enzymes. B. W. Town, E. D. Wills and A. Wormall. (*Nature*, 1949, **164,** 233.) Hexokinase, urease, yeast decarboxylase, succinic dehydrogenase and trypsin are all strongly inhibited by concentrations of suramin similar to those maintained in the blood plasma of man and other animals for at least several hours after the intravenous injection of a normal dose (1 to 2 g.) of the drug. No evidence is yet available to show whether or not enzyme inhibition plays any part in the action of suramin on trypanosomes, but the possibility of such an effect cannot be excluded. Antrycide, on the other hand, in concentrations as high as M/500, has no inhibitory effect on urease, succinic dehydrogenase and the system of yeast juice enzymes required for the fermentation of glucose, and the hydrolysis of casein by trypsin is not significantly inhibited by M/2400 antrycide. Some of these enzymes are actually mildly stimulated by high concentrations of antrycide. These results do not show that suramin and antrycide exert their trypanocidal actions by different mechanisms, but they would furnish proof of such a difference if it should be eventually established that the inhibition of glucose-metabolising and/or proteolytic enzymes is an essential feature of the trypanocidal action of suramin. S. L. W.

Sympathin. G. B. West. (*Nature*, 1949, **163,** 721.) Recent studies on the substance or substances liberated at the nerve endings when sympathetic nerve fibres are stimulated show that in some cases this is adrenaline while in others the indications are that it is noradrenaline. In cats, under chloralose and cocaine, after double vagotomy, the author was able to demonstrate that in at least two cases the nature of sympathin (as shown by effects produced in different parts of the animal) depends on the length of time the nerve has been sectioned before being stimulated. It appears that sectioning of some sympathetic nerves may be linked with the inhibition of methylation of the primary amine, so that the stimulation produces more noradrenaline and less adrenaline. In these cases the normal adrenergic mediator may be a mixture of varying proportions of noradrenaline and adrenaline. Blood analyses at various stages may serve to identify the substance or substances liberated. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

Essential Oils, A Method for Comparing the Antibacterial Activity of. M. P. Schroeder and A. M. Messing. (*Bull. nat. Form. Comm.*, 1949, **17,** 213.) The antibacterial activity of certain water-insoluble substances against 14 different micro-organisms was evaluated by the following method. A 12·5 mm. filter-paper disc was placed at the centre of an agar plate, previously seeded with the test organism, 0·1 ml. of a dilution of the substance in alcohol was placed on the disc, and the zone of inhibition was measured after incubation for 24 and 72 hours. The production of a significantly large zone of inhibition after 24 hours, maintained after 72 hours' incubation was the criterion used in assessing bacteriostasis. The test was repeated for several

[*Continued on page* 272

BOOK REVIEWS

SYNTHETHISCHE METHODEN DER ORGANISCHEN CHEMIE. W. Theilheimer. Repertorium 3. Pp. viii + 412. Basel and New York: S. Karger, 1949. 40 Swiss Francs.

The third volume of this excellent literature summary has now appeared, and, as foreshadowed in the preface to Volume II, covers the years 1946-1947 (with a supplement to 1948) mainly from the point of view of American literature. It contains references and abstracts of 771 reactions, and the index, which covers Volumes I and II in addition, can be used adequately without reference to the classification system. As the literature of Organic Chemistry is somewhat voluminous, gaps inevitably arise in any compilation, but the reviewer was surprised that he has been unable to find any reference, either in the present volume or the previous one, to the elegant method of alkali metal—liquid ammonia—alcohol reduction introduced by A. J. Birch in 1944. Omissions, however, are few, and the general standard is excellent. Although in German, the knowledge of the language required for such abstracts is small, and as the majority of papers are of English-speaking origin there would appear, from the point of view of English-speaking users, to be little case for translating this volume. The binding and the layout is extremely good, and—a point which makes for ease of reference—each particular reaction is outlined in formulæ: a feature unhappily absent from many books.

DAVID W. MATHIESON.

THE MAMMALIAN ADRENAL GLAND, by G. H. Bourne. Pp. 214 + Bibliography + Index and 15 special plates. Oxford University Press (Geoffrey Cumberledge), London, 1949, 30s. 0d.

It is amazing that so much can be written about such a small part of the mammalian anatomy, but when one considers that this book contains descriptions of the adrenal glands of more than 250 species, of which more than half are described for the first time, one realises what a fine achievement this volume represents. From the rat and mouse to the whale, giraffe or elephant, the descriptions are compact and well covered. The author is an expert in histology and this aspect of the account has taken a prominent place, as has also the embryological side. The book really is a record of work done by the author while he was a research student in the University of Western Australia. The constant reminder of the dual origin of the mammalian adrenal body is given by the description of the presence of accessory adrenal tissue scattered through the body cavity of the majority of mammals. The author himself states that he has found these additional adrenal bodies with cortex and medulla in one type of Australian cat. Variability of the medulla appears to be no less than that of the cortex of different mammals. Body weight/adrenal weight ratios were not possible for all species of mammals examined, and this is certainly a disadvantage. One cannot criticise such a fine thesis in any stern manner, but the reviewer would like to make three points: (1) the historical section ends, rather abruptly, about 1941; (2) more stress should have been laid on the pioneer work of Elliott (1904-5) and also on the preparation of cortical extracts; and (3) no reference has been made to the presence of noradrenaline in the medulla. The volume will be always a masterpiece for specialised reference.

G. B. WEST.

PHARMACOPŒIAS AND FORMULARIES

THE BRITISH PHARMACEUTICAL CODEX, 1949

A Review of Inorganic and Physical Chemistry

By A. MIRIMANOFF

Professor of Galenical Pharmacy in the University of Geneva.

L'augmentation continue du nombre des médicaments implique pour les Pharmacopées un choix de substances souvent très délicat à établir. L'élimination ou la non acceptation d'un médicament par une instance officielle n'empêche certes pas son emploi par le médecin ou l'industriel. Il en résulte qu'une foule de substances courent le risque d'être utilisées à des fins thérapeutiques en échappant à des exigences officielles quant à leur pureté et à leur efficacité.

Le " British Pharmaceutical Codex " a compris le danger qui pouvait ainsi menacer la santé publique, et son édition de 1934, en particulier, a établi des normes (standards) pour cette vaste catégorie de médicaments.

La présente édition (1949) contient une mine de renseignements décrits sous une forme aussi concrète que possible; aussi convient-il de souligner que l'ensemble des caractères d'identité, des critères de pureté et des données analytiques sont réunis sous la rubrique " standards." Un coup d'œil suffit au lecteur pour rechercher un point particulier, ce qui facilite grandement la tâche de l'analyste. Très pratiques également les autres alinéas: " action and uses, sterilisation, storage, dose, etc."

En ce qui concerne la chimie inorganique et la chimie physique, seul domaine auquel est consacrée cette brève analyse, on peut faire les remarques suivantes:

(a) " GENERAL MONOGRAPHS "

Le nombre des articles représente en général environ le double de celui de la B.P. Ainsi, par exemple, 15 composés du calcium (8), 38 dérivés du sodium (20), etc. Très utile la description des propriétés chimiques et pharmacologiques de deux substances voisines ou parfois confondues dans leur action. Exemple: le comportement différent du kaolin et du trisilicate de magnésium envers le suc gastrique, les sels ferreux et ferriques, les composés du bismuth pour l'usage interne et externe.

Au point de vue de l'établissement du standard, le Codex s'est inspiré le plus souvent des méthodes de la Pharmacopée, critères d'identité et de pureté, ainsi que pour le dosage. Remarquons pour la bentonite des critères dérivant de la chimie des colloïdes: gel formation et " swelling power," définis par un procédé simple mais quantitatif, qui nous paraissent d'une certaine originalité.

Excellent standard pour certains sels organiques du calcium, comme le lévulinate, que le praticien aurait de la peine à établir lui-même. Même remarque pour les dérivés de l'acide glycérinophosphorique et les persels (perborate, persulfate).

(b) LA VI^{e} PARTIE (" FORMULARY ")

Apporte des données nouvelles, au point de vue de l'application de la physicochimie à la pharmacie.

Collyria. Ce paragraphe apporte des renseignements qu'on chercherait en vain dans la B.P. Au sujet de la pression osmotique de la sécrétion lacrymale, le Codex la ramène à sa juste valeur, exprimée approximative-

ment par 0,9 pour cent d'une solution de NaCl. La notion erronée d'une concentration plus élevée (1,4) est rencontrée fréquemment à l'étranger.

Guttae ophtalmicae. Même remarque que ci-dessus. A noter l'usage d'une " solution for eye-drops " contenant deux substances fungistatiques (methyl et propyl hydroxybenzoate).

Injectiones. Le moyen est donné au pharmacien de réaliser—comme pour les collyres—des solutions isotoniques sans difficulté; une table de correction valable pour les substances le plus fréquemment employées se trouve à la suite de la formule établie sur la base de mesures cryoscopiques.

Si les *Emulsiones* n'apportent rien de bien nouveau au point de vue standard chimique, les *Liquores* nous paraissent mieux définies que généralement, car elles sont pourvues d'un standard simple, mais précis, qui doit pouvoir rendre service au pharmacien praticien.

Solvellae. La plupart de ces formules, dont la base est en général une association d'acide borique et de chlorure de sodium, sont régies par un standard exigeant une proportion définie des composants et une méthode de dosage.

Au point de vue de la chimie minérale, les autres formes pharmaceutiques du formulaire n'apportent rien de particulier.

En résumé, le " British Pharmaceutical Codex " fournit un complément et des explications très utiles en ce qui concerne les médicaments de la B.P. Il codifie avec précision, grâce à l'établissement d'un standard, de nombreuses substances couramment employées en thérapeutique, tout en définissant leur usage et leurs propriétés. Sans parler ici de la foule de renseignements contenus dans les autres parties de l'ouvrage (antisera, vaccines, etc.), il codifie également de nombreuses formules thérapeutiques.

S'il n'a pas introduit de méthodes à proprement parler originales, l'esprit dans lequel cet ouvrage a été rédigé est remarquable par sa concision et sa clarté. Il n'est pas exagéré de considérer ce Codex comme réunissant, sous une forme résumée, les données du National Formulary américain, du Commentaire de la Pharmacopée helvétique et de l'Officine de Dorvault.

A ce titre, l'édition 1949 du " British Pharmaceutical Codex " est appelée à rendre service non seulement aux pharmaciens et aux médecins de l'Empire britannique, mais au monde médical tout entier.

ABSTRACTS (*continued from page* 269)

dilutions of each antibacterial substance. Control tests, omitting the antibacterial substances showed that the action of the alcohol is too small to affect the results. Replacement of the alcohol by ether or acetone did not significantly affect the result, in contrast to the phenol coefficient method, where the solvent has a relatively large effect on the antibacterial action. Cassia oil (2·5 per cent.) inhibited the growth of 11 out of 14 test organisms and showed a greater activity against the coli-typhoid-dysentery group than against pyogenic bacteria and *B. subtilis.* Pine oil (25 per cent.) was more active bacteriostatically than Australian ti tree oils in the same concentration. Cinnamaldehyde (2·5 per cent.) was effective against the test organisms, except *B. subtilis* and *Ps. æruginosa.* Hydrocinnamaldehyde differed little in activity from cinnamaldehyde at the same concentration, but was effective against all the organisms. Cinnamic acid (5 per cent.) had an antibacterial effect of a low order, benzaldehyde was bacteriostatic at a concentration of 25 per cent. or more, and acetaldehyde had no activity at a concentration of 10 per cent. G. B.