REVIEW ARTICLE

THE MODE OF ACTION OF ANTISEPTICS

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The study of antibacterial agents falls into two parts, evaluation[1–4] and mode of action. From the latter point of view antibacterial agents may be conveniently divided into three categories, (i) antimetabolites, (ii) antibiotics, (iii) a group of compounds which in this context may be called non-specific and includes such diversified substances as the phenols, phenoxetol, cationic surface-active compounds, certain dyes[5,6], mercuric salts[7], formaldehyde[8], chelating agents[9] and halogens[10,11].

It is proposed to discuss only phenols and their derivatives and cationic surface-active compounds in this review; recent reviews dealing with group (i) are given in references[12–13] and those of group (ii), references[14–18].

Phenols and Their Derivatives

Phenol itself was isolated from coal-tar by Runge and introduced into medicine in 1865 by Lister. Systematic work on its mode of action began in 1872, when it was discovered that phenols were solvents for proteins. Thus Ritthausen[19] and Osborne[20] found that zein dissolved in melted phenol. Kjedahl[21] showed that gliadin was soluble in *p*-cresol, from which it could be precipitated by many organic solvents, while Reichel[22] found that when serum was warmed with anhydrous phenol a clear solution was obtained which underwent no apparent change on boiling. Cooper[23] extended these studies and found that molten phenol dissolved natural and heat coagulated egg albumen. *m*-Cresol was also found to dissolve many proteins.

Meyer[24] showed that the antibacterial action of phenols was parallel to their distribution between protein and water, while Reichel[22] observed that heat coagulated serum and egg white absorbed phenol from aqueous solutions in amounts which were directly proportional to the phenol concentrations, and that the process was reversible. He also showed that *Pseudomonas aeruginosa* absorbed phenol and that the addition of NaCl increased the absorption by both bacilli and heat coagulated serum, and also increased the bactericidal action of phenol.

Cooper[26] concluded that the absorption of phenols by bacteria was the initial stage of their germicidal action. The subsequent action was not the result of a chemical union with the bacterial proteins, but was apparently associated with the de-emulsification of the colloidal system within the cell shown by the precipitation of proteins when a certain phenol concentration was attained. Richardson and Reid[26] showed that the observed action of phenols could be related to the oil : water partition coefficient. The relation of activity to both oil : water and protein : water

distribution coefficients are reconcilable with the findings of Knaysi[27] and other workers that the cell-membrane of some bacteria may be lipoprotein in nature, while lipoid material and protein are both known to be constituents of bacterial protoplasm. Bancroft and Richter[28] reviewing the chemistry of disinfection concluded that germicidal action is similar to narcosis in higher organisms and concluded also that phenol acts by directly coagulating cell colloids. They were able to observe directly the coagulation of the cell proteins of *Bacillus megaterium* and *Aerobacter aerogenes* by phenol.

Fogg and Lodge[29] summarising a series of studies on the effects of sublethal concentrations of various antibacterial agents on *A. aerogenes*, confirmed that the antibacterial activity of phenols could be related to the distribution coefficient between an aqueous system and olive oil. Those phenols in which the ratio of the concentration in oil to the concentration in the aqueous system was high had the greater antibacterial activity. The criteria of activity were the effect on cell division, growth rate and on the lag phase of growth. Because these factors were affected to the same extent it was concluded that there was a general mechanism by which phenols killed bacteria, namely the coagulation of cellular protein. Phenols were also found to differ from substances such as sulphanilamide, acriflavine or methylene blue in that it was not possible to train or adapt the test organism to grow in inhibitory concentrations of the phenols. This was taken as further evidence that phenols exert their antibacterial effect by a non-specific mechanism. Labes[30] favoured a chemical rather than a physical mechanism and suggested that phenols exerted their action by combining with hydroxy groups in the cell protein. This may well be one of the mechanisms by which protein coagulation or precipitation is effected.

Pulvertaft and Lumb[31] found that antiseptics at bacteriostatic concentrations caused the lysis of certain bacterial cultures. The organisms tested varied in the extent to which lysis occurred, advanced lysis being found with staphylococci, pneumococci, *Bacillus subtilis* and several strains of *Escherichia coli*; less-marked lysis was found with *Shigella dysenteriae*, very little lysis was found with *Streptococcus haemolyticus* and a non-haemolytic streptococcus. With some organisms although lysis was encountered at a low concentration of the antiseptic it did not occur at the higher concentration. For example, at a phenol concentration of 0·045 per cent a culture of *E. coli* underwent complete lysis, whereas at 0·54 per cent no lysis occurred. A suggested explanation of these facts was that at the lower concentration lytic enzymes present in the cell were activated by phenol. At the higher concentration, the lytic enzymes were themselves inhibited and thus no lysis occurred. The lytic phenomenon was not seen in older cultures.

Hotchkiss[32] stated that when bacterial cells were treated with tyrocidine or certain detergents a leakage of phosphorus- and nitrogen-containing substances occurred. Gale and Taylor[33] studied the kinetics of the leakage of individual amino acids from the internal environment of *Streptococcus faecalis*. Amino acid release was estimated by measuring the

carbon dioxide produced in the presence of the specific amino acid decarboxylase. Tyrocidine, Aerosol OT, cetrimide and phenol were examined. Phenol, in a final concentration of 10 mg./ml. (1 per cent) caused in 40 minutes a leak of glutamic acid almost as great as that released by boiling the cells. A concentration of 0·33 per cent phenol caused a leakage of about one quarter of the amount released by 1 per cent phenol. A concentration of 2 per cent phenol also caused a leak, but the glutamic acid decarboxylase was itself inhibited at this concentration of phenol. The leakage of lysine could not be followed satisfactorily as lysine decarboxylase was sensitive to the phenol concentrations used, although an evolution of carbon dioxide was observed before the decarboxylase was inactivated. Previous experiments had determined that a concentration of 2 per cent of phenol sterilised a culture containing 10^8 cells/ml. in 30 minutes and a concentration 0·25 per cent prevented the growth of an innoculum of 10^6 cells/ml. after 48 hours incubation at 37°. It was concluded from these experiments that phenol owes its disinfectant action to an effect on the bacterial cell wall whereby essential constituents of the internal environment are released.

Deere[34] in his studies on the phenomenon of lactose fermentation in *E. coli. mutabile* noticed that those which did not ferment lactose could be made to do so by exposure to thymol or toluene, or by drying. These treatments were thought to increase the permeability of the cells to the substrate, since they were not deficient in the "lactose" enzyme. Maurice[35] studied the effect of phenol on the permeability of *E. coli* to basic dyes. He found that as the dye was absorbed, the optical density of the suspension increased and by following optical density changes he was able to follow the course of absorption. He found that phenol increased the rate of penetration of the dyes into the bacterial cell, and also that calcium ions were able to inhibit this effect.

More recently Haydon[36] observed the effect of phenol on the electrophoretic mobility of *E. coli* and concluded, as similarly did Gale and Taylor[33], that phenol caused leakage of metabolites from the cell; also lysis and death were related. Whether lysis caused death or death resulted in lysis was not known. Lysis and leakage similar to that produced by detergents or phenol are known to be produced by heating the cells, thus lysis is a result of death by heat in this instance.

Quastel and Whetham[37] studied the ability of the monohydric alcohols (C_1 to C_8) to function as substrates for *E. coli*. It was found that methyl alcohol and the alcohols from butyl, C_4, to octyl, C_8, were unable to function as substrates and furthermore they inhibited or retarded the dehydrogenation of other substrates. Ethyl and propyl alcohols exhibited a dual role acting as substrate at low concentrations and at higher concentrations as inhibitors of the dehydrogenation of other substrates. The observed inhibitory effect was thought to be due to adsorption at the surface of the organism. It was noted also that the amount of inhibition increased with the chain length of the alcohol. The inhibitory action of phenol and a number of organic solvents on dehydrogenases was also investigated. The method was to place the substrate

in phosphate buffer, bacterial suspension, methylene blue and inhibitor in a Thunberg tube and then to measure the time to reduce the dye. A comparison of these reduction-times gave a measure of the relative inhibitory power of the substances tested. *cyclo*Hexanol and *cyclo*hexene were found to be much more toxic to succinic dehydrogenase than phenol. Both viability and the metabolic activity of the cells should be investigated since Cathcart and Hahn[38] have shown that acetone-killed bacterial cells reduce methylene blue.

The mechanism of action of inhibitors was extended by Quastel and Wooldridge[39]. They treated the washed suspensions of *E. coli* in phosphate buffer with the inhibitor for a given period. The cells were then washed free from inhibitor and resuspended in phosphate buffer, and their dehydrogenase activity measured. The results thus indicate irreversible decreases in dehydrogenase activity brought about by the various inhibitors. They found that glucose, glycerol and mannitol dehydrogenase were susceptible to all the inhibitors examined; lactate and formate dehydrogenase were more resistant (Table I).

TABLE I

THE REDUCTION TIMES IN MINUTES FOR METHYLENE BLUE AFTER EXPOSURE TO VARIOUS INHIBITORS

Substrate	Inhibitor and exposure time				
	Phenol 1 per cent 5 mins*	Propanol 30 mins	Toluene 5 mins	*cyclo*Hexanol 5 mins	Control untreated
Succinate	44·0	∞	21·5	∞	14·2
Lactate	7·5	2·5	8·7	∞	7·7
Formate	4·2	25·7	4·7	49	4·0
Glucose	∞	∞	∞	∞	11·2
Glycerol	∞	∞	∞	—	5·2
Mannitol	∞	∞	∞	∞	7·2

* Subculture showed a few discrete colonies.

Quastel and Wooldridge[39,40] believed that the site of dehydrogenation reactions was at the cell surface and that the reason for the selective action of toluene in inhibiting the dehydrogenations of the sugars was that the responsible enzymes were associated with lipoid material or were lipoidal in nature; although it was also considered that the permeability of the cell might be affected, the role of an altered permeability was not considered to be significant. Cook[41] later showed that the velocity of acetate oxidation by *E. coli*, measured in the Barcroft respirometer, was slightly faster in the toluene-treated cell than in the untreated cell and suggested that this effect was due to an increase in cell permeability. He was also able to show that toluene-treated cells still retained their ability to oxidise lactate, formate and succinate when molecular oxygen as well as methylene blue were the final hydrogen acceptors. Formate was oxidised to completion and succinate was converted to fumarate in 82 per cent yield and lactate to pyruvate in 76 per cent yield.

No further work on the action of antiseptics on enzymes appeared until 1937, when Bach and Lambert[42,43] studied the effect of antiseptics and solvents on certain dehydrogenases of *Staphylococcus aureus*. Their method was to expose the washed cells to the antiseptic for 30 minutes

at 40° both in the presence and absence of the substrate. Toluene, benzene and *cyclo*hexanol were used in saturated aqueous solutions. The cells were then washed three times with water and their dehydrogenase activity measured by the Thunberg method. With the lactic dehydrogenase[42] it was found that toluene, benzene, *cyclo*hexanol, acetone and phenol never produced total destruction of this enzyme; also the presence of lactate reduced the apparent inhibition of the dehydrogenase. In contrast iodine 1×10^{-4}, mercuric cyanide 1×10^{-6}, and copper sulphate 1×10^{-2} completely destroyed the enzyme and no protection was afforded by the substrate.

The work was extended[43] to the glucose, succinic, formate, butanol, pyruvic, fumaric and glutamic dehydrogenases. The glucose, formate and butanol systems, like the lactate were only partially inactivated by benzene, toluene, acetone or phenol treatment, while systems activating succinate, fumarate, pyruvate and glutamate were completely destroyed. Results for benzene and phenol are given in Table II. Except for the system glucose: acetone, the presence of the substrate exerted a protective effect. Iodine, mercuric cyanide and copper sulphate completely inactivated all these enzymes whether substrate was present or not.

TABLE II

THE EFFECT OF INHIBITORS ON CERTAIN DEHYDROGENASES OF *Staph. aureus*

Inhibitor	Suspension	Substrate Reducing time, minutes							
		Lactate	Glucose	Formate	Butanol	Succinate	Fumarate	Pyruvate	Glutamate
Phenol 0·1 per cent	A	2·5	7	7·25	9	40	26	33	29
	B	4	11	17·5	12·5	∞	∞	∞	∞
	C	2·3	6	8·75	9·75	∞	65	∞	20
Benzene saturated aqueous solution	A	3	6·5	28	10	52	25	17	30
	B	3	7	52	12	>152	127	19	125
	C	3	3	13	20	—	—	—	—

Suspensions. A. Untreated. B. Treated with inhibitor for 30 minutes at 40°. C. As B but substrate present.

Sykes[44] also investigated the action of substances used in medicine as antiseptics on the succinic dehydrogenase of *E. coli*. His method differed from that of Quastel and Wooldridge, and Bach and Lambert in that the bacteria were not washed after treatment with the antiseptic. The cells were treated with the antiseptic at room temperature and after an interval, substrate, buffer and methylene blue were added. The tubes were then evacuated and filled with nitrogen, placed in a water bath at 37° and the time at which 90 per cent reduction of the dye was seen, compared with a similar tube containing only 10 per cent of the methylene blue. For phenol, viable counts were made on the suspensions after treatment; with other antiseptics subcultures were made to test for residual viability. Sykes concluded that the concentration of *p*-chlor-*m*-cresol, hexylresorcinol, *p*-butylphenol, amyl-*m*-cresol, phenol and ethyl, *iso*propyl, *n*-butyl

and *n*-amyl alcohols required to completely inhibit the succinic dehydrogenase of *E. coli* was always slightly in excess of the minimum lethal concentrations.

Dagley and colleagues[45] grew cells of *A. aerogenes* in a synthetic medium containing glucose, potassium dihydrogen phosphate, ammonium sulphate, and magnesium sulphate, and found that a progressively increasing lag-phase was introduced by increasing the doses of phenol. This lag could be abolished or reduced by the addition of a culture filtrate of the synthetic medium in which the organisms had been growing, or by L-leucine, DL-methionine, or L-glutamic acid and also by α-ketoglutaric or succinic acids. On the other hand other amino and carboxylic acids were found to increase the lag period, DL-aspartic or fumaric acids being examples. The bacteriostatic effect of phenol was thought to be due to its inhibition of the synthesis of metabolites essential for rapid cell division. Roberts and Rahn[46] selected one substrate, acetate, and investigated the action of germicides on its oxidation and dehydrogenation by *E. coli*. As catalase was thought to be implicated in using acetate, the effect of the germicides on this enzyme was also investigated (Table III).

The effects of sublethal concentrations of phenol were found to be irreversible by dilution with water. The general effect emerges from a

TABLE III

THE EFFECT OF PHENOL ON CERTAIN METABOLIC ACTIVITIES OF *E. coli*

Enzyme system	Per cent inhibitions in phenol concentrations of		
	0·075 per cent	0·15 per cent	1·2 per cent
Acetate oxidation	20	48	96
Acetate dehydrogenation ..	0	0	96
Catalase activity	9	10	38
Effect on growth	retarded	inhibited	lethal

consideration of the data with phenol that enzyme systems differ in their sensitivity to phenol and that even at lethal concentrations some oxidase and dehydrogenase activity persists. The catalase activity is apparently inhibited only to about 38 per cent.

The action of 2:4-dinitrophenol (DNP) and certain other nitrated and halogenated phenols on the metabolic reactions of microorganisms are of considerable interest. Shoup and Kimler[47] found that DNP at first stimulated and then depressed the rate of respiration of certain luminous bacteria. Stimulation was not however found with all substrates tested; thus Krahl and Clowes[48] and Genevois and Creach[49] detected no stimulation in the respirator of yeast with lactate, pyruvate or glycerol as substrates.

Attempts to explain the mechanism of the stimulatory action of DNP have been made by several workers. Stenlid[50] working with young wheat roots believed that dinitrophenols render the substrates more accessible to the enzymes by breaking down cellular compartmentation, while

Vandendriessche[51] thought that the stimulation was due to a specific stimulation of phosphohexokinase. Loomis and Lipmann[52] made the fundamental discovery that in a cell-free homogenate from rabbit kidney oxidising glutamic acid, *m*-dinitrophenol prevented the phosphorylation while oxidation was unaffected or even slightly stimulated. These workers believed that the general mechanism of dinitrophenol stimulation was due to the "uncoupling" of a phosphorylation and oxidation in a known biochemical reaction, and the term "uncoupling reagent" was coined for compounds which exhibited this property.

It is well known that washed suspensions of bacteria do not always oxidise a substrate to completion. With *E. coli* and *Bacterium alcaligenes*, Cook and Stephenson[53] showed that although formate was oxidised to completion, the following substrates were oxidised only to the extent shown: lactate 66, glucose 66, pyruvate 60 and acetate 75 per cent. Barker[54] found incomplete oxidation of certain substrates by suspensions of the alga *Prototheca zopfii*, the figures being glycerol 29, glucose 30, ethanol 46 and acetate 50 per cent. Giesberger[55] found a similar situation with different species of spirillum and made the suggestion that the unmetabolised fraction of the substrate might be assimilated. Clifton[56] confirmed the incomplete oxidation of substrate by *E. coli* and extended it to *Pseudomonas calco-acetica*. He found, however, that in the presence of sodium azide (NaN_3) or DNP, oxidation proceeded to completion, the inference being that these reagents prevented assimilation and the substrate was wholly oxidised. A more detailed study of the phenomenon was made by Clifton and Logan[57] who confirmed that washed suspensions of *E. coli* do not oxidise acetate, lactate, glycerol, fumarate, succinate and glucose to completion. The oxidation of glucose, pyruvate, acetate and glycerol proceeded to completion in the presence of DNP or NaN_3. The oxidation of fumarate and succinate was inhibited at very low concentrations of the reagents. Later experiments attempted to prove that assimilation had in fact occurred, and Siegel and Clifton[58] demonstrated an increase in cell weight, but this did not account for the discrepancy between the calculated and measured increase in cell weight for the substrates investigated, but the degree to which a 100 per cent carbon recovery may be demonstrated is obviously dependent on the accuracy of measuring all the metabolic products.

Other workers have shown that DNP and NaN_3 are not universal uncoupling reagents. Burris and Wilson[59] using washed suspensions of the root nodule bacterium *Rhizobium trifolii* noticed that in the presence of DNP, although increased O_2 consumption of the suspensions with glucose did occur, oxidation did not proceed to completion. Further, when added after all the glucose had disappeared from the external medium, DNP caused a further consumption of oxygen which suggested that one of the actions of DNP was to cause intracellular material to be oxidised.

Pickett and Clifton[60] observed that the assimilation of glucose by yeast cells under aerobic conditions, although inhibited by DNP, was not accompanied by an increase in oxygen consumption. Hotchkiss[61] using washed suspensions of staphylococcus cells noticed that DNP stimulated

oxygen consumption and decreased the assimilation of inorganic phosphate. Gale[62] found that the assimilation of free glutamic acid by *Staph. aureus* required energy which could be made available by the co-fermentation of glucose. DNP inhibited the assimilation of the amino acid, but this was not due to inhibition of glucose fermentation. With *Str. faecalis* an increase in the internal concentration of glutamic acid was observed. This was shown to be due to the interference within the cell of the normal glutamic acid metabolising system by the DNP. Rothstein and Burke[63] studied the effect of DNP on the endogenous CO_2 production of "starved" yeast cells and found that in the absence of the phenol no measurable quantities of CO_2 were produced; in the presence of phenol, however, an appreciable production of CO_2 was induced; the source of the CO_2 and ethanol produced, was intracellularly stored glycogen, and the main action of the DNP was to cause this reserve material to be fermented.

Simon[64,65] made a detailed study of the action of phenol and certain nitrated phenols on the respiration, assimilation and fermentation of glucose by a yeast isolated from a sample of commercial baker's yeast. 3:5-Dinitro-*o*-cresol in concentrations of 10^{-5}M stimulated the amount of oxygen used by as much as 1·7 times. At high concentrations the consumption of oxygen was inhibited and aerobic fermentation appeared, which reached a peak at about 10^{-4}M and then was progressively inhibited. In the absence of inhibitors the use of oxygen by washed yeast suspensions with glucose is about only half that required for complete oxidation according to the equation $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$. But in the presence of 2×10^{-5}M of the cresol, an oxygen consumption corresponding to 93 per cent oxidation was obtained. It was assumed that the action of the cresol at concentrations rising to 10^{-5}M was to prevent assimilation of glucose, and this accounted for the increased use of oxygen, that proportion of glucose assimilated by normal cells being now oxidised by molecular oxygen. *o* and *p*-Nitrophenol gave similar results. Phenol, although it did not stimulate respiration, did inhibit assimilation.

Simon[66] believed from an analysis of his result that dinitrocresol acts as an uncoupling agent and that both oxidative assimilation and the rate of glycolysis are controlled by the level of energy-rich phosphate. He concluded that there was little doubt that both the stimulation of respiration and the inhibition of oxidative phosphorylation is profoundly influenced by nitrophenols. The results obtained with phenol provided no clear evidence of any effect on phosphorylation.

The general effect of nitration or chlorination of phenol is to increase its bactericidal properties but the additional uncoupling effect on metabolism should not be overlooked. Higher concentrations are, of course, necessary to kill bacteria than those which cause the uncoupling effects. Suter[67] gives extensive data relating structure and bactericidal properties of phenols and substituted phenols.

Hugo[68,69] studied the effect of phenol and phenoxetol on the oxidation of certain substrates by washed suspensions of *E. coli*. It was shown that

phenol and phenoxetol 0·1 to 0·2 per cent caused a stimulation (10 to 20 per cent) of the rate of oxygen consumption when glucose, mannitol and lactose were used as substrate; these same concentrations caused a marked inhibition (10 to 15 per cent) of the rate of oxygen consumption when lactate, pyruvate, acetate or succinate were the substrates. No changes in the viable population and no uncoupling effect could be demonstrated. It was thought that the enzymes mediating the stimulated reaction were situated within the cell and the first action of phenol and phenoxetol was to increase the permeability thus facilitating the access of substrate to enzymes within the cell. The marked inhibition of lactate and succinate activity could be interpreted if the enzymes responsible for their oxidation were located at the surface of the cell. Partial confirmation was obtained[70] by comparing the action of these two antiseptics on a disrupted preparation of *E. coli* which was capable of oxidising glucose and lactate. No stimulation of glucose oxidation was obtained with the disrupted preparation and the oxidation of lactate appeared less sensitive in the disrupted preparation than in the intact cell. Disruption of the cell would have the effect of destroying the status quo of enzyme location, thus the diffusion barrier represented by a cell wall or cell membrane would no longer function, and a reaction stimulated by an increase in the permeability of the barrier would not be expected to undergo stimulation in a disrupted preparation. Similarly, enzymes located at a cell surface and therefore immediately exposed to the action of an adverse environment might appear less susceptible when the cells have been disrupted. A review dealing with the special problems of enzyme location in microbial cells was made by Alexander[71], and of enzyme isolation by Hugo[93].

Cationic Surface-active Compounds

Miller, Baker and Harrison[72] showed that a very low concentration of an alkyldimethylbenzylammonium chloride inhibited the respiration and glycolysis of pure cultures of organisms found in the early lesions of human teeth. They used washed suspensions of these organisms and measured the respiration in the Warburg apparatus. They extended their work[73,74] to a systematic study of the effects of anionic, cationic and non-ionised synthetic detergents on the aerobic and anaerobic respiration of glucose by washed suspensions of *Staph. aureus*, *Staph. albus*, *Micrococcus tetragenus*, *E. coli*, *Proteus vulgaris*, *Salm. paratyphi*, *Sarcina lutea*, *Pseudomonas aeruginosa*, *A. aerogenes*, *Shig. dysenteriae* and a lactobacillus and concluded that all the cationic detergents were effective inhibitors of respiration at concentrations of 1 : 3000 and that the Gram-negative and Gram-positive organisms were equally affected. They also noted that some of these detergents stimulated bacterial metabolism at subinhibitory concentrations, a phenomenon they found much more commonly amongst the anionic detergents. Later these workers showed that depression of metabolism was roughly parallel with killing. The test of time to kill was based on an end point method capable only of detecting all alive, or all dead.

Kuhn and Bielig[75] suggested that quaternary ammonium compounds

could react with protein causing in this way the death of the cell, possibly by a disorganisation of the cell membrane. Following up Kuhn and Bieligs' suggestion, Hotchkiss[32] was able to show the leakage of nitrogen- and phosphorus-containing compounds from staphylococci when treated with a variety of surface-active compounds including dodecylamine, and alkyldimethylbenzylammonium chloride and the polypeptide antibiotic tyrocidine. Gale and Taylor[33] made a detailed study of the action of tyrocidine and an anionic (Aerosol O.T.) and a cationic (cetrimide) detergent on the leakage of free amino acids from the internal environment of *Str. faecalis*. They prepared cell-free amino acid decarboxylases of high specificity which enabled individual amino acids to be estimated[76]. Gale and Taylor concluded that the lytic action of tyrocidine, cetrimide, Aerosol O.T. and phenol was sufficient to explain the disinfecting action of these substances. Similar results were obtained with *Staph. aureus* and *Saccharomyces cerevisiae*.

Salton[77] made a detailed study of the "leakage" phenomenon using cetrimide and six different organisms. He measured quantitatively the leak from the cell of material absorbing in the ultra-violet region of the spectrum at 260 mμ, inorganic phosphorus, total phosphorus, pentose, glutamic acid, purines, pyrimidines and their derivatives. This work revealed a simple relation to exist between the leak of 260 mμ absorbing material, glutamic acid and inorganic phosphorus from *Staph. aureus* and *Bacillus pumilus*. A similar relation was found for *E. coli* except that glutamic acid was not released from this organism. Treatment of suspensions with sufficient cetrimide to sterilise them released amounts of cell constituents comparable to those released by placing the cells in boiling water. When smaller amounts of cetrimide were used, a quantitative relation was found to exist between the amount of detergent present, the proportion of cells killed and the amount of 260 mμ absorbing material released. The form of the curve relating the uptake of this detergent from solutions containing it in varying amounts was shown in the case of *Staph. aureus* and *E. coli* to be that of a typical adsorption isotherm, and the maximum amounts of cetrimide adsorbed varied for each of six bacterial species tested.

Salton[78] also grew *Staph. aureus* and *Pseudomonas fluorescens* on a medium containing ^{32}P. An increased leak of ^{32}P was demonstrated when the cells were treated with cetrimide. Further evidence for the cytolytic damage was obtained by Salton, Horne and Cosslett[79], who studied electron micrographs of *Staph. aureus*, *Str. faecalis* and *E. coli* treated with cetrimide, and found that using concentrations of 90 μg./ml. the cytoplasm shrunk away from the cell wall. With concentrations of 900 μg./ml. the cell wall was stripped off. Meisel and Umanskaya[80] had observed, with the ordinary optical microscope, that the protoplasm of yeast cells shrunk away from the cell wall when treated with cetylbenzalkonium chloride.

The change in electrophoretic mobility of bacterial cells has been used to investigate the bacterial surface and the effect of substances on the surface charge[81]. Dyar and Ordal[82], studied the electrophoretic mobility

of ten microorganisms and the effect of 2-methyl-7-ethylundecanol-4-sulphate (STS) and cetylpyridinium bromide (CPC) on mobility. They found that with CPC the following general picture was obtained with all their bacterial species, viz., decrease, reversal and stabilisation of the surface charge, although the degree of the change and the concentration of CPC to produce it varied with the species of bacteria tested. In contrast, when anionic detergents were used, considerable differences were seen in the responses of the bacterial species. For example, using *Ps. aeruginosa* the mobility showed a small but definite increase, while using two strains of *E. coli* no change in mobility was recorded between 0 and 10^{-3}M STS. With *Spirillum volutans* at a concentration of STC of 10^{-5}M the mobility showed a marked increase.

McQuillen[83] reinvestigated the effect of cetrimide on the electrophoretic mobility of *Staph. aureus*, *E. coli* and *Str. faecalis*. McQuillen's findings agreed with those of Dyar and Ordal for the Gram-negative *E. coli*; that is, the mobility of the organism was decreased, became zero and finally its direction was reversed with increasing concentrations of cetrimide. The behaviour of the Gram-posititive organism studied by McQuillen differed from that found by Dyar and Ordal[82], who had reported that the Gram-positive *Staph. aureus* showed the same type of behaviour as the Gram-negative *E. coli*. McQuillen found that with Gram-positive organisms after a small initial decrease in mobility there was an abrupt rise above 50 μM to a maximum at 100 μM followed then by a decrease in mobility so that these organisms bore only a small residual positive or negative charge in the presence of 250 μM cetrimide. This maximum at 250 μM is seen when the cell surface is saturated with the detergent, measured by the adsorption techniques. McQuillen concluded that for the Gram-negative organism the increasing quantities of the detergent cation combine with the negatively charged organism resulting in the reduction and eventual reversal of the net negative charge. For Gram-positive organisms this explanation was not adequate. As stated above, it had been found that the maximum of the mobility concentration curve coincided with saturation of the cells with the detergent. To attempt to explain this mobility maximum several hypotheses were suggested. A removal of an external layer from the cell may expose a layer with a greater inherent negative charge; or a general reorientation of the surface layers of the cell may in some way reveal a greater number of more negatively charged groups. Thirdly, material leaking from the cell may be reabsorbed on to the surface of the cell and thus again increase the apparent net negative charge. McQuillen felt it was not possible from the evidence obtained to suggest any particular site on the cell surface at which cetrimide might appear to be specifically absorbed.

Further evidence for the occurrence of leakage from bacterial cells treated with a surface-active cationic germicide was obtained by Eggenberger[84]. It was found that on the addition of dodecylammonium chloride to thrice washed *Staph. aureus* cells suspended in conductivity water, an increase in the equivalent conductivity of the system was noted, presumably due to the leak of electrolytes from the cells. When autoclaved

bacteria replaced the living suspension, the increase conductivity on exposure to the surface-active agents could not be detected, suggesting the heat treatment also caused a leak of electrolyte and further leakage could not be induced by the detergent. This was later confirmed by Salton[77] who demonstrated a leak of 260 mμ absorbing-material from bacteria that had been kept at 100° for 10 minutes. Thus it was concluded that the leak of electrolytes from bacterial cells could be effected by heat treatment as well as by treatment by surface-active agents. The data suggested to Eggenberger and colleagues that the released material could not have been entirely inorganic ions but, because of the increases in equivalent conductivity obtained, must have been due to some other material.

Ordal and Borg[85] studied the effect of cetylpyridinium chloride and sodium dioctylsulphosuccinate on the oxidation of lactate by *E. coli* and *Staph. aureus* using both molecular oxygen and methylene blue as hydrogen acceptors. These workers found that the lactate methylene blue system of *Staph. aureus* was far more susceptible to the action of both agents than was the same system in *E. coli*. When molecular oxygen was the final hydrogen acceptor, lactate oxidation by *Staph. aureus* was inhibited by both compounds in contrast to *E. coli* to which only the cationic (pyridinium) compound was inhibitory. It was concluded from this work that the terminal oxidation enzymes of lactate oxidation of *E. coli* are more susceptible than those responsible for the reaction in which an artificial carrier was the final hydrogen acceptor, and it was suggested that the cytochrome system responsible for the mediation of the reduction of molecular oxygen with *E. coli* was either more susceptible or more accessible to the action of the surface-active agent.

Sevag and Ross[86] made a systematic study of the action of the cationic detergent, benzalkonium, on certain enzyme systems of bakers' yeast. It was found that at concentration of 0·1 per cent the 545 to 565 and 605 to 625 mμ absorption bands of cytochrome c in yeast cells were reversibly reduced in intensity. Quantitative measurements of the reduction in intensity of the bands were not made, their value being estimated by inspection in a hand spectroscope. The cytochrome-cytochrome oxidase system was investigated by following the oxidation of *p*-phenylenediamine colorimetrically and manometrically. Inhibition of this system was found to be complete at a benzalkonium concentration of 1 : 35,000 and a yeast-benzalkonium ratio between 10 : 1 and 20 : 1. The oxidation of glucose by molecular oxygen was inhibited to the extent of 91 per cent at yeast-benzalkonium ratios of 30 : 1. When methylene blue replaced oxygen as the final acceptor, inhibition was 97 per cent for yeast-benzalkonium ratios of 30 : 1 to 40 : 1. Further experiments attempted to relate the inhibition of growth with inhibition of respiration of glucose in phosphate buffer. At benzalkonium concentrations of 1 : 55,000 to 1 : 220,000 a stimulation of oxygen consumption of 54 to 60 per cent was obtained, the inhibition of growth was stated to be from 34 to 83 per cent.

Roberts and Rahn[87] measured the effect of disinfectants on the dehydrogenation and oxidation of acetate and the catalase activity in *E. coli*. Enzymic activities in the three reactions were followed by measuring the

rate of decolorisation of methylene blue with the Thunberg method, the rate of oxygen consumption in the Warburg respirometer and the amount of undecomposed hydrogen peroxide remaining in the reaction system. The authors were seeking to test whether the killing of bacteria was due to the inhibition of energy-furnishing enzymes and concluded that with phenol and the two cationic compounds, retardation of growth occurred at a concentration which had little effect on energy production measured by these three biochemical reactions. At bacteriostatic concentrations the amount of enzyme inactivation varied, the acetate oxidation system appearing to be the most susceptible. At lethal concentrations, acetate oxidation and dehydrogenation was retarded to the extent of 96 per cent, but catalase activity was again less susceptible.

Krebs[88], in a general study of the decarboxylation of glutamic acid and its monoamide, glutamine, by washed suspensions of *Clostridium welchii* noted that cetrimide accelerated the decarboxylation of these two compounds as measured manometrically by the rate of CO_2 evolution, while an anionic detergent, sodium dodecylsulphate, even at 0·05 per cent concentration, inhibited this decarboxylase. The histidine decarboxylase on the other hand was inhibited by cetrimide, but not affected by sodium dodecylsulphate. Krebs suggested that one explanation of the acceleration was that the permeability of the bacterial cells to the substrate was increased. To test this hypothesis Hughes[89] measured the effect of cetrimide on the rate of decarboxylation of glutamate and glutamine using cell-free preparations of *Cl. welchii*, *Proteus morganii*, *E. coli* and *Str. faecalis*. Contrary to the expectation, cetrimide accelerated the decarboxylation of glutamine and glutamic acids by *Cl. welchii*, *P. morganii* and *E. coli* to the same extent whether intact cells or cell-free extracts were used, and Hughes concluded that the main action could not be due to the effect on permeability. At low substrate concentrations, however, the degree of acceleration with intact cells was slightly higher than with extracts, and it was suggested that under these conditions the acceleration might be due to an increase of cell permeability. An examination of the remaining experimental data suggested that cetrimide increased the apparent affinity of the enzyme for its substrate. This in turn could be accounted for by (i) increased local concentration of substrate in the vicinity of the enzyme, (ii) alteration in the properties of the enzyme by reacting with an added substrate, or (iii) removal of an enzyme inhibitor. Of these hypotheses the third was thought to be the only one which explained the stimulation of decarboxylase and glutaminase activity. The possible nature of the inhibitor was not discussed. No stimulation was observed with decarboxylases for other amino acids when five bacterial and two plant sources of these enzymes were tested.

Knox and colleagues[90] studied the effect of five quaternary ammonium and pyridinium detergents on certain metabolic reactions of *E. coli*. These were, lactate oxidation and dehydrogenation, glucose and hexose diphosphate oxidation and glycolysis, the oxidation of pyruvate, formate, alanine and succinate, arginine decarboxylation and finally aldolase activity. In the experiments on the inhibition of glucose oxidation the

kill per cent was estimated at the end of the experiment by means of a viable count on the contents of the Warburg flask. In certain instances cell-free enzymes were prepared and the effect of the detergents on the activities of the cell-free enzymes compared with the effect on the intact cells. The concentration of each of the five detergents expressed as μg./mg. of bacterial nitrogen to produce 50 per cent kill and 50 per cent inhibition of glucose and lactate oxidation are summarised in Table IV.

TABLE IV

EFFECT OF DETERGENTS ON VIABILITY AND SUBSTRATE OXIDATION USING *E. coli*

Detergent	Concentration to produce		
	50 per cent kill	50 per cent inhibition glucose oxidation by intact cells	50 per cent inhibition of lactate oxidation by intact cells
1	40 μg./ml.	27 μg./ml.	—
2	95 ,,	88 ,,	180 μg./ml.
3	120 ,,	110 ,,	—
4	140 ,,	140 ,,	—
5	260 ,,	225 ,,	—

1. 1-*n*-Hexadecylpyridinium chloride (Ceepryn). 2. Benzalkonium. 3. Cetrimide. 4. *N*-(Nonylnaphthyl-methyl)-pyridinium chloride (Emcol 888). 5. *N*-(Lauryl-colamino-formyl-methyl)-pyridinium chloride (Emulsept).

The figures for the viable count may be low as dilutions were plated out into Endo's agar and counts made after 24 hours, while it is customary to count after 48 or even 36 hours incubation, and no attempt was made to neutralise the cationic detergent or to overcome the clumping that occurs with this class of antiseptic. With these reservations, the Table shows a relation between killing and glucose oxidase inhibition. The detergent: bacterial nitrogen ratio for 50 per cent inhibition of lactate oxidation to benzalkonium was 180 μg./mg. N for intact cells, a higher figure than the corresponding value for glucose (88 to 90 μg./mg. N). A "considerable stimulation" of lactate oxidation by sub-bactericidal amounts of this detergent was reported. No actual figures were given for the extent of stimulation, nor was this observation discussed. The arginine decarboxylase activity of intact cells was found to be remarkably resistant to benzalkonium, and at certain detergent concentrations marked stimulation of its activity noted.

In this instance the arginine decarboxylase activity persists and is even stimulated at bactericidal concentrations of the detergent; this stimulation was not shown to such a marked extent with a cell-free arginine decarboxylase. The authors interpreted the stimulation to be due to an increase in the permeability of the intact bacterial cell to the substrate, caused by the benzalkonium. A lactic oxidase preparation which catalysed the oxidation by molecular oxygen of lactic acid to pyruvic acid was prepared by grinding the cells for 3 hours in a Booth-Green mill and centrifuging the resulting slurry after diluting with water. It was concluded that the specific inhibition of detergent sensitive enzymes can account for the metabolic inhibition, cell death and increased permeability observed in bacteria with bactericidal amounts of cationic detergents.

It is difficult to see how metabolic inhibition and cell death can be accounted for by the fact that a lactic oxidase is inhibited by detergents to the same extent unless this reaction is shown to be essential for the metabolism of the cell. It is even more difficult to see how this enzyme sensitivity can account for the increased permeability shown although the stimulated lactate oxidation by intact cells in the presence of subinhibitory concentrations may be attributable to a change in the permeability of the cell to substrate or coenzyme. This is the reason given for stimulated arginine decarboxylase activity.

Knivett[91] while studying the catabolism of arginine by washed suspensions of *Str. faecalis* noted that despite the complete disappearance of this amino acid only 70 to 80 per cent could be accounted for by the known products of the reaction, which were postulated to be ornithine, carbon dioxide and ammonia. Ultimately, citrulline was found in the reaction mixture and this accounted for the discrepancy. Cell-free preparations or acetone or cetrimide treated cells were found to convert arginine to citrulline and carbon dioxide. With cetrimide-treated cells the reaction is rapid and proceeds to completion. Citrulline was found to be attached very slowly to intact cells. If adenosine triphosphate is added to the reaction system, cetrimide and acetone treated cells can then convert citrulline to arginine, carbon dioxide and ammonia.

Postgate[92] has demonstrated the leak of a cytochrome component from *Desulphovibrio desulphuricans*, on treatment of the cells with cetrimide.

Conclusion

Until recent years so little was known about the antibacterial action of phenols and their chlorinated and nitrated derivatives that it was described in general terms as non-specific, or they were even less helpfully known as general protoplasmic poisons or protein precipitants.

It was the application of what is, in terms of modern methods, the relatively crude methods of micro-respiration which provided the clue to the precise mechanism of action of the substituted phenols. In turn these methods have been applied to the cationic surface-active compounds. It may be expected that more modern methods will prove equally successful. In particular some of the problems of cell interface reactions may be expected to resolve to the more physico-chemical methods which have been applied to single-cell studies, and it may be that interference and phase-contrast microscopy will find a direct application. There is scope for systematic investigation of the mode of action of the newer antiseptics now being introduced into medicine, and it can be expected that interesting and unsuspected mechanisms may be discovered.

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RESEARCH PAPERS

VALERIANA OFFICINALIS LINN., ITS POLYPLOID FORMS AND THE STRUCTURE OF THEIR RHIZOMES AND ROOTS

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From the Museum of the Pharmaceutical Society of Great Britain

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Skalinska[1] has noted that recent work has revealed the occurrence in Europe of three chromosomal forms of *Valeriana officinalis* Linn. The diploid form has been reported from the Continent only[2], whereas tetraploid and octoploid forms occur in Britain as well as upon the Continent. The present communication is a record of a histological study of tetraploid and octoploid forms, authentic specimens of which were provided by Skalinska. The work was further extended by examining a number of specimens grown for commercial use and others consisting of the dried rhizome and roots sold as "valerian root" of commerce for the preparation of medicines.

Materials

The materials used are as follows:—

1. Authentic samples of *Valeriana officinalis* Linn., tetraploid and octoploid forms, from Mme. Skalinska of the Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey.
2. Samples of fresh rhizome from Harold Deane, Esq., of Messrs. Stafford Allen and Co., Long Melford, Suffolk.
3. Fresh samples grown by T. E. Wallis at Mill Hill, London, N.W.7, for several successive seasons; the parent plant was obtained from H. Deane, Esq., of Long Melford.
4. Sample from Switzerland, Botanical Garden, Zurich.
5. Samples of leaves and flowers from herbarium sheets in the Herbarium at Kew Gardens, through Dr. C. R. Metcalfe.
6. Samples from the Museum of the Pharmaceutical Society of Great Britain.
7. Five commercial samples of "Valerian, B.P." from different wholesale houses in England.

Description of the Rhizome

The fresh rhizome grows vertically in the ground, it is bluntly cylindrical-obconical, about 3 cm. long and 2 cm. wide at the crown. Horizontal stolons arise in the axils of the scale leaves and bear other rhizomes at their extremities, some of the stolons are very short, so that three or four rhizomes often become grouped together to form a rather dense mass. The longer stolons may attain a length of about 10 cm. and a diameter

* The subject-matter of this communication formed part of a Thesis by one of us (P.K.S.) accepted for the degree of Ph.D. in the University of London.

of 5 mm.; they frequently ascend at the tips and grow out to form aerial stems; they have intermodes about 5 mm. long near the parent rhizome, but as much as 15 mm. in the more distal part; scale leaves and sometimes also adventitious roots occur at the nodes from some of which branch stolons may arise and these also may grow out to form aerial shoots. Very numerous pale-buff adventitious roots arise from all parts of the surface of the rhizome except the actual summit; they are about 15 to 20 cm. long and 2 to 3 mm. thick; the majority travel horizontally for a distance of about 10 cm. and then curve downwards; roots from the lowest part of the rhizome are not directly vertical but somewhat oblique. The apical 2 to 5 cm. of each root is more slender and has numerous fibrous branches.

The fresh rhizome has no valerianic odour but when broken or squeezed, a slight aromatic odour is perceptible; it has a slightly pungent aromatic taste.

Commercial valerian, sold as a drug, is dull brownish grey and consists of the dried rhizomes with stolons and and attached roots. To facilitate drying the larger rhizomes are cut longitudinally, more rarely transversely, into 2 or 4 pieces and show the same general characters as the fresh rhizomes. They are, however, much shrunken and in the pieces cut longitudinally the exposed pith may show about 4 to 9 transverse diaphragms separated by spaces or lacunae (see Fig. 1, *G* and *H*). The roots are so numerous that there is hardly any free surface between their bases at the surface of the rhizome; they are about 2 mm. in diameter, brittle and much curved and twisted and are longitudinally wrinkled. The smaller rhizomes are entire and many of the roots are broken off and lie loose in the drug. The stolons are reddish brown, much shrunken and about 2 mm. in diameter. Numerous small circular scars are present on the rhizomes and also upon the nodes of the stolons where roots have been broken off. The drug is hard and breaks with a short horny fracture; it has a strong characteristic odour and a sweetish, camphoraceous and ultimately slightly bitter taste.

Origin of the Lacunae

The microscopy of the rhizome has been well described by several pharmacognosists, the most detailed account being that of Moll and Janssonius[3]. An item of interest which does not appear to have been described is the mode of origin of the large horizontal disc-shaped lacunae in the pith. The large parenchymatous pith contains an abundance of starch; the cells measure, R and T* 48 to **75** to 115 μ and L 61 to **80** to 86 μ. In the middle part of the pith of older rhizomes there is a vertical series of lacunae. Each lacuna originates by the development of isolated cells without visible contents, followed by their multiplication and finally by the rupture of their cells walls (see Fig. 2). The large space or lacuna thus formed extends almost across the pith in the position

* When recording measurements, the letters L, R and T are used to indicate measurements in a longitudinal, radial and tangential direction, respectively, the directions having reference to the axis of the relevant plant member.

of one of the short internodes of the rhizome; between the lacunae diaphragms of parenchyma remain in the position of the nodes; young rhizomes are devoid of lacunae and diaphragms. The formation and position of these structures is shown in Fig. 1, *H*.

Sclereids of the Pith

The rhizomes of the tetraploid and octoploid forms are closely similar in their structure. Though the cells of the pith and cortical parenchyma are rather larger in octoploid than in tetraploid forms there is not sufficient

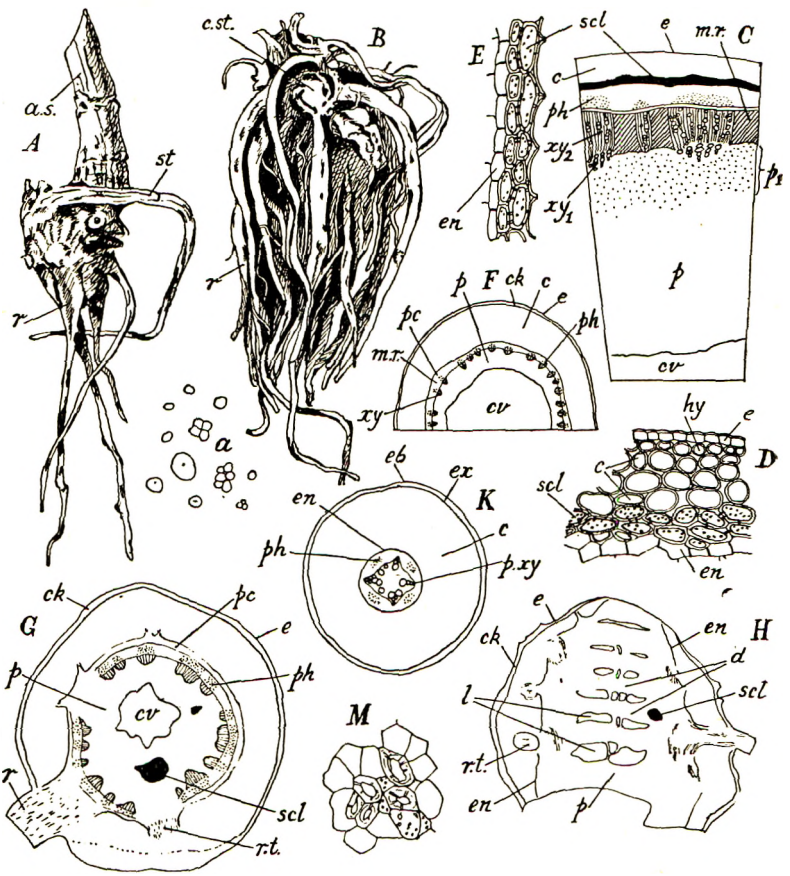

FIG. 1. *Valeriana officinalis* Linn. *A* and *B* dried commercial rhizomes, × 1; *C*, diagram of a transverse section of an aerial stem, × 6; *D*, outer tissues of stem in T.S., × 50; *E*, sclerenchyma and endodermis of stem in L.S., × 50; *F*, diagram of T.S. of a stolon, × 5; *G*, diagram of T.S. of a rhizome, × 5; *H*, diagram of L.S. of a rhizome, × 5; *K*, diagram of T.S. of a root, × 25; *M*, small group of sclereids from pith of a rhizome, × 35; *a*, starch, × 200; *a.s.*, aerial stem; *c*, cortex; *ck*, cork; *c.st.*, scar left by stolon; *cv*, cavity; *d*, diaphragm; *e*, epidermis; *eb*, epiblema; *en*, endodermis; *ex*, exodermis; *hy*, hypodermis; *l*, lacuna; *m.r.*, medullary ray; *p*, pith; p_1, lignified part of pith; *pc*, pericycle; *ph*, phloem; *p.xy*, protoxylem; *r*, root; *r.t.*, root trace; *scl*, sclereids; *st.*, stolon; *xy*, xylem; xy_1, primary xylem; xy_2, secondary xylem.

difference to be of real diagnostic value. The starch grains also have a maximum size of 12 to 14 μ in octoploid and of 8 to 10 μ in tetraploid plants. The difference of sizes of these cells and starch granules is of the same order as the error of measurement, so that they are useless as criteria for differentiation of the varieties. There is, however, one feature of the pith which possesses a well-defined diagnostic significance; this is the presence of groups of sclereids in the diaphragms of the pith of tetraploid plants (see Fig. 1, *G*, *H* and *M*) and their absence from octoploids. These sclereids are strongly lignified, pitted and heavily thickened, individual sclereids are isodiametric and measure about 30 to **50** to 60 μ in diameter. See Fig. 1, *M*.

These sclereids are briefly referred to by Karsten and Beneke[4] and by Gilg, Brandt and Schürhoff[5], but these authors do not say where they occur in the rhizome. They have been described and figured by Tschirch and Oesterle[6], by Flück and Haller[7] and by Flück, Schlumpf and Siegfried[8], all of whom note their occurrence in the diaphragms of the pith. Tschirch and Oesterle also state that when found in the powder, they indicate the presence of rhizome. It is also evident that all these authors have examined tetraploid plants.

Histology of the Root

(a) *Roots from Plants of the Current Season* (see Fig. 3)

Externally is a piliferous layer consisting of small subrectangular cells measuring about R and T 6 to **30** to 40 μ and L 40 to **60** to 102 μ. The outer walls are dome-shaped, yellowish, cuticularised and slightly lignified, the thickening of the wall showing striations due to layering of the secondary deposit; they may be prolonged as papillae or as short or long unicellular root-hairs which are from 20 to **53** to 82 μ long and 12 to 20 μ wide.

The exodermis over the greater part of the root consists of a single layer of large polygonal tabular cells; sometimes becoming locally increased, in the one to 3 centimetres nearest to the rhizome, to 2 to 4 layers over about one-eighth to one-quarter of the circumference, Figure 3, *E*. The cells have thin, suberised and lignified walls and are fairly uniform in size measuring R 20 to **41** to 53 μ, T 32 to **60** to 70 μ and L 32 to **60** to 82 μ. The cells of the exodermis contain globules of volatile oil, but occasional cells, scattered at irregular intervals, are nearly cubical in shape, about 35 to 40 μ across, and contain granular contents, which stain with Sudan-red and also stain deep brown with iodine and sulphuric acid.

The cortex consists of about 15 to 28 layers of cells; the outer 2 or 3 layers, just within the exodermis, are collenchymatous and smaller in size than the cells in the middle part of the cortex. Collenchymatous cells are prominent in the sections cut from the portion nearer to the rhizome where the roots have become fully developed. All the cells of the cortex contain abundance of starch and the walls are cellulosic. The measurements of the outer collenchymatous cells are R 16 to 40 μ, T 28 to 50 μ and L 8 to **88** to 135 μ; the larger cells of the middle region measure R 33 to **53** to 70 μ, T 40 to **60** to 70 μ and L 48 to **112** to 155 μ,

while those immediately outside the endodermis are about the same size as the collenchymatous cells. There are a few isolated cells whose contents become brownish with iodine and sulphuric acid, but these cells were not found in all sections.

The endodermis consists for the greater part of one layer of prominent regularly arranged cells with bright casparian strips; at places, over a very limited area, the endodermis consists of two layers of cells. The

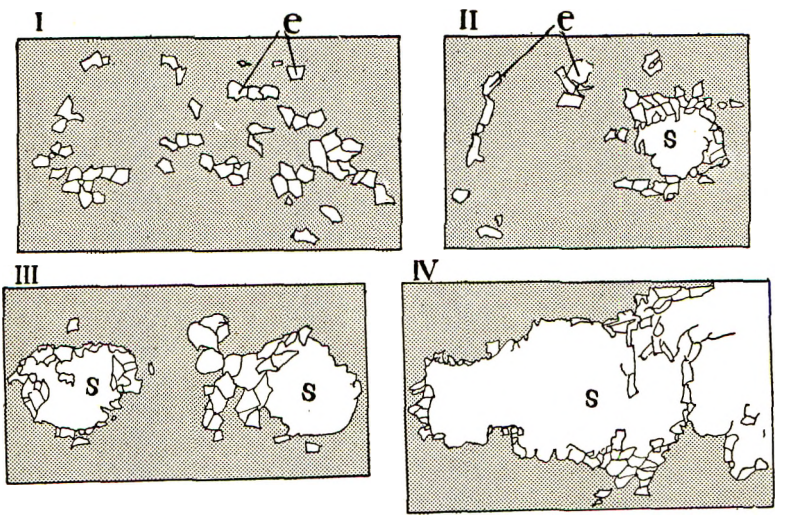

FIG. 2. *Valeriana officinalis* L., Rhizome. I, II, III and IV successive stages in the development of the lacunae between the diaphragms of the pith; all × 25. The shaded areas consist of parenchyma containing starch, e, cells without visible contents.

cell walls are suberised and take a reddish or brownish stain with Sudan-red. A very few small droplets of oil have been found in some sections, and there are some cells with granular contents which may take a black stain with iodine, but become brown on the further addition of sulphuric acid. The dimensions of the cells are R 12 to **20** to 28 μ, T 20 to **45** to 49 μ and L 32 to 50 μ. By treatment with iodine and sulphuric acid the endodermal layer becomes more prominent, the walls take a brown colour and passage cells can be seen opposite to the protoxylem groups and occasionally in other positions also.

The pericycle consists of 1 to 3 layers of large parenchymatous cells, which are sometimes slightly collenchymatous. The walls are of cellulose and the cells measure R 16 to **20** to 24 μ and T 24 to 32 μ.

The roots are at first mostly tetrarch, but sections taken from the portion nearest to rhizome show a polyarch structure with up to 19 bundles which may be discrete or more or less united into groups by slight secondary development of xylem vessels. Generally nearest to the rhizome, due to the secondary growth of xylem, the protoxylem comes to lie at the middle of each xylem group. If sections are taken at every 2·5 cm. of a long root, beginning from the rhizome, the number of bundles

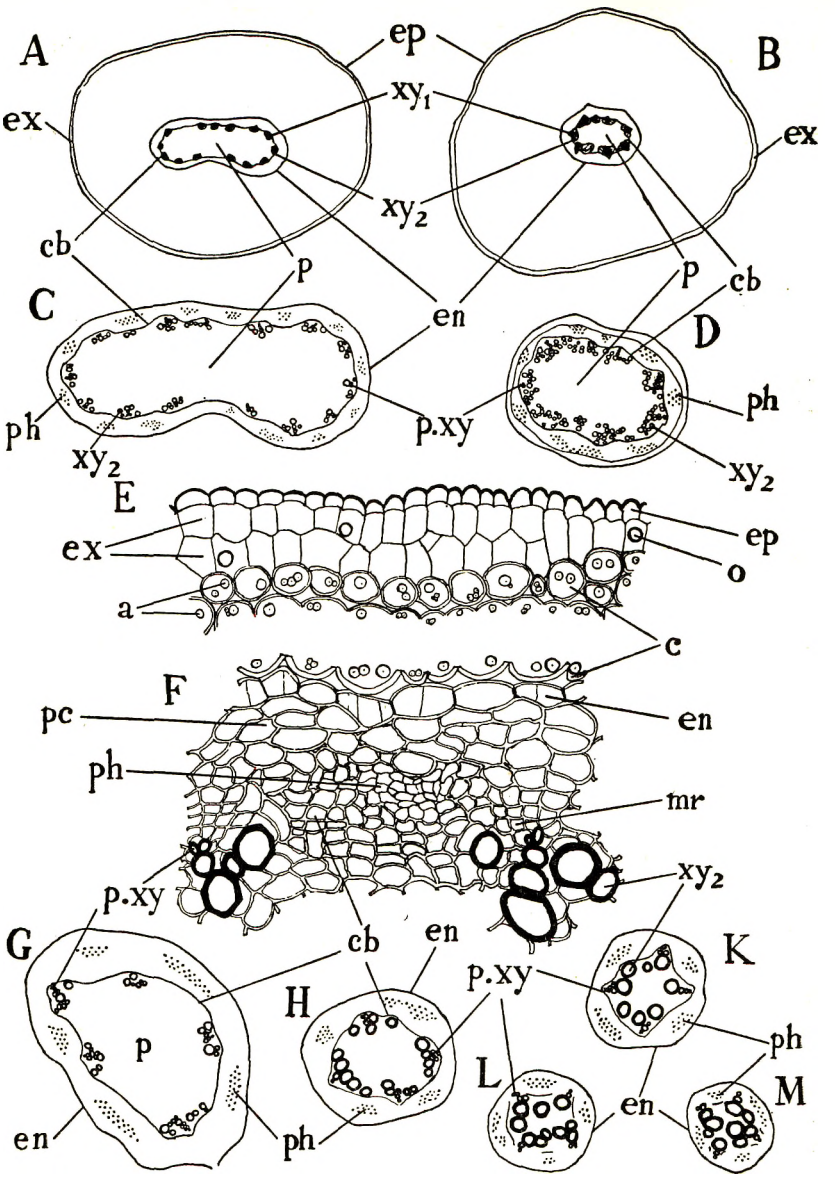

FIG. 3. *Valeriana officinalis* L., Root. *A*, diagrammatic transverse section of a long root, showing an oval stele × 10; *B*, diagrammatic transverse section of an externally similar root showing a nearly circular stele × 10. Both *A* and *B* were cut within 1·0 cm. of the rhizome. *C*, and *D*, steles from *A*, and *B*, respectively, both × 25. *E*, portion of the outer cortex from the same root as *G*, showing part of the exodermis where much of it has become 2 layered × 100. *F*, details of two xylem bundles and one phloem bundle from *A*, × 200. *G*, *H*, *K*, *L*, and *M*, diagrams of the steles of 5 successive transverse sections of the same root cut at distances of 1, 2, 3, 5 and 7 inches respectively measured from the rhizome, showing the gradual diminution in size of the pith until it practically disappears, all × 50. *a*, starch; *cb*, cambium; *c*, cortex; *en*, endodermis; *ep*, epiblema; *ex*, exodermis; *mr*, medullary ray; *o*, oil globule; *p*, pith; *pc*, pericycle; *ph*, phloem; *p.xy*, protoxylem; xy_1, primary xylem; xy_2, secondary xylem.

becomes gradually less till near the tip it is only four. Excluding the very small vessels of the protoxylem, the majority of the vessel-elements measure R and T 20 to **36** to 50 μ and L 164 to **205** to 287 μ. The phloem groups also increase by secondary development and nearest to the rhizome the phloem groups with the medullary rays between them form an almost continuous narrow ring outside the xylem. The medullary rays extending from the tips of protoxylem groups to the cortex are very clearly seen when stained with iodine and sulphuric acid since they take a contrasting blue colour. Starch grains in small numbers are found in the pericycle and phloem, but not in all the cells. In the older parts of the roots, the cambiform tissue consists of 3 to 4 layers of cells, the walls of which become light blue and the contents yellow with iodine and sulphuric acid.

Towards the tip of the root where it is usually tetrarch, there is no pith, though there may be one or two parenchymatous cells between the vessels of opposite bundles, Figure 3, *M*. In older parts of the root, as the number of xylem bundles increases, the parenchymatous cells also multiply so that near the rhizome a well marked pith is usually present; see Figure 3, *G*. The cells of the pith measure from R and T 24 to 32 μ and about L 40 μ, they have cellulose walls and contain starch in smaller amount than the cells of the cortex. Some of the root sections show steles of oval shape while others are nearly circular, Figure 3, *A* and *B*. Foder and Kichler[9] have stated that the Japanese variety of valerian can be distinguished from the European by the shape of the central cylinder; that of the Japanese valerian is oval while in European valerian it is round. However, since the roots of several of samples from England possess oval steles in the 4 cm. nearest the rhizome, this alleged distinction is not valid.

As there are two forms of British valerian, having octoploid and tetraploid nuclei respectively, the structure of roots from three tetraploid and four octoploid samples was examined to search for any anatomical features by which the two forms may be distinguished. No clearly marked differences in cell structure of the roots from the two forms could be discovered by which they can be certainly distinguished. However, one can obtain an indication of the degree of polyploidy by determining the maximum size of the starch grains present in the roots. For this purpose, softened roots (stored in glycerol 1 volume, water 3 volumes) were scraped with a needle and the starch so removed was mounted in lacto-phenol; the largest grains were selected by eye in a systematic search of the mount under a 16 mm. objective and as each was noted, its size was accurately determined under a 4 mm. objective. Proceeding in this way one finds that starch of the tetraploids has a maximum size of 18 μ, while that of the octoploids attains a maximum of 30 μ.

(b) *Roots from Rhizomes of Last Season, after the Aerial Plant has Fully Developed, Flowered and Fruited and Died Down*

Transverse sections of roots from old rhizomes showed a typical secondary development of the xylem and other tissues. At the centre

is a pith resembling that of the roots from daughter rhizomes; or sometimes a large central hollow has replaced much of the pith which is surrounded by a wide band of xylem having a width almost 1½ times the diameter of the pith. Medullary rays traverse the secondary xylem, one opposite to each protoxylem group, and the cells of the rays have become both thickened and lignified; the parenchyma of the xylem is also thickened and lignified so that a very hard core of xylem is formed. Outside the xylem is a cambium and a narrow band of phloem. The cortex is almost twice the width of the ring of xylem and is similar in structure and contents to that of the roots attached to rhizomes of the present season, see Figure 4. The great majority of the roots on the old parent rhizome show these features, but there are a very few roots, apparently formed during the current season, which resemble those of the daughter rhizome, as one would expect. Starch is equally abundant in the cortex of both old and young roots. The roots from tetraploid and octoploid plants are similar in structure and distribution.

The structure of the roots is described somewhat fully because Tschirch[10] has stated that two types of roots are present in valerian; Neuber[11] and Tschirch[12] have suggested that the roots should be named "hold-fast" and "storage or nourishing" roots respectively. Tschirch and Neuber further suggest that the presence of two distinct types of root is to be found in many plants. In valerian, however, it seems to be obvious that the difference is merely one of the age and that two physiologically differentiated types do not exist (see Fig. 4).

This conclusion is in agreement with the finding of Flaskamper[13], who states that his investigations show that the occurrence of "nourishing" and "hold-fast" roots in the sense of Tschirch's thesis cannot be maintained. Tschirch, in his "Handbuch", remarks in a note that "The objections of Flaskamper against the Heterorizie are overruled, the hold-fast roots are not an older stage of the nourishing root", but he offers no further evidence.

In commercial valerian there are few parent rhizomes bearing the remains of the large aerial stem. This is probably due to the fact that these old rhizomes become shrunken and tend to decay. We have, however, been able to find a very few parent rhizomes and the roots conform to the descriptions given above. Another point also deserves comment; Moll and Janssonius[3] make a distinction between roots growing vertically and those growing horizontally. We have been unable to confirm this view, but find no fundamental difference in the structure of the xylem of roots growing in these two directions.

Stolon (see Fig. 5)

The epidermal cells of the stolon measure about R 12 to 16 μ, T 16 to **28** to 36 μ and L 40 to 60 μ; the outer walls are slightly dome-shaped, thickened and have a striated cuticle. A phellogen arises in the outermost layer of the cortex and forms up to 3 or 4 layers of cork cells in the older stolons. The cells walls of the cork are thin, suberised and measure R 20 to **36** to 41 μ, T 41 to **45** to 49 μ and L 53 to **61** to 69 μ.

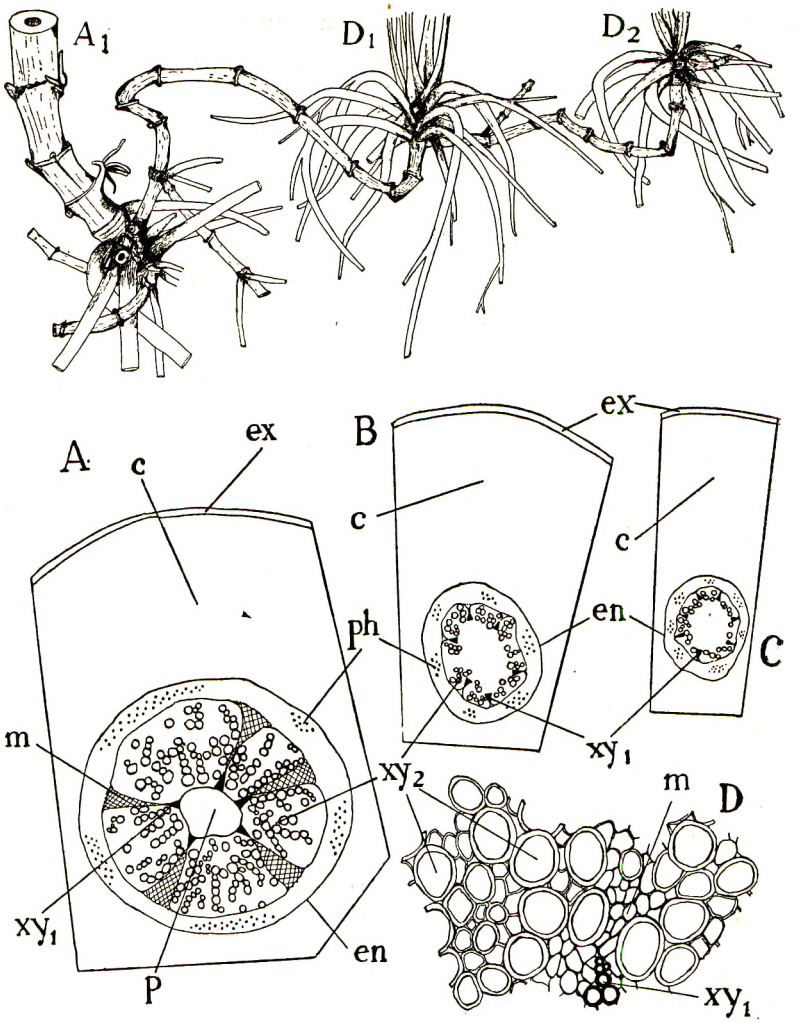

FIG. 4. Three plants of *Valeriana officinalis* Linn., octoploid, attached to one another by means of stolons. A_1 is a parent plant, with a thick woody aerial stem; D_1 is the first daughter plant, which arose from the parent plant during the succeeding season; D_2, another daughter plant grown the same year as D_1, but much younger. All $\frac{2}{3}$ natural size. The drawing shows how each stolon terminates in a new plant and turns at its extremity into an almost vertical direction when doing so. *A*, *B* and *C* are diagrammatic transverse sections from the roots of the plants A_1, D_1 and D_2, respectively, all × 25. *D*, portion of a xylem bundle from *A*, showing a primary xylem group together with some secondary xylem × 100. *c*, cortex; *en*, endodermis; *ex*, exodermis; *m*, medullary ray; *p*, pith; *ph*, phloem; xy_1, primary xylem; xy_2, secondary xylem.

The cortex consists of about 12 to 16 layers of parenchyma, the cells of the outer and innermost regions being somewhat smaller than those of the middle region; the walls are of cellulose and the whole tissue is filled with starch granules. Individual cells measure about R 20 to **40** to 82 μ, T 28 to **60** to 102 μ and L 70 to **94** to 106 μ.

The endodermis consists of one to two layers of cells which are 4–5-sided in transverse section, having suberised and slightly lignified walls and having the dimensions R 20 to **36** to 41 μ, T 28 to **41** to 48 μ and L 61 to **82** to 139 μ; some of the cells contain droplets of oil but there are no other visible contents.

The pericycle consists of 3 to 4 layers of small but thick-walled cellulosic cells which measure R and T 12 to 28 μ and L up to 48 μ. The stele contains a ring of 15 to 25 collateral bundles with wide medullary rays between them. There is a well-marked cambium which is continued across the medullary rays. The xylem vessels are strongly lignified and the vessel elements measure L 205 to 328 μ and R and T 16 to 32 μ, often being slightly wider tangentially. The pith occupies about one-half to three-fifths of the diameter of the stolon and consists of cellulosic parenchyma; usually there is a large hollow in the centre. The cells of the pith measure about R and T 28 to 65 μ and L 125 to 145 μ; they contain abundant starch grains which are mostly simple, but some are 2– to 3–compound, individual starch grain measure about 6 to 12 μ. In stolons showing marked secondary growth, the xylem bundles become closely approximated, eventually forming a closed ring, the medullary rays as well as the outer part of the pith becoming lignified.

No marked difference could be observed between the stolons of the octoploid and tetraploid forms, excepting that a hollow is more consistently present in the pith of the stolons of octoploid plants than in those of the tetraploid plants.

Aerial Stem (see Fig. 1, *C*, *D* and *E*)

The epidermis consists of slightly elongated polygonal tabular cells which measure about R 17 to 20 μ, T 20 to **33** to 43 μ and L 40 to **59** to 66 μ. The outer wall of the epidermal cells is more strongly thickened than the other walls; it is cuticularised but not lignified, being insoluble in cold sulphuric acid and giving no reaction with phloroglucin and hydrochloric acid. In the younger parts of the stem there are numerous trichomes mostly unicellular, but occasionally two celled. The unicellular trichomes are about 116 μ long and 26 μ wide at the base; the two-celled trichomes are longer and measure about 155 to 693 μ long and up to 36 μ wide; the surface of the trichomes exhibits numerous elongated cuticular warts and the base of some of them is slightly constricted. The older basal region of the stem bears a few trichomes only.

The cortex consists of 5 to 10 layers of rounded parenchymatous cells measuring about R 30 to **50** to 66 μ, T 53 to **73** to 76 μ and L 86 to **116** to 132 μ; the smallest cortical cells are those of the hypodermis; the dimensions of the cells of the remaining tissue gradually increase towards the interior as far as the layer of sclereids. The walls are of cellulose and starch is abundant in the young stem, but is absent from the cortex of the very mature woody stem close to the rhizome; the one or two layers of the cortex immediately adjacent to the endodermis gradually become thickened and lignified, with simple pits in the walls; so that in the oldest parts of the stem this layer forms a complete ring of

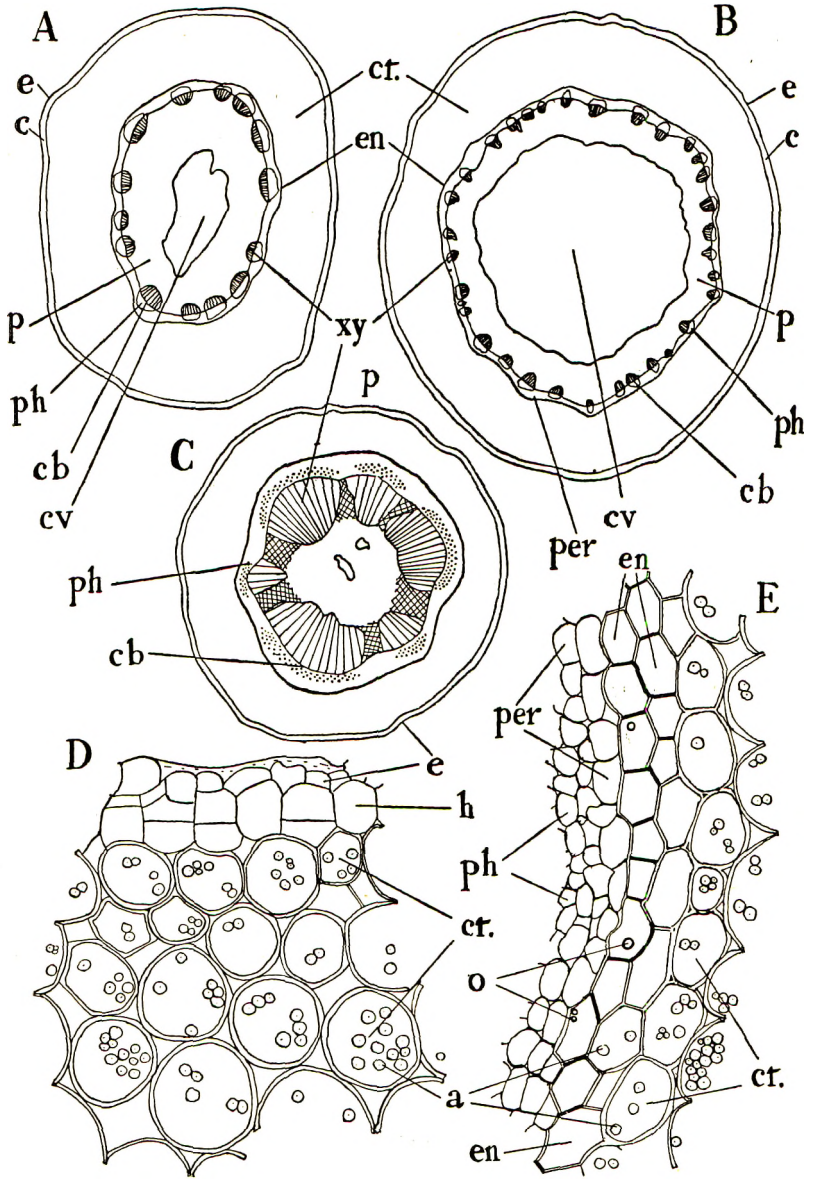

FIG. 5. *Valeriana officinalis* Linn., Stolon. *A*, diagrammatic transverse section of a younger stolon branching from the older stolon *C*. *B*, diagrammatic transverse section of a thick stolon from a young and vigorous rhizome. *C*, diagrammatic transverse section of an old stolon in its second year from a parent plant bearing a large aerial stem. *A*, *B*, and *C*, all × 12. *D*, epidermis and outer cortical layers from the stolon *B*. *E*, part of a transverse section of the stolon *B*, showing the endodermis and adjacent layers. *D* and *E*, both × 200. *a*, starch; *c*, cork; *cb*, cambium; *ct*, cortex; *cv*, cavity; *e*, epidermis; *en*, endodermis; *h*, hypodermis; *o*, oil; *p*, pith; *per*, pericycle; *ph*, phloem; *xy*, xylem.

sclerenchyma. These sclerenchymatous cells measure about R 36 to **46** to 50 μ, T 66 to **79** to 83 μ and L 53 to **70** to 149 μ; they contain no visible cell contents.

The endodermis consists of one or in some places of two layers of cells measuring about R and T 23 to **33** to 43 μ and L 66 to **83** to 99 μ; the cell walls are slightly lignified and cuticularised in the oldest part; they show a distinct casparian strip.

The pericycle consists of a layer of parenchymatous cells measuring about R and T 16 to 23 μ.

In the younger part of the stem there is a circle of numerous collateral bundles separated by wide medullary rays. In the hard and woody part of the stem nearest to rhizome secondary xylem has developed strongly, especially outside the primary xylem groups. The medullary ray tissue formed by the cambium becomes lignified and only a few narrow strips of secondary xylem and phloem are formed in the interfasicular region. The primary xylem groups stand out prominently when the section is treated with phloroglucin and hydrochloric acid, because they project into the pith and the parenchymatous cells between the vessels have not become lignified. All the parenchyma of the secondary xylem is lignified. The elements of the xylem vessels measure about R and T 17 to **40** to 56 μ and L 148 to **198** to 495 μ.

In the centre there is a large pith which is sometimes partially or almost entirely replaced by a central hollow. The cells measure R 116 to **149** to 165 μ, T 109 to **116** to 132 μ and L 132 to **149** to 205 μ; in the young stem they have cellulosic walls and contain starch. The starch grains of both cortex and pith are mostly 2– to 4–compound; single grains measure 3 to 8 μ. Starch is not found in the very thick woody part of the stem nearest to the rhizome and here also the cells of the outer part of the pith are lignified.

Sclereids in the Powder

The structure of the sclerenchymatous layer of the innermost part of the cortex of the aerial stem has been made a subject of special study by Flück and Haller[7] with the purpose of devising a criterion by which the amount of aerial stem present in powdered valerian can be determined. Since these cells and similar ones in the bases of the leaf-stalks are easily distinguished from the sclereids of the pith of tetraploid rhizomes, they can be accurately counted in powdered valerian. A certain amount of sclerenchyma occurs in some stolons, but it is different in form and, since the amount of stolon in commercial valerian is very small—about 0·5 to 1·0 per cent—any error it may introduce is negligible. Flück and Haller find 6·83 sq. cm. of sclerenchyma of stem base and leaf stalk per g. of aerial stem and they propose to exclude more than 5 per cent of stem bases from the drug by imposing a limit of not more than 0·35 sq. cm. of sclereids from the aerial stem per g.

Octoploid Valerian Plants

Evidence that the plant grown at Mill Hill from a rhizome supplied by Mr. H. Deane of Long Melford was an octoploid form was obtained

by measuring the diameters of its pollen grains, as suggested by Blakeslee[14] and also the lengths of its stomata, as used by Karpechenko[15] and quoted by Darlington[16]. These same data were successfully used by Rowson[17] when studying polyploid forms of belladonna and stramonium. Skalinska[18] examined cytologically valerian plants of this same strain and found them to be octoploid; she gave measurements for the pollen grains which she recorded as having a mean diameter of 56 μ with a maximum of 72 μ and 87 μ[1]. Skalinska did not state the mountant in which she examined the pollen grains; preparations mounted in lactophenol were therefore made from specimens in the Herbarium at the Royal Botanic Gardens, Kew, using plants which she had determined cytologically as tetraploid and octoploid. Leaves from the same specimens were cleared in solution of chloral hydrate (chloral hydrate 5, water 2) so as to determine the dimensions of the stomata, values which had not been recorded by Skalinska. The results obtained from these herbarium plants are given in the accompanying Table and the maximum diameters of the pollen grains agree with those recorded by Skalinska.

TABLE I

RECORDS OF LENGTHS OF THE LARGEST STOMATA OBSERVED AND OF THE GREATEST DIAMETERS OF POLLEN GRAINS OF SKALINSKA'S TETRAPLOID AND OCTOPLOID FORMS OF *Valerian officinalis* Linn. PRESERVED IN THE HERBARIUM AT THE ROYAL BOTANIC GARDENS, KEW, SURREY. ALL MEASUREMENTS ARE IN MICRONS

Tetraploid			Octoploid		
No. of plant	Stomata	Pollen grain	No. of plant	Stomata	Pollen grain
T_1	32 to 36	57 to 65	O_1	50 to 52	65 to 70
T_2	36 to 40	53 to 57	O_2	50 to 53	67 to 70
T_3	40 to 42	50	O_3	50	78 to 86

The measurements made of pollen grains from the Mill Hill plant mounted in lactophenol gave a maximum of 78 μ with one larger grain measuring 86 μ; the stomata from leaves of the same plant measured in preparations cleared with solution of chloral hydrate had a maximum length of 50 μ; these dimensions agree with the figures obtained from the octoploid plants of Skalinska's specimens.

These results confirm the validity of the conclusions drawn from the maximum dimensions of starch grains from the roots and from the presence or absence of sclereids in the pith of the rhizomes.

Maximum figures have been quoted in this work because their use has been strongly advocated by Macleod in his book, *The Quantitative Method in Biology*, 2nd edition, 1926, where he summarises observations made during a lifetime of research. His maximal measurements and the application he has made of them give very strong evidence of the utility of maximum values for characterising particular species, even when they are very closely related.

Summary

1. A description is given of the fresh rhizome of *Valerian officinalis* Linn., with its stolons, offsets and adventitious root system.

2. The development of lacunae in the pith of the rhizome is shown to originate from cells having no visible contents by their multiplication and final breakdown to form spaces.

3. The occurrence of sclereids in the pith of the rhizome is shown to be characteristic of the tetraploid plant.

4. The anatomy of the roots is described and the differentiation into "holdfast" and "nourishing" roots is rejected, and it is suggested that these two postulated forms are really older and younger stages of the same roots.

5. The starch granules of tetraploid roots have a maximum size of 18 μ while those of octoploid roots have a maximum of 30 μ.

6. The anatomy of the stolon and of the aerial stem is described and figured and attention is directed to the proposed use of the area of lignified sclerenchyma in the powder as a means of excluding more than 5 per cent of aerial stem.

8. Data based on pollen grains and stomata are given for distinguishing tetraploid and octoploid plants.

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ALKALOIDS OF *RAUWOLFIA* SPECIES

PART II.* THE ESTIMATION OF RESERPINE IN SAMPLES OF RAUWOLFIA BY MEANS OF COUNTERCURRENT DISTRIBUTION

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SEVERAL instances of the use of countercurrent distribution for the separation of alkaloids in various species of *Rauwolfia* have been reported[1–5], and the method has been useful both in the initial resolution of extracts into groups of alkaloids[3], and in the purification of individual substances intractable to other approaches[4]. While exploring the possibilities of this technique, the distribution characteristics of reserpine have been investigated in a variety of systems and applied to the estimation of this alkaloid in small samples of several species of *Rauwolfia*.

A number of methods have been described for the assay of reserpine, but some of these are designed for use with pharmaceutical preparations in which reserpine preponderates, and are less suitable when dealing with natural alkaloidal complexes. Sakal and Merrill[6] have, however, assayed reserpine in crude extracts by ultra-violet spectrophotometry after an initial separation by paper ionophoresis in 5N acetic acid, while Dechene[7] has described a fluorimetric method which was applied to an extract of *R. serpentina*. In the latter case it was not established that the extraction procedure separated reserpine completely from other fluorescent alkaloids, while it has been our experience that reserpine and rescinnamine are not adequately separated by paper electrophoresis in 3N acetic acid. High capacity paper chromatography, with 2 per cent acetic acid in propylene glycol as stationary phase and 1 : 1 benzene *cyclo*hexane as mobile phase enabling as much as 5 mg. of reserpine to be run without bad streaking, has also been used for a preliminary separation of reserpine from crude extracts before assay by ultra-violet absorption[8], while a more recently published method[9] makes use of liquid-liquid partition chromatography on a Celite column followed by hydrolysis and estimation of the resulting trimethoxybenzoic and trimethoxycinnamic acids.

A study was made of the distribution of reserpine in several two phase systems consisting of (*a*) various organic solvents with aqueous acetic acid, and (*b*) an ether-chloroform (3 : 1) mixture with aqueous buffer solutions. Using the latter system, the log of the partition coefficient varied in a linear manner with pH over the range pH 2·4–3·2, giving a partition coefficient of 1 at pH 3·1. At this pH, the partition isotherm was linear over the concentration range 0·1–0·4 per cent w/v reserpine in the solvent phase. A 24 transfer distribution of a sample of reserpine in a Gilson-Wright semi-automatic countercurrent apparatus yielded a distribution curve (Fig. 1) symmetrical about the centre point, the amount

* Part I, *Chem. and Ind.*, 1955, 1481.

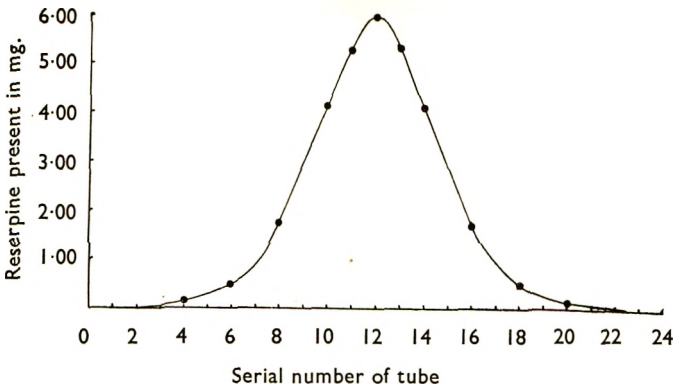

FIG. 1. A 24-transfer countercurrent distribution of pure reserpine.

of solute in each tube being determined by ultra-violet absorption spectroscopy. Under similar conditions, a 14 transfer distribution of a reserpine-rich fraction from *R. vomitoria* in a smaller apparatus yielded a distribution curve in which the central reserpine peak was over-lapped on either side by other absorbent material (Fig. 2). By making three additional transfers and discarding three issuing fractions, the resolution was improved sufficiently to obtain a distinct reserpine peak (Fig. 3), which could then be used to estimate the amount of this alkaloid present. The essentially homogeneous nature of the material comprised within this peak was checked by paper chromatography and electrophoresis. The technique was applied to several different rauwolfia samples with the results given below. It was found desirable to use a chloroform-soluble fraction, and this was conveniently prepared from the root powder by a method similar to that described by Hochstein, Murai and Boegemann[3] for *R. heterophylla.*

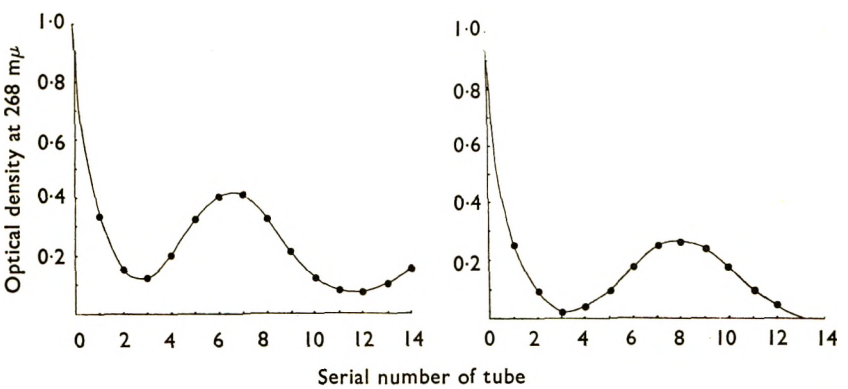

FIG. 2. A 14-transfer countercurrent distribution of a fraction from *R. vomitoria* showing incomplete resolution of reserpine.

FIG. 3. Effect of withdrawing 3 further fractions from 15-tube apparatus giving adequate resolution for estimating reserpine in *R. vomitoria.*

Experimental

Countercurrent Distribution of Reserpine

The solvent system used was as follows.

The mobile phase was composed of chloroform (250 ml.) diluted to 1 litre with ether.

The stationary phase consisted of buffer solution of pH 3·1 containing 16·3 g. of citric acid ($C_6H_8O_7 \cdot 1H_2O$) and 16·1 g. of disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) in 1 litre of water.

These solutions were mutually saturated before use and the stationary phase put in 25 tubes of a semi-automatic Gilson-Wright apparatus with a lower phase capacity of 20 ml. per tube. After carrying out one transfer with 20 ml. of mobile phase to ensure equilibrium, 20 ml. of a solution of reserpine (100 mg.) in chloroform (12·5 ml.) diluted to 50 ml. with ether was introduced and distributed through the apparatus using about 50 tube-inversions and a settling time of 2 minutes per transfer. After 24 transfers, 1 ml. of ammonia (sp.gr. 0·880) was added to each tube, the apparatus given 50 tube-inversions and aliquots of 5 ml. withdrawn from each upper phase and diluted to 50 ml. with pure ethanol. The optical density of each sample was measured at 268 mμ using as blank solvent a portion of equilibrated mobile phase (5 ml.) similarly diluted with ethanol.

This experiment was repeated with a 15 tube manually operated apparatus of similar design using 30 tube-inversions per transfer. Using the previously determined value of log ϵ 4.21 for the reserpine maximum at 268 mμ, the estimated quantity of reserpine in the apparatus was 38·7 mg. (97 per cent of the amount taken).

Preparation of a Reserpine-rich Fraction from Rauwolfia Root

The following example, using a commercial sample of *R. vomitoria*, illustrates the general procedure adopted in all estimations.

A suspension of the milled root-powder (50 g.) in methanol (500 ml.) was gently refluxed on the steam bath for 2 hours. After filtration, the extraction was repeated three times giving eventually a nearly colourless solution. The combined extracts were concentrated at reduced pressure to about 15 ml. and the residual solution added to N acetic acid (30 ml.) to give a turbid solution from which fatty material was removed by two washings with *n*-hexane (25 ml.). The clear liquid was treated at 5–10° with ammonia to pH 8–9, the tan-coloured precipitate collected, washed with water and combined with an additional quantity extracted by chloroform from the filtrate. The total weak bases (1·4 g.) were now extracted alternately with chloroform and N acetic acid, the extracts shaken together and the chloroform layer separated, washed with a little ammonia and evaporated, affording a brown powder (0·69 g.).

Countercurrent Analysis of Reserpine-rich Fractions

To estimate the reserpine content, about 100–130 mg. of the brown powder was accurately weighed into chloroform (6·25 ml.) and the solution diluted with ether to 25 ml. After centrifuging to remove a light

flocculent precipitate, a part (20 ml.) of the clear supernatant liquid was pipetted into the 15-tube countercurrent apparatus. The procedure described for pure reserpine was now followed except that 17 transfers were given, withdrawing three fractions from the outlet. After adding ammonia to each tube and shaking, aliquots of the upper phases were taken and optical densities measured at 268 mμ. The reserpine content was calculated from the densities of the tubes contained within the peak (see Fig. 3), and the combined upper layers of these tubes then evaporated to dryness. The residue was compared with pure reserpine (*a*) by ascending paper chromatography on Whatman No. 20 paper using 10 per cent acetic acid in 5 per cent aqueous sodium acetate just saturated with di(*n*-butyl)ether[10], and (*b*) by paper electrophoresis on Whatman No. 31 Extra Thick paper in 3N acetic acid at 400 volts at 10 milliamps for about 4 hours. The papers were examined for fluorescence under ultra-violet light.

Results and Discussion

The method described above was applied to a commercial sample of *Rauwolfia serpentina* and to two distinct samples of *R. vomitoria*. Since these materials were also extracted on a larger scale in 5 kg. batches, it is possible to compare the estimated reserpine contents with the quantities actually isolated and these figures are given in Table I. The isolation of reserpine by several alternative routes was examined, and the yields quoted are those obtained by the most quantitative procedure encountered, viz. extraction with 10 per cent acetic acid of a water-washed methanolic root extract, followed by transference of reserpine from the acetic acid into chloroform and finally chromatography on alumina[11].

TABLE I

Comparison of estimation results with yields of reserpine by large scale extraction

Material	Weight of reserpine in 5 kg. of sample	
	Estimated by countercurrent	Actually isolated
R. serpentina	2·7 g.	2·6 g.
R. vomitoria (Sample I) ..	7·0 g.	7·0 g.
R. vomitoria (Sample II) ..	12·2 g.	10·9 g.

The method is thus capable of indicating the approximate yield of reserpine which may be expected from rauwolfia samples of the species mentioned, and is conveniently operated, since a sufficient degree of resolution is obtainable with comparatively few transfers. The combined use of paper electrophoresis and paper chromatography under the conditions described has been found to discriminate between a large number of rauwolfia alkaloids, including for example, reserpine and rescinnamine, and thus provides a fairly rigorous indication of the homogeneity of the material used for assay. In practice, reserpine provided the sole strongly fluorescing spot, and the one other barely discernible spot which could be

detected in two cases was of such a low order of intensity that it is not likely to have interfered with the estimation of reserpine within the limits of accuracy required. In the case of other species of *Rauwolfia* in which the reserpine content may be of an altogether lower amount, the method as described can give some indication of this amount, but the contributions of any overlapping alkaloids are likely to be proportionately greater. An experiment carried out with a Brazilian sample of *R. sellowii*, for example, gave a much reduced reserpine peak from which it was possible to calculate that the reserpine content was less than 0·004 per cent. A more accurate figure could not be derived since paper electrophoresis revealed that two other substances were present within the peak. Hochstein has reported[12] a yield of 0·002 per cent of reserpine in this species.

In cases of inadequate resolution in 17 transfers, the accuracy of the procedure could presumably be increased by carrying out more transfers but this has not been found necessary within the scope of the work reported.

Summary

1. The behaviour of reserpine in a countercurrent apparatus when distributed between a number of systems and especially ether-chloroform (3:1)/buffer of pH 3·1 has been examined.

2. This is the basis of a convenient method for the estimation of reserpine in small samples of *Rauwolfia serpentina* and *Rauwolfia vomitoria*, and the results have been compared with the yields of reserpine isolated from larger quantities of the same material.

The authors wish to thank Dr. D. F. Muggleton for carrying out the paper electrophoresis and most of the spectrophotometric measurements, and Mr. R. F. Silver for technical assistance.

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INFLUENCE OF α-COCAINE ON SOME PHARMACOLOGICAL EFFECTS OF TYRAMINE AND ADRENALINE

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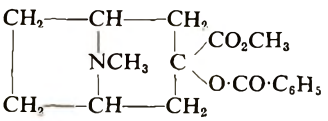
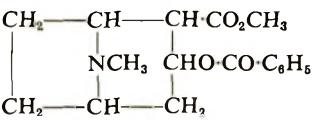
α-Cocaine (I) is a structural isomer of cocaine (II).



(I)



(II)

In previous work α-cocaine was found to have the same inhibitory action as cocaine on amine oxidase from cat liver and nictitating membrane, and rather more inhibitory action than cocaine on amine oxidase from rabbit liver and rabbit uterus[1]. On the other hand, the isomer only occasionally potentiated the action of adrenaline in isolated preparations of the rabbit or in the spinal cat; usually it reduced it or caused no change.

Tyramine is known to be more rapidly destroyed by amine oxidase than adrenaline. Therefore, experiments have been done to compare the influence of α-cocaine on the pharmacological effects of tyramine and adrenaline.

Methods

Observations were made on cats and dogs under chloralose anaesthesia (80 mg./kg.) and on rats under urethane anaesthesia (1·75 g./kg. subcutaneously). The blood pressure was recorded in the carotid artery. The contractions of the nictitating membrane were recorded by attaching it to an isotonic lever fitted with a frontal writing point; the contractions were magnified 10 times. All the drugs were injected into the femoral vein. The isolated spleen preparation was made as described by Höller and Kiehtreiber[2].

The drugs used were tyramine hydrochloride, tyramine ethanolsulphonate*, cocaine hydrochloride and α-cocaine hydrochloride.

Results

α-Cocaine and tyramine. Both potentiation and reduction of the effect of tyramine on the blood pressure and nictitating membrane of the cat were observed. A typical experiment in which reduction was noted is shown in Figure 1. Between A and B 17 mg./kg. α-cocaine was injected intramuscularly in four different places, and 58 minutes later the effect of tyramine on both blood pressure and nictitating membrane was reduced, as shown in B. Between B and C 13 mg./kg. cocaine was injected intramuscularly in four different places, and 18 minutes later this

* Kindly supplied by Messrs. Burroughs Wellcome & Co. Ltd.

caused a complete abolition of the effect of tyramine, as shown in C. The tone of the nictitating membrane was increased after cocaine, but the contraction of the membrane was reduced to a small spike. The injection of cocaine caused arrest of the respiration and the blood pressure fell. The record in C was taken during artificial respiration.

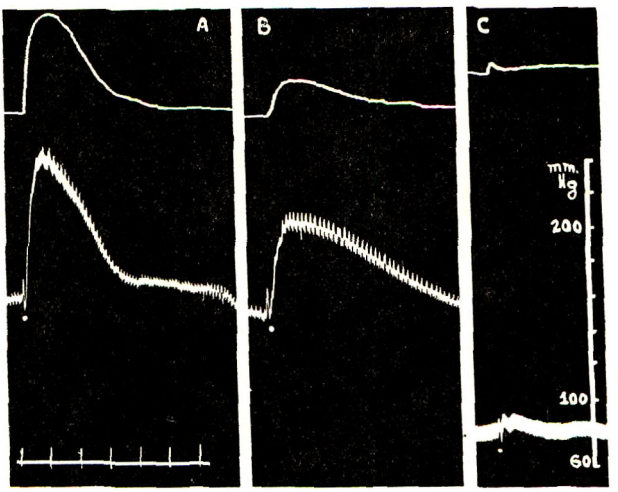


FIG. 1. Cat, 4·6 kg., chloralosed, vagi cut. Upper record: contractions of the nictitating membrane. Lower record: blood pressure. At white dots: 2 mg. tyramine hydrochloride i.v. Between A and B 80 mg. α-cocaine hydrochloride i.m. Between B and C 60 mg. cocaine hydrochloride i.m. B was taken 58 min. after α-cocaine and C was taken 18 min. after cocaine. Time: 1 min. intervals.

In another experiment the effect of tyramine on the nictitating membrane was potentiated after α-cocaine, as shown in Figure 2. Between A and B 18 mg./kg. α-cocaine was injected intramuscularly and 55 minutes later the effect of tyramine on the nictitating membrane was increased, whereas the effect on the blood pressure was slightly prolonged. Between

TABLE I

EFFECT OF α-COCAINE ON THE RESPONSE TO TYRAMINE

Species	Dose, mg./kg.	Blood pressure	
		Reduction or no change	Potentiation
Cat	17–29	6/9	3/9
Dog	7·1–8·8	4/5	1/5
Rat	6–26	3/5	2/5

B and C 13 mg./kg. cocaine was injected intramuscularly and 15 minutes later the effect of tyramine was almost completely abolished, as shown in C.

The results with tyramine in cats, dogs and rats are shown in Table I. Both α-cocaine and cocaine were tested for comparison in 8 cats and 3 dogs. In all these experiments, no matter whether the isomer caused

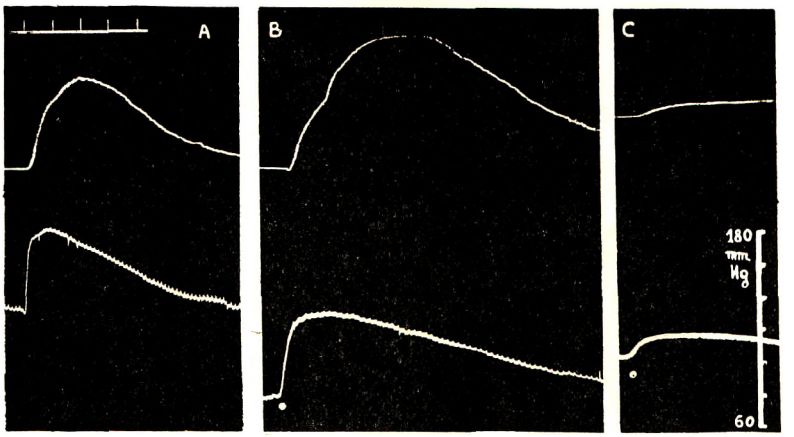


FIG. 2. Cat, 3·3 kg., chloralosed, vagi cut, artificial respiration. Upper record: contractions of the nictitating membrane. Lower record: blood pressure. At white dots: 2 mg. tyramine hydrochloride i.v. Between A and B 60 mg. α-cocaine i.m. Between B and C 40 mg. cocaine hydrochloride i.m. B was taken 55 min. after α-cocaine and C was taken 15 min. after cocaine. Time: 1 min. intervals.

a reduction or a potentiation of the effect of tyramine, cocaine (6–18 mg./kg.) caused invariably a significant reduction or even abolition of the effect of tyramine.

α-Cocaine, adrenaline and noradrenaline. It was found that in the spinal cat α-cocaine did not modify the actions of adrenaline or noradrenaline on the blood pressure and nictitating membrane in doses five times larger than potentiating doses of cocaine[1]. In the present experiments similar observations were made on 10 dogs and 2 cats under chloralose anaesthesia. In 8 out of 10 dogs and in 1 out of 2 cats α-cocaine caused a reduction or no change in the actions of adrenaline and noradrenaline on the blood pressure and nictitating membrane. A typical experiment is shown in Figure 3. Between A and B α-cocaine was injected intramuscularly in a dose of 15 mg./kg. and 70 minutes later the action of adrenaline and noradrenaline on the nictitating membrane were reduced as shown in B, whereas their action on the blood pressure was almost unchanged.

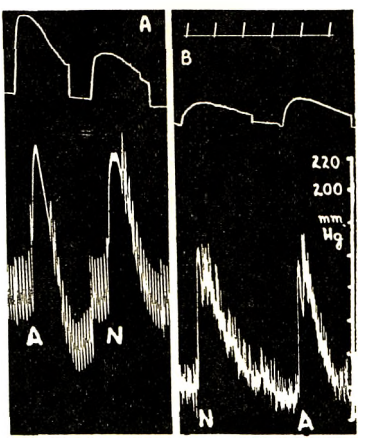


FIG. 3. Dog, 10·5 kg., chloralosed, vagi cut. Upper record: contractions of the nictitating membrane. Lower record: blood pressure. At A 200 μg. adrenaline hydrochloride i.v. At N 200 μg. noradrenaline bitartrate i.v. Between first and second tracing 15 mg./kg. α-cocaine hydrochloride i.m. B was taken 70 min. after injection of α-cocaine. Time: 1 min. intervals.

In 2 out of 10 dogs and in 1 out of 2 cats α-cocaine (10 to 17 mg./kg.) caused a potentiation of the effects of adrenaline and noradrenaline, particularly if time was allowed. Nevertheless, this potentiation was much less pronounced than after cocaine. A typical experiment is shown in Figure 4. In this animal α-cocaine caused no change in the response to adrenaline and noradrenaline until 50 minutes after injection of α-cocaine. Later, the effect of adrenaline and noradrenaline both on

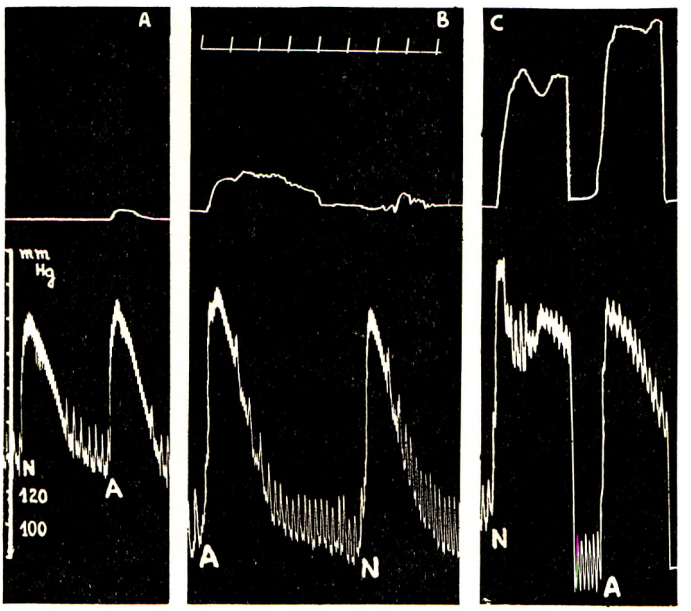


FIG. 4. Dog, 7·15 kg., chloralosed, vagi cut, artificial respiration. Upper record: contractions of the nictitating membrane. Lower record: blood pressure. At A 40 μg. adrenaline hydrochloride i.v. At N 40 μg. noradrenaline bitartrate i.v. Between first and second tracings 14 mg./kg. α-cocaine i.m. Between second and third tracings 7 mg./kg. cocaine hydrochloride i.m. The second tracing was taken 118 min. after α-cocaine. The third tracing was taken 10 min. after cocaine. Time: 1 min. intervals.

the blood pressure and on the nictitating membrane was potentiated, as shown in B which was taken 118 minutes after α-cocaine was injected. Between B and C 7 mg./kg. cocaine hydrochloride was injected intramuscularly and 10 minutes later the response to adrenaline was potentiated much more, as shown in C.

α-Cocaine and adrenaline on the isolated rabbit spleen. The reduction of the effect of adrenaline by α-cocaine which has been observed on the rabbit uterus, intestine and perfused ear was also seen on the isolated rabbit spleen. The effect of α-cocaine on the response of the isolated rabbit spleen to adrenaline was tested in six preparations. In all of them the isomer caused a reduction of the effect of adrenaline, whereas cocaine in the same doses invariably caused potentiation. A typical

experiment is shown in Figure 5. In A, α-cocaine was added to the bath to make the concentration 7×10^{-5} g./ml. The effect of adrenaline was significantly reduced and after repeated changes of the bath fluid returned to its original level. At C cocaine hydrochloride was added to the bath making the concentration 7×10^{-5} g./ml. The effect of adrenaline was much greater in the presence of cocaine, the potentiation persisting even after repeated washing. In B, 58 minutes after adding cocaine, the effect of adrenaline had returned to normal.

In one experiment the reduction of the adrenaline effect by α-cocaine was in linear relationship to the log of the dose of the isomer.

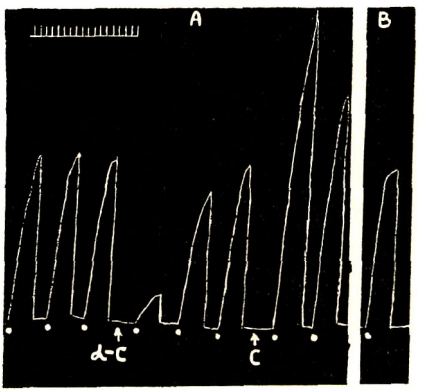


FIG. 5. Contractions of the isolated rabbit spleen in 30 ml. bath. Each white dot represents the addition of 20 μg. adrenaline hydrochloride into the bath. At vertical lines the bath was washed out. At α-C, addition of 2 mg. of α-cocaine and at C 2 mg. of cocaine hydrochloride into the bath. B was taken 58 min. after the last contraction in A. Time: 1 min. intervals.

DISCUSSION

In the present experiments α-cocaine caused potentiation or reduction of the effect of tyramine on the blood pressure and nictitating membrane in cats and dogs under chloralose anaesthesia. α-Cocaine is known to be an inhibitor of amine oxidase *in vitro*[1] and tyramine is known to be a specific substrate for this enzyme. Another amine oxidase inhibitor, *iso*propylisoniazid (Marsilid), potentiates the effect of tyramine on the nictitating membrane[3] and blood pressure[4] of the cat, and increases the toxicity of tyramine[5]. Thus, potentiation of the effect of tyramine by α-cocaine might be explained in terms of amine oxidase inhibition, although the present experiments do not exclude other possibilities.

It has been shown that cocaine depresses the action of tyramine and other sympathomimetic amines on the nictitating membrane of the cat in a way similar to denervation[6,7], i.e., cocaine reduced the action of tyramine by paralysing the postganglionic sympathetic fibres. Presumably this effect of cocaine depends on its local anaesthetic activity. However, the local anaesthetic activity of α-cocaine, when examined by intradermal injection into guinea pigs or by application to the lumbar nerve plexus in frogs, is only one-fifth to three-fifths that of cocaine. Thus, reduction of the action of tyramine on the nictitating membrane by α-cocaine does not seem to depend only on the anaesthetic activity of the isomer. On the other hand, Trendelenburg[8] found that amounts of cocaine which potentiated the response of the nictitating membrane to preganglionic stimulation reduced the action of tyramine at the same time.

It is possible therefore that the depression of the action of tyramine by α-cocaine is due to factors other than local anaesthetic activity.

In the majority of experiments α-cocaine was found to reduce or to leave unchanged the actions of adrenaline on the nictitating membrane and blood pressure of dogs and cats, but in 2 out of 10 dogs and in one cat α-cocaine potentiated the response to adrenaline and noradrenaline, particularly if time was allowed after injecting the α-cocaine. This finding is in agreement with the results of Balzer and Holtz,[4] obtained with another amine oxidase inhibitor, *iso*propylisoniazid (Marsilid).

The isolated rabbit spleen preparation described by Höller and Kiehtreiber[2] was found useful for demonstrating modification of adrenaline activity. In this preparation α-cocaine reduced the motor effect of adrenaline, whereas cocaine invariably caused a potentiation. Cocaine, however, may depress the action of adrenaline. Thus Burn and Tainter[9] found that cocaine diminished the action of adrenaline on the isolated uterus of the cat, and that it sometimes augmented and sometimes diminished the action of adrenaline on the isolated heart. Therefore, a difference between α-cocaine and cocaine on the isolated rabbit spleen does not necessarily mean a difference in the mode of action. Both may potentiate and both may depress, the effect seen being the algebraic sum of potentiation and depression. On the spleen potentiation predominates with cocaine, while depression predominated with α-cocaine.

Summary

1. α-Cocaine caused either potentiation or reduction of the action of tyramine on the blood pressure and nictitating membrane in cats and dogs under chloralose, and in rats under urethane anaesthesia. Potentiation of tyramine effects was explained in terms of amine oxidase inhibition. Reduction of tyramine effects probably does not depend only on the local anaesthetic activity of α-cocaine.

2. In the majority of experiments α-cocaine was found to reduce or to leave unchanged the effects of adrenaline and noradrenaline on the blood pressure and nictitating membrane in cats and dogs. Potentiation was observed occasionally.

3. In the isolated rabbit spleen preparation α-cocaine was found to reduce the effect of adrenaline, whereas cocaine invariably caused a potentiation.

I am indebted to Dr. R. Foster of the Department of Pharmacology, Oxford, for supplying α-cocaine.

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MICROCHEMICAL IDENTIFICATION OF SOME LESS COMMON ALKALOIDS

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During recent years new techniques have been developed for the identification of alkaloids and older techniques have been modified[1,2], but the tendency has been to describe each new development as applied to the same group of twenty or thirty well known alkaloids. The result is that of the thousands of alkaloids now known, micro tests suitable for medico-legal work have been described for fewer than a hundred. The toxicological chemist must be in a position to give positive identification to any alkaloidal substance that he isolates, and not merely to a selected few of classical forensic interest. This paper describes crystal and colour tests for 40 alkaloids which, although less well known, are all substances that may either be obtained commercially or extracted with comparative ease from common plants; most of these compounds are referred to by Henry[3] or Manske and Holmes[4]. Of the more recently discovered ones, aquaticine was isolated from *Senecio aquaticus* by Evans and Rees Evans[5], and demecolcine (desacetylmethylcolchicine) from *Colchicum autumnale* by Santavy and Reichstein[6].

Experimental Procedure

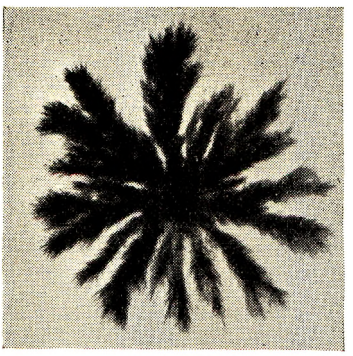
Microcrystalline tests:—The hanging-microdrop technique described by Clarke and Williams[7] was employed. The alkaloids were dissolved in 1 per cent acetic acid or 1 per cent hydrochloric acid except in the following cases: piperine was dissolved in ethanol, theobromine in a mixture of 1 volume of concentrated hydrochloric acid and 2 volumes of water, and reserpine in a mixture of 50 volumes of ethanol, 50 volumes of water, and 1 volume of glacial acetic acid.

TABLE I

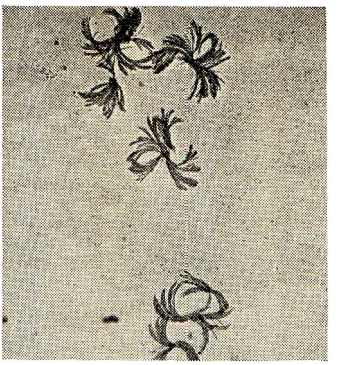
Reagents

Ammonium thiocyanate ..	5 per cent solution
Di-sodium methyl arsonate ..	5 per cent solution
Gold cyanide	Dissolve 5g. gold chloride in 100 ml. water, and add solid sodium cyanide till the precipitate redissolves
Picrolonic acid	Saturated solution
Platinum bromide	5 g. platinum chloride and 10 g. sodium bromide in 100 ml. water
Potassium cyanide	5 per cent solution
Styphnic acid	5 per cent solution
Trinitrobenzoic acid	Saturated solution

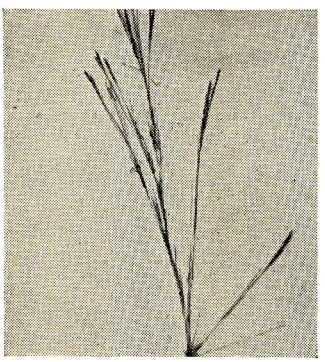
In spite of every effort to restrict the number of reagents employed, it was found necessary to use several solutions in addition to those previously described[7,8]. Details of these are given in Table I. In the course of this work many other reagents have been tested, but have been discarded as of no value.



A. Ergometrine (1·0 μg.) with trinitrobenzoic acid.

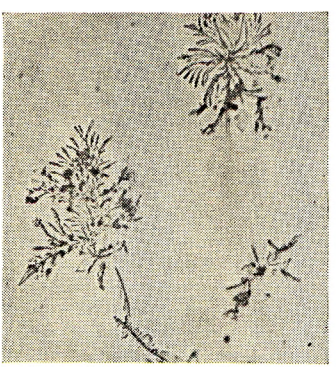


B. Berberine (0·5 μg.) with picric acid.

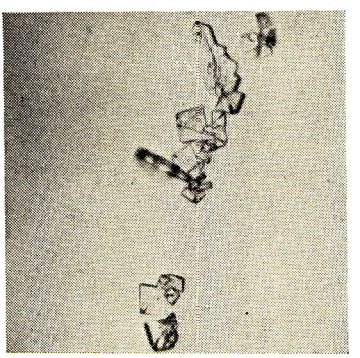


C. Cytisine (0·25 μg.) with potassium mercuric iodide.

D. Demecolcine (0·5 μg.) with gold chloride.



E. Reserpine (0·5 μg.) with ammonium thiocyanate.



F. Dimethyltubocurarine (0·25 μg.) with potassium iodide.

FIG. 1.

TABLE II

Alkaloid	Reagent	Crystals	Sensitivity μg.
Agmatine	Picric acid	Small irregular rhomboids	0·025
	Platinum iodide	Rods and rhomboids	0·025
α-Allocryptopine	Gold cyanide	Feathery rosettes*	0·25
	Potassium iodide	Dense rosettes*	0.5
Aquaticine	Potassium bismuth iodide	Small branching needles	0·025
	Sodium carbonate	Plates or serrated blades	0·1
Benzoyl ecgonine	Gold chloride	Long plates and needles	0.05
	Potassium permanganate	Branching needles	0·25
Berberine	Picric acid	Curved needles	0.025
	Gold cyanide	Feathery rosettes	0·025
Betaine	Platinum iodide	Rectangular plates	0·5
	Gold chloride	Rods and plates	1·0
Bicuculline	Sodium carbonate	Dense rosettes*	0·05
	Potassium chromate	Dense rosettes*	0·25
Boldine	Mercuric chloride	Dense rosettes	0·25
	Gold cyanide	Long needles*	0·5
Chelidonine	Sodium carbonate	Serrated plates	0·1
	Zinc chloride	Irregular prisms	0·25
Coniine	Potassium bismuth iodide	Rhomboids	0·025
	Platinum iodide	Plates	0·25
Corynanthine	Mercuric chloride	Bunches of small plates	0·25
	Potassium iodide	Plates and dense rosettes	0·5
Cryptopine	Picric acid	Rosettes of needles	0·1
	Zinc chloride	Rosettes of very small needles	0·1
Cytisine	Potassium tri-iodide (1)	Short rods	0·025
	Potassium mercuric iodide	Fans of needles	0·1
Demecolcine	Gold chloride	Feathery rosettes	0·1
	Platinum chloride	Dense rosettes	0·5
Dimethyl tubocurarine	Potassium chromate	Bunches of irregular plates	0·025
	Potassium iodide	Plates	0·1
Ecgonine	Potassium bismuth iodide	Hexagons*	0·1
	Platinum iodide	Small rectangular plates*	0·25
Ergometrine	Picric acid	Clumps or fans of rods	0·5
	Trinitrobenzoic acid	Dendrites	0·5
Ethyl hydrocupreine	Ammonium thiocyanate	Needles	0·25
	Sodium phosphate	Rosettes of rods	1·0
Ethyl papaverine	Zinc chloride	Bunches of plates	0·05
	Potassium iodide	Rosettes of curved blades	0·05
Galegine	Picric acid	Curved needles	0·05
	Styphnic acid	Stout needles	0·05
Gramine	Gold bromide	Rosettes of feathery needles	0·025
	Potassium bismuth iodide	Prisms, often rectangular*	0·025
Harmine	Gold chloride	Rods	0·025
	Potassium iodide	Needles	0·025
Hordenine	Potassium bismuth iodide	Needles or rhomboids	0·05
	Gold bromide	Long needles*	0·1
Hydroquinidine	Gold bromide/hydrochloric acid	Branching needles	0·025
	Gold chloride	Branching needles	0·025
Hydroquinine	Platinum bromide	Bunches of plates	0·05
	Mercuric chloride	Large needles	0·025
α-Lobeline	Sodium carbonate	Rosettes	0·025
	Potassium cyanide	Rosettes	0·025
Lupanine	Potassium mercuric iodide	Bunches of feathery needles*	0·025
	Gold bromide	Bunches of plates	0·05
Lycorine	Picric acid	Plates	0·5
	Potassium cyanide	Stout needles	0·5
Mescaline	Potassium bismuth iodide	Dense rosettes	0·025
	Styphnic acid	Small needles	0·025
Methyl ergometrine	Picric acid	Rosettes or fans of needles	0·5
	Trinitrobenzoic acid	Feathery rosettes	0·5
Piperine	Sodium carbonate	Long plates	0·25
	Trinitrobenzoic acid	Small needles	0·1
Protopine	Picric acid	Minute dense rosettes	0·025
	Picrolonic acid	Rosettes of hair like needles	0·025
Protoveratrine 'A'	Di-sodium methyl arsonate	Dense rosettes	0·5
	Sodium carbonate	Oily rosettes	0·25
Protoveratrine 'B'	Di-sodium methyl arsonate	Dense rosettes	0·5
	Picric acid	Oily dendrites*	1·0
Reserpine	Potassium cyanide	Small rosettes*	0·1
	Ammonium thiocyanate	Plates*	0·1
Theobromine	Gold bromide	Hair like needles†	0·025
	Platinum iodide	Small rods and plates	0·1
Theophylline	Gold bromide/hydrochloric acid	Needles†	0·25
	Mercuric chloride	Sheaves of broad needles	0·25
Trigonelline	Gold chloride	Fine needles	0·25
	Potassium tri-iodide (1)	Plates	0·5
Tropine	Gold bromide	Rhomboids	0·025
	Picric acid	Needles	0·025
Tubocurarine	Zinc chloride	Rosettes of small plates*	0·5
	Sodium phosphate	Very small needles*	0·5
Yohimbine	Sodium carbonate	Rosettes of rods	0·05
	Potassium cyanide	Rosettes of rods	0·05

*Crystals form very slowly †Crystals not stable

TABLE III

COLOUR TESTS

Alkaloid	Colour	Sensitivity µg.
Formaldehyde-sulphuric acid test 'Marquis'		
α-Allocryptopine	Mauve	0·1
Berberine	Yellow-green	0·25
Bicuculline	Orange	1·0
Boldine	Green—purple—green	0·1
Chelidonine	Faint green	0·5
Corynanthine	Brown—purple-brown	0·5
Cryptopine	Blue—green	0·1
Demecolcine	Yellow	0·25
Ethyl papaverine	Faint brown	1·0
Ergometrine	Brown	0·25
Gramine	Grey-brown	0·25
Harmine	Orange—grey	0·025
Hordenine	Brown—green	0·5
Lobeline	Red-violet—brown	0·1
Mescaline	Orange	0·1
Methyl ergometrine	Brown	0·25
Piperine	Orange—brown	0·5
Protopine	Blue—green	0·025
Reserpine	Blue—grey-green—brown	0·1
Ammonium vanadate test		
α-Allocryptopine	Purple—yellow	0·25
Berberine	Blue-green—mauve	0·025
Bicuculline	Red	0·25
Boldine	Green—brown	0·25
Chelidonine	Yellow—green	0·5
Corynanthine	Blue—green	0·25
Cryptopine	Violet—blue—green	0·25
Demecolcine	Purple—yellow	0·25
Ethyl papaverine	Green	1·0
Ergometrine	Grey-green—grey-purple	0·5
Gramine	Violet—yellow-green	0·05
Harmine	Blue—green	0·025
Lobeline	Grey	0·5
Mescaline	Orange	0·25
Methyl ergometrine	Grey-green—grey-purple	0·5
Piperine	Red-brown—green	0·25
Protopine	Violet—blue—green	0·05
Reserpine	Blue—brown-purple	0·1
Yohimbine	Blue—green	0·1
Ammonium molybdate test		
α-Allocryptopine	Green—violet—yellow-green	0·1
Berberine	Brown—grey	0·025
Bicuculline	Blue-green—yellow-green	0·25
Boldine	Blue—blue-green	0·1
Chelidonine	Green—blue—green	0·1
Corynanthine	Blue—green	0·1
Cryptopine	Green—violet—green	0·1
Demecolcine	Green—yellow	0·25
Dimethyl tubocurarine	Blue—green	0·5
Ethyl papaverine	Green—blue—green	0·1
Ergometrine	Green—brown	0·25
Galegine	Blue	1·0
Gramine	Blue—yellow-green	0·025
Harmine	Yellow—grey	0·025
Hordenine	Blue—green—yellow	0·25
Lobeline	Grey—pink	1·0
Lupanine	Faint blue	1·0
Lycorine	Green—blue—yellow	0·25
Mescaline	Green-blue	0·05
Methyl ergometrine	Green—brown	0·25
Piperine	Red-brown	0·25
Protopine	Green—violet—green	0·025
Reserpine	Blue	0·1
Tubocurarine	Blue-green	0·5
Yohimbine	Blue—green	0·025
Selenium dioxide test		
α-Allocryptopine	Violet—red-brown	0·1
Berberine	Brown—purple	0·05
Bicuculline	Yellow—brown	1·0
Boldine	Green—brown	0·25
Chelidonine	Yellow—brown—orange	0·25
Corynanthine	Blue-green—green	0·25

TABLE III—*continued*

Alkaloid	Colour	Sensitivity μg.
Selenium dioxide test—*continued*		
Cryptopine	Violet	0·1
Demecolcine	Yellow	0·25
Dimethyl tubocurarine	Brown—orange	0·5
Ethyl papaverine	Grey	0·1
Ergometrine	Green—brown	0·25
Galegine	Orange	1·0
Gramine	Grey-green—grey-brown	0·25
Harmine	Green—yellow	0·05
Lobeline	Yellow-brown	1·0
Lycorine	Brown	0·5
Mescaline	Yellow-brown	0·1
Methyl ergometrine	Green—brown	0·25
Piperine	Brown—green	0·1
Protopine	Purple—brown	0·05
Reserpine	Olive	0·1
Tropine	Greenish-brown—purple-brown	0·5
Tubocurarine	Brown—orange	0·5
Yohimbine	Blue—green	0·1
Vitali's test		
α-Allocryptopine	Yellow/brown/brown	0·5
Berberine	Brown/brown/violet	0·025
Bicuculline	Yellow/brown/light-brown	0·5
Boldine	Brown-orange/brown/dark-brown	0·1
Chelidonine	Yellow/brown/brown	0·5
Corynanthine	Yellow/yellow/red-violet	0·1
Cryptopine	Yellow/brown/brown	0·5
Demecolcine	Yellow—purple/yellow/red	0·25
Ethyl papaverine	— /yellow-brown/brown	1·0
Ergometrine	Yellow-brown/yellow-brown/purple-brown	0·1
Gramine	Yellow/orange/red-brown	0·1
Harmine	Green/grey/orange—pink	0·025
Hordenine	— / — /orange	0·1
Lycorine	Yellow/orange/orange	0·5
Mescaline	Violet/brown/brown	0·25
Methyl ergometrine	Yellow-brown/yellow-brown/purple-brown	0·1
Piperine	Yellow/yellow/ —	1·0
Protopine	Yellow/brown/dark-brown	0·5
Reserpine	Orange/orange/brown	1·0
Yohimbine	Yellow/yellow/violet	0·1

The results obtained are shown in Table II. In order to save space, only two tests have been given for each alkaloid. It must, however, be realised that many alkaloids form crystalline derivatives with a number of reagents. Unless this fact is borne in mind, some confusion may arise in applying these tests. Thus both tubocurarine and dimethyl-tubocurarine form small plates with zinc chloride solution; but only the latter forms crystals with potassium chromate and potassium iodide. Some typical crystals are shown in Figure 1.

Colour tests:—These are carried out with microdrops on opal glass as previously described[7]. The results obtained are given above in Table III. In the case of Vitali's test the colours shown are those given on addition of the nitric acid, after evaporation, and on addition of the ethanolic potash respectively.

In both crystal and colour tests, final identification rests on the comparison of the results obtained from the test material with those obtained from a control solution made from a known sample of the suspected alkaloid. If a number of controls of varying dilution are employed, the objection raised by Pedley[9] that the crystals vary with concentration is largely overcome. In addition, a rough approximation of the strength of the test solution may be obtained.

Discussion

Although the classical crystal and colour tests are being abandoned in favour of physicochemical methods such as paper chromatography and ultra-violet spectrophotometry, these latter techniques are really more suited to preliminary screening than to positive identification. Thus if there is no peak at 287 mμ[10] morphine cannot be present; but a positive result does not mean that it must necessarily be so. Similarly an R_F value of 0·25[11] indicates that strychnine is possibly present, but cannot be considered as proof of its presence without confirmatory evidence. As the microcrystal and colour tests described above may easily be applied to substances eluted from paper chromatograms[12], this method forms a convenient means of providing such additional evidence.

All the tests described above were carried out with pure alkaloids but the technique has been used on numerous occasions to identify substances isolated from animal or plant material by a modified Stas-Otto process.

Summary

Crystal and colour tests are described for the identification of 40 of the less common alkaloids.

I wish to express my thanks to Professor E. C. Amoroso for the interest he has taken in this work, and to acknowledge most gratefully gifts of alkaloids from Professor H. Berry; Dr. D. G. Harvey; Mr. E. T. Rees Evans; Messrs. Burroughs Wellcome and Co.; Messrs. L. Light and Co. Ltd.; Messrs. Ciba Laboratories Ltd.; Dr. H. Holgate and Messrs. Sandoz Products Ltd. I am also much indebted to Miss A. Stanley for technical assistance.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Aminosalicylic Acid, Sodium Aminosalicylate and Commercial Preparations, Non-aqueous Titration of. L. G. Chatten. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 556.) For the assay of *p*-aminosalicylic acid, a sample of 80 to 100 mg. is dissolved in 50 ml. of acetone using a magnetic stirrer, and titrated with a 0·1N solution of potassium hydroxide in methanol, using thymol blue as indicator and titrating to the blue end point. Tablets should be powdered and a sample of powder stirred for 10 minutes with 40 ml. of acetone. After filtering and washing the residue with acetone, the filtrate and washings are mixed and titrated as above. The procedure recommended for sodium *p*-aminosalicylate is similar except that a 40 to 50 mg. sample is dissolved in 50 ml. of anhydrous methanol and titrated with 0·05N perchloric acid in dioxan, using thymol blue and titrating to the peach end point. In both cases the colour change is sharp and coincides with the potentiometric end point. The method is rapid, accurate and less troublesome than the method of titration with nitrite and an external indicator. G. B.

Aureomycin Hydrochloride and Tetracycline Hydrochloride in Aureomycin Hydrochloride, Assay for. F. S. Chiccarelli, P. Van Gieson and M. H. Woolford Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 418.) Aureomycin may be determined by measurement of the blue fluorescence formed when an alkaline solution is excited by filtered ultra-violet radiation. Small quantities of tetracycline, if present, do not interfere in the determination. Tetracycline, when examined in alkaline solution has an absorption maximum at 380 mμ; solutions containing at least 0·2 mg. of aureomycin per ml. in 0·25N sodium hydroxide exhibit a shelf at 380 mμ in the absorption spectrum, proportional in height to the concentration of tetracycline present, and this is the basis of a method for the determination of small quantities of tetracycline in aureomycin. G. B.

Carbon Disulphide in Piperazine Compounds, Determination of. R. E. Booth and E. H. Jensen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 535.) Carbon disulphide may be liberated from its piperazine compound by acid hydrolysis, separated by extraction with chloroform and determined spectrophotometrically as follows. A sample equivalent to about 0·12 g. of piperazine-carbon disulphide equimolecular complex is placed in a 200-ml. pressure bottle and shaken for one minute with 100 ml. of chloroform and 10 ml. of 14N sulphuric acid. The bottle is closed and heated in a water bath at 70° for at least 10 minutes. After cooling in water a quantity of the chloroform solution is withdrawn from below the acid layer through a chloroform-washed plug and the light absorption measured at 319 mμ against a chloroform blank. The concentration of carbon disulphide in g./litre is calculated from the absorption index of carbon disulphide in chloroform (1·11). The assay has also been applied successfully to 2-methyl and 2:6-dimethylpiperazine-carbon disulphide, and to a commercial product consisting of piperazine-carbon disulphide in an inert diluent. G. B.

Tetracycline Hydrochloride and Aureomycin Hydrochloride in Tetracycline Hydrochloride, Assay for. M. H. Woolford Jr. and F. S. Chiccarelli. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 400.) Small amounts of aureomycin present in tetracycline cannot be estimated fluorimetrically because the fluorescence is quenched by the relatively large amount of tetracycline. The proposed method depends upon the determination of tetracycline by measurement of the ultra-violet absorption at 380 mμ in alkaline solution. Under these conditions aureomycin is rapidly destroyed and quantities up to 20 per cent do not affect the result. The quantity of aureomycin may then be calculated from the ultra-violet absorption of an acid solution at 355 mμ, after correcting for the absorption due to tetracycline at this wavelength. G. B.

Three Tetracyclines, Identification Tests for. A. Fouchet. (*Ann. pharm. franç.*, 1956, **14**, 281.) Place 2 ml. of a 50 per cent solution of zinc chloride in a small porcelain dish and heat until a skin forms. Add a very small quantity of the substance under test and continue heating for 1 minute. Aureomycin gives a garnet-red residue similar in colour to chromic acid, which dissolves in water acidified with acetic acid to give a solution the colour of dichromate. Tetracycline gives a yellow precipitate, soluble in water to give a solution the colour of potassium chromate. Oxytetracycline yields a violet (amethyst)-coloured residue, soluble in water acidified with acetic acid. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Oxytocin, Synthesis and Biological Activity of a New Potent Analogue of. R. A. Boissonnas, St. Guttmann, P. A. Jaquenoud, J. P. Waller, H. Konzett and B. Berde. (*Nature, Lond.*, 1956, **178**, 260.) The preparation of a structural analogue of oxytocin by replacing the *iso*leucyl group by a valyl group is described. 'Valyl-oxytocin' was assayed by several methods, the international post-pituitary standard powder being used for comparison throughout. The effect on the blood pressure in the chicken and on the isolated uterus was equivalent to that of 3 I.U./ml. However, by measuring the milk ejection pressure of the rabbit mammary gland, the effect was equivalent to that of 15 I.U./ml. On the cat uterus *in situ*, the effect was equivalent to that of 16 I.U./ml., whereas on the cat uterus *in vitro* it was equivalent to that of 6 I.U./ml. In non-anaesthetised rats, 'valyl-oxytocin' had an antidiuretic effect equivalent to that of 0·03 I.U./ml. In spinal cats its pressor activity was approximately equivalent to that of 0·015 I.U./ml. The relationship between the pressor activity and the uterine activity of 'valyl-oxytocin' is therefore even more in favour of the uterine activity than is the case with natural and synthetic oxytocin.

A. H. B.

BIOCHEMICAL ANALYSIS

Barbiturates in Biological Material, Detection, Estimation and Identification of. P. M. G. Broughton. (*Biochem. J.*, 1956, **63**, 207.) A rapid method is described for the determination and identification of barbiturates in biological material. For estimation in blood, extract 5 to 10 ml. three times with 30 ml. of chloroform, pass the combined extracts through a filter paper into a separating funnel and extract with 5 to 10 ml. of 0·45N sodium hydroxide. Separate and clear the aqueous layer by centrifuging. With urine, gastric contents etc. take 10 to 25 ml., acidify with sulphuric acid, extract as above, but wash the

chloroform extracts with 5 ml. of phosphate buffer before filtering to remove salicylates which may be present. Tissues may be extracted by homogenising with the chloroform. The clear sodium hydroxide extract is now hydrolysed by heating 5 ml. in a calibrated tube for 15 minutes in a water bath, cooled and made up to 5 ml. For measurement 2 ml. quantities of the extract are added to 2 ml. of 0·45N sodium hydroxide and to 2·0 ml. of 0·6M–H_3BO_3–KCl. The extinction E_N and E_B of these two solutions are measured from 227 to 265 mμ, paying particular attention to the wavelengths at which maxima, minima and isosbestic points occur. The criteria for the spectrophotometric detection of barbiturates are, maximum at 238 to 240 mμ in borate, maximum at 252 to 255 mμ and minimum at 234 to 237 mμ in sodium hydroxide, isosbestic points at 227 to 230 and at 247 to 250 mμ. The greatest differences between E_N and E_B are at 260 mμ (positive) and 236 mμ (negative). The determination of barbiturate depends on the fact that, at 260 mμ, $E_N = E_B$ for extracts containing no barbiturate. Partial identification of barbiturates, particularly between short, intermediate and long acting, can be made by measuring their decomposition in alkali. Solutions in 0·45N sodium hydroxide are heated in a boiling water bath and the percentage, R, of barbiturate remaining after a given time is found by measuring $E_N - E_B$ at 260 mμ before and after hydrolysis. R varies from 31·8 for phenobarbitone to 98·2 per cent for pentobarbitone after 15 minutes hydrolysis. A complete analysis can be made in one hour. G. F. S.

Parathion in Biological Material, Isolation and Identification of. A. Fiori. (*Nature, Lond.*, 1956, **178**, 423.) Most of the methods used for the isolation of parathion from biological material have defects which result either in a small yield of the material or in a very impure material. Among the identification methods, the Averell and Norris reaction has proved non-specific while ultra-violet spectrophotometry and paper chromatography are affected by the impurities present in the extracts. In devising a new system of isolation and identification, a preliminary extraction is made by treating the tissue with a mixture of ethanol, trichloroacetic acid and water. After centrifugation the supernatant fluid is filtered, the ethanol evaporated off and the aqueous solution is passed through a column of acid alumina. No visible rings formed but the parathion was completely adsorbed, while many impurities remained in the aqueous solution. The parathion is then eluted from the alumina with ethyl ether. The ether is filtered and evaporated. The residue is dissolved in benzene or ethanol and chromatographed. Development is carried out with the ascending method using 5 per cent ethyl ether in water-saturated light petroleum as solvent. The spots of the parathion and of its breakdown product, *p*-nitrophenol, are detected by two ultra-violet lamps, one at 3600 Å and the other at 2537 Å. With the 3600 Å lamp the *p*-nitrophenol absorbs strongly, while the parathion absorbs weakly. With the 2537 Å lamp the parathion absorbs strongly; 0·5 μg. being easily detectable. The parathion has an R_F value of 0·98 and *p*-nitrophenol has a value of 0·30. M. M.

CHEMOTHERAPY

Oils, *In Vitro* Antibacterial Activity of. J. C. Maruzzella and M. B. Lichtenstein. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 378.) Oils were tested against 10 organisms by a method in which a disk of filter paper moistened with the oil was placed on a seeded nutrient agar plate and incubated, the zone of inhibition being measured after 24 hours at 37°. Of 110 volatile oils examined, eucalyptus, birch tar, cinnamon and cedar leaf oils and balsam of tolu showed the greatest

antibacterial activity. Birch tar, parsley seed, valerian, eucalyptus, pennyroyal and rosemary oils and balsams of tolu and Peru were effective against 8 or more of the test organisms. Cajuput, copaiba, garlic, hops, expressed laurel, myrrh, niaouli, nutmeg, origanum and savory oils showed no antibacterial activity. Of the test organisms used, *Bacillus subtilis* was the most sensitive and *Aerobacter aerogenes* the most resistant to the effect of volatile oils. G. B.

PHARMACY

Antacids, *In Vitro* Study of. J. J. Hefferren, G. Schrotenboer and W. Wolman. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 564.) Seven commercial antacid tablets were examined by four methods of testing *in vitro*. The preparations included dried aluminium hydroxide gel, magnesium trisilicate, aminoacetic acid, calcium carbonate, dihydroxy aluminium aminoacetate, dihydroxyaluminium sodium carbonate and hydroxyaluminium magnesium aminoacetate. The tests methods employed included those of Hammarlund and Rising, and Dale and Booth, in which a test dose is placed in the test medium, and fresh medium added at intervals. In the method of Johnson and Duncan, part of the test medium is removed from time to time and fresh medium added. This method and a modification of it were also used. Results obtained by the various methods differed widely, but the same order of effectiveness was established for all the preparations irrespective of the test method employed. In a comparative study of various artificial gastric juices and pooled human gastric juice for testing purposes, it was shown that the addition of mucin to simulated gastric juice U.S.P. gave a product with a titration curve and antacid response similar to human gastric juice. G. B.

Antibiotics, Comparative Release of, from Ointment Bases. H. J. Florestano, M. E. Bahler and S. F. Jeffries. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 538.) The extent to which antibiotics are released from ointments was assessed by measuring their potency by the agar cup-plate method against a number of organisms common in cutaneous infections (*Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*). Bacitracin, polymyxin B sulphate, neomycin sulphate and mixtures of these antibiotics were incorporated in two ointment bases and examined by this method. In all cases the release of antibiotic was considerably greater from a water-miscible base "Fuzene"—containing liquid paraffin 10, white soft paraffin 10, Glycowax S-932 ("polyhydric alcohol fatty acid") 10, polyethylene glycol 400 distearate 10 and polyethylene glycol dilaurate 60, than from a greasy base consisting of soft paraffin, mineral oil and lanolin.

G. B.

B.C.G. Vaccine, Freeze-dried. J. Ungar, P. Farmer and P. W. Muggleton. (*Brit. med. J.*, 1956, **2**, 568.) A method is described for the production of a viable B.C.G. vaccine by freeze-drying a suitable suspension of cells in 8·3 per cent dextran with 7·5 per cent glucose. The B.C.G. organisms for vaccine production can be grown in deep culture in Sauton's medium with 0·025 per cent triton WR 1339—a non-ionic polyoxyethylene ether—without affecting their biological properties. By the incorporation of this wetting agent in the culture medium a deep growth of the B.C.G. develops as unclumped cells which disperse well on shaking. This makes it possible to dispense with the milling process necessary to produce a homogeneous suspension from a surface growth and which may result in the retention of small clumps and render bacteria more vulnerable to the stress of freeze-drying. The bacteria, which are uniformly

dispersed, can be easily harvested in the centrifuge and the deposit resuspended in dextran-glucose solution. The freeze-dried vaccine produced in this way is a safe and reasonably constant product. Owing to its long storage life it can be accurately standardised before issue on the basis of its viable-cell content, and each batch can also be checked for virulence. In addition each batch can be tested for its tuberculin-converting power and for its freedom from toxicity as judged by the absence of excessive local lesions after injection into guinea pigs. A study of the keeping properties of the dried vaccine has shown that it has a life of at least 12 months when stored below 20°. S. L. W.

Hydrophilic Ointment Bases, Release of Medication from. D. Y. Barker, J. E. Christian and H. G. DeKay. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 601.) Radio-active iodine (^{131}I) was obtained from sodium radio-iodide solution and incorporated with iodine and potassium iodide in the ointment bases under examination. The activity, expressed as counts/minute, was determined on an accurately-weighed sample of each ointment. Petri dishes of nutrient agar were prepared but the medium was not seeded with a test organism. One cup containing a weighed amount of ointment was placed on each agar plate. The dishes were incubated at 37° for 24 hours, after which strips of agar were removed and the radioactivity measured. Corrections were applied for background and decay, and the proportion of iodine released to each part of the plate calculated. Compared with the usual method of examining ointments by measuring zones of inhibition on agar plates seeded with a suitable test organism, the radioactive tracer method has the advantage of measuring the actual quantity of medicament released from the ointment and its distribution in the agar. Tests were carried out using hydrophilic ointment U.S.P.XIV, and similar ointments in which the sodium lauryl sulphate was replaced by other surface-active agents. In all cases the iodine was found to have diffused throughout the agar medium. The maximum release of iodine was obtained when the concentration of surface-active agent was 1 per cent for sodium lauryl sulphate Ethomid C/15 or Brij 35, or 5 per cent for G-7596-J. The same trend was observed using the seeded plate technique, but whereas the U.S.P.XIV ointment gave the smallest zone of inhibition, the radioactive tracer method revealed that the release of iodine from this base was the greatest. G. B.

Silicone-Rubber Tubing in Blood Transfusion. J. F. Wilkinson, G. G. Freeman, N. New and R. B. Noad. (*Lancet*, 1956, **271**, 621.) The results of 296 transfusions of whole blood, packed cells, and serum through seven types of silicone-rubber tubing are reported. In the course of these transfusions only 3 reactions (1·01 per cent occurred). This incidence of reactions is of the same order as has been observed with natural rubber tubing under similar conditions at the same hospital. Because of its resistance to repeated sterilisation, its relatively long life in good condition, its transparency, and its physiological inertness, silicone rubber is considered superior to natural rubber for blood-transfusion work and is recommended for this purpose. Silicone-rubber tubings were found to be in satisfactory condition for further use after over 40 transfusions, while natural rubber tubings under similar conditions showed much deterioration after 6 to 12 sterilisations. The most satisfactory compositions of silicone rubber so far examined are a dimethylpolysiloxane gum filled with either an amorphous precipitated silica or a pyrogenic silica. S. L. W.

Tablet Granulations, Porosity of. W. A. Strickland Jr., L. W. Busse and T. Higuchi. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 482.) The true density of tablet granulations was determined by preparing tablets with punches

set at a little over 7000 kg./sq. cm., so as to remove all void space, weighing the tablets and calculating the volume from their dimensions. The apparent density was determined by weighing the granules in air and in mercury at various pressures, and extrapolating to low pressures of mercury at which all intergranular spaces are filled but no mercury has entered the granules. Void space was low (about 3 per cent) in an acetylsalicylic acid granulation prepared by precompression and in granular potassium bromide (about 5 per cent). Granules prepared by moist granulation with starch paste contained about 30 per cent of void space. G. B.

PHARMACOLOGY AND THERAPEUTICS

Amiphenazole in Obstetric Analgesia. J. M. Holmes. (*Lancet*, 1956, **2**, 765.) Morphine grains $\frac{1}{2}$ and amiphenazole 30 mg. were given intramuscularly to 20 patients in strong labour. Six of the patients were delivered within 2 hours, and 5 of the babies cried immediately. Nine were delivered from 5 to 7 hours after the injection, and 6 of the babies had difficulty in breathing and became cyanosed; and 9 babies of the 20 patients receiving the mixture showed definite but reversible respiratory depression. From this trial it was concluded that the action of amiphenazole is less prolonged than that of morphine and its duration of effective action is probably about 4 hours. 30 other patients were given morphine grains $\frac{1}{4}$ and amiphenazole 30 mg. If the cervix became fully dilated within 8 hours, a further dose of amiphenazole 30 mg. was given intramuscularly. Only 4 of the babies became cyanosed and showed evidence of respiratory depression. Each of these depressed babies received amiphenazole 3 mg. intravenously into the umbilical vein and respiration was fully established within 6 minutes of birth. Of the 50 patients in the trial only 1 developed vomiting attributable to morphine, and 6 others, who normally vomited after morphine, experienced mild nausea only. Slowing of maternal respiration was not observed. Narcosis was greater than after pethidine but much less than after morphine used alone; there was more amnesia than after pethidine. Uterine contractions became less frequent but were not reduced in strength, and the progress of labour, and the cervical dilatation, appeared to be accelerated. No adverse side-effects were observed. The author concluded that amiphenazole much reduces the undesirable qualities of morphine without significantly reducing its analgesic action on the mother, and in the doses recommended can reduce, though not completely eliminate, neonatal apnoea caused by morphine. S. L. W.

Antihistamines, Physical Properties and Pharmacological Activity. N. G. Lordi and J. E. Christian. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 300.) The ionisation constants, solubilities at pH 7·4 at 37·5° and relative surface activities at pH 7·4 were determined for a series of 16 commercial antihistamines. The constants of procaine and papaverine were also determined for purposes of comparison. There was no apparent correlation between any of the properties investigated and the antihistamine activity. It is concluded that antihistamine activity is a highly specific effect, the influence of the physical properties of the drug being concerned with access to the site of action, forces which tend to bind the drug to the receptor, and persistence of effect. It was observed that the least soluble antihistamines are among the least toxic and have the slowest onset of action and the most prolonged effect. G. B.

Benactyzine Derivatives, Potentiating Effect of, on Anaesthesia in Mice. C. H. Holten and V. Larsen. (*Acta pharm. tox. Kbh.*, 1956, **12**, 346.) The prolonging effect of a series of compounds, related to benactyzine, adiphenine and

diphenhydramine, on hexobarbitone anaesthesia in mice has been studied. The compounds to be tested were given intraperitoneally to groups of ten mice at ascending dose levels and thirty minutes later all groups and a control group were given 100 mg. per kg. of hexobarbitone intraperitoneally. By comparing the prolongation of the sleeping times to the controls and relating this to the log dose, the index of activity was calculated. Most of the compounds were also tested for spasmolytic effects on spasms induced on the guinea pig isolated ileum with histamine, acetylcholine or barium chloride, and also for acute toxicity in mice. The results showed that the activities of a number of benzilic esters of amino alcohols, especially ethylaminoethanol, dimethylaminoethanol, diethylaminoethanol (benactyzine) and diethylamino*iso*propanol were very high and the compounds also had strong anticholinergic effects. Derivatives of benactyzine in which the tertiary alcoholic group in the benzilic acid moiety of the molecule was altered showed the chlorine compound to be as active as benactyzine. When the tertiary alcohol group was etherified, activity was somewhat decreased, while esterification decreased the effect considerably. Diethylaminoethyldiphenylpropyl acetate (SKF 525-A) also had a considerable action. Adiphenine and trasentin had very little effect. Diphenhydramine and related compounds had a moderate effect, while chloro-substitution in the phenyl group increased the activity without increasing toxicity. Of the common drugs, methadone, pethidine and chlorpromazine were active. Chlorpromazine was as active as benactyzine while reserpine was considerably more active. In contrast the quaternary ammonium compounds were inactive. G. F. S.

Carbutamide (BZ55) in Diabetes, Clinical Trial of. L. J. P. Duncan, J. D. Baird and D. M. Dunlop. (*Brit. med. J.*, 1956, **2**, 433.) Carbutamide (*N*-butyl-*N'*-sulphanilylurea), a hypoglycaemic compound which is active when given by mouth, was studied in patients over the age of 45 suffering from mild diabetes who were not more than 10 per cent over their ideal weight but whose hyperglycaemia and glycosuria could not be eliminated by dietary restrictions alone. The trial was completed in 44 patients of whom 22 were studied in hospital for 20 days and 22 were kept in hospital for 8 days. Carbutamide was started on the 8th and 4th day respectively, dosage being adjusted in accordance with the clinical response. Diets were individually prescribed, and for each patient the same meal each day contained a constant calculated quantity of calories, carbohydrate, fat and protein. Blood sugar was determined 3 times daily and glucose tolerance was measured during the initial control period and again after 5 or 10 days treatment. Haemoglobin determinations and white cell counts were made, and liver function tested. The initial dose given to the first 12 patients was 3 g. of the drug in 0·5 g. tablets before breakfast. The remaining 32 received 4·5 g. If the response was satisfactory the daily dose was gradually reduced over a few days to 1·5 g. and if it continued to be satisfactory the patients were discharged on a daily dose of 1–1·5 g. Of the first group of 22 patients 16 responded favourably; symptoms were abolished, blood-glucose levels were satisfactory and glycosuria was reduced by at least 75 per cent of the control values. 6 patients were unresponsive to the drug. In the second group also 16 patients responded satisfactorily; 3 showed some but not adequate response and 3 were entirely unresponsive even to large doses of the drug. The 32 responsive patients were observed as out-patients for periods of from 4–18 weeks. All remained free from diabetic symptoms; in 2 the dose had to be reduced to 0·5 g. daily because of hypoglycaemic symptoms. In 12 patients who were given dummy tablets the blood glucose level and glycosuria began to increase within 2 weeks; good control was again achieved

within 1 week of recommencing treatment. Side effects consisting of mild headache, malaise and drowsiness occurred in a few patients receiving the larger initial dose but disappeared on reducing the dose to maintenance levels. One case of drug fever occurred necessitating temporary cessation of treatment. In 3 cases a sulphonamide rash appeared but regressed spontaneously. No interference with liver function was noted. There was a definite granulocytopenia during the initial 2 weeks of treatment but in most cases the count returned to normal on the maintenance dosage. There was evidence of a toxic effect on the blood platelets and there is a possibility that thrombocytopenia may occur with alarming frequency. The drug may provide a welcome alternative to insulin in a limited group of middle-aged or elderly non-obese patients whose diabetes cannot be satisfactorily controlled by dietary restrictions alone but further study is necessary before it is used generally. H. T. B.

Carbutamide (BZ55) in Diabetes, Trial of. F. W. Wolff, G. A. Stewart, M. F. Crowley and A. Bloom. (*Brit. med. J.*, 1956, **2**, 440.) A detailed study was made of the use of carbutamide in the treatment of 45 diabetics. The group included a preponderance of middle-aged or elderly female patients. In 34 the need for insulin had previously been established; 7 were new cases who would normally have received insulin and 4 were patients who had been controlled for several years by diet alone but were now showing signs of needing insulin. The patients were kept in hospital for 2 weeks and then observed twice weekly. For the first 5 days they were maintained by diet alone without insulin. On the next two days breakfast was omitted and on the second day carbutamide was given in a dose of 55 mg./kg. body weight (2·5 to 3·5 g. per patient). On the second day of treatment the patients were given 2 g. of the drug and on subsequent days 1 g. daily as in-patients for 5 days and continued as out-patients if the response was satisfactory. Sugar and sulphonamide levels in the blood and urine were determined 3- or 6-hourly. In 19 patients the blood-sugar levels fell to within normal limits and glycosuria disappeared either during the period in hospital or during the follow-up period. In the remaining 26 patients administration of the drug caused a significant drop from the fasting blood-sugar levels but failed to prevent hyperglycaemia and glycosuria; increasing the dose three-fold did not reverse the trend. In 5 patients a typical sulphonamide skin rash occurred. The response to carbutamide was not related to the duration of the diabetes but the successful cases generally gave a history of diminishing insulin requirements. As the drug exerted no control over the post-prandial rise in blood-sugar its action is probably on the mechanism controlling the utilisation of endogenous glucose. H. T. B.

Carbutamide (BZ55) in Treatment of Diabetes. J. M. McKenzie, P. B. Marshall, J. M. Stowers and R. B. Hunter. (*Brit. med. J.*, 1956, **2**, 448.) Carbutamide was used for the treatment of 20 patients with mild diabetes. 16 needed small doses of insulin and had never known ketosis; 4 required insulin to avoid ketosis. Detailed studies were made of 10 in-patients. They were treated for an initial period by dietary restrictions alone until no further improvement could be obtained or hyperglycaemia was increasing. A high loading dose of carbutamide was then given over 3 days followed by maintenance on 0·5 g. every 12 hours. The blood-sugar levels were reduced in all cases, the higher the initial fasting level the greater the hypoglycaemic effect produced. In 3 of these patients, when the drug was replaced by a dummy tablet relapse occurred after 3, 9 and 15 days respectively. Of the remaining 10 patients, 5 failed to respond. Four of them needed insulin to avoid ketosis. Supplementing the insulin by oral carbutamide gave better control but owing to the

fluctuations in the blood-sugar levels the effect of carbutamide was not statistically significant. Studies of arteriovenous blood-sugar differences and of changes in serum inorganic phosphorus levels provided no evidence that carbutamide increased the effectiveness of insulin. H. T. B.

Carbutamide (BZ55) in Treatment of Diabetes. I. Murray and I. Wang. (*Brit. med. J.*, 1956, **2**, 452.) Carbutamide was tried in a group of 23 diabetic patients who were over 45 years of age at the time of onset of the disease and who were of approximately ideal weight. 17 of them had never had insulin. No ketonuria was present at the start of the trial but it had been noted previously in 7 patients. Two young diabetics were also included in the trial. The patients were given dummy tablets for the first two weeks, and then tablets of carbutamide for two weeks. Dosage was an initial dose of 2·5 g. on the first day, then 1·5 g. on the second day and 1 g. daily thereafter. In 11 patients the mean blood-sugar level at noon was reduced to less than 180 mg./100 ml. and their glycosuria was markedly improved; they were regarded as successes. In 6 patients there was partial success, the glycosuria being markedly reduced but the blood-sugar level at noon remained above 180 mg./100 ml. There was no response in 6, all of whom had previously received insulin. The two young diabetics also failed to respond. Two patients did not respond while they were suffering from sepsis but gave a considerable response when the sepsis cleared. H. T. B.

Carbutamide (BZ55) in Treatment of Diabetes. G. Walker, W. L. B. Leese and J. D. N. Nabarro. (*Brit. med. J.*, 1956, **2**, 451.) Carbutamide was tried in 24 patients ranging in age from 38 to 82 years in whom diabetes had been recognised for from 1 to 42 years. Insulin had been given at some time to 10. All except 2 had glycosuria when carbutamide was started; 6 had mild diabetic symptoms and 5 had diabetic retinitis. None had ever had any significant degree of ketosis. Insulin was stopped in the case of patients receiving it; they were put on a low calorie diet and carbutamide was not started until the blood-sugar exceeded 200 mg./100 ml. Treatment was started with 2·5 g., followed by 1·5 g. the next day and usually 1 g. daily thereafter; it has been continued for 7 months in 2, 6 months in 4 and 3 or more months in most of the remainder. With 2 exceptions the blood-sugar levels are now satisfactory. Side effects included an irritant rash in 2 patients, making it necessary to stop treatment. One patient had a scaly eruption which disappeared in spite of continuing treatment. One patient developed mild hypothyroidism after treatment for 5 months with 1 g. daily; it responded to treatment with L-thyroxine. H. T. B.

Catechol Amines in Lymph, Rate of Elimination of. O. Celander and S. Mellander. (*Acta Physiol. scand.*, 1956, **37**, 84.) Lymph was collected from the thoracic duct of cats and man. Known amounts of adrenaline or noradrenaline were added to either heated or non-heated lymph or to Tyrode solution. The solutions were incubated at 38° between 0·5 and 4 hours; the pH of all the solutions being between 7·3 and 7·6. Assay of the catechol amines remaining after incubation was made on the isolated intestine of the rabbit. It was found that, in both cat and human lymph, the rate of destruction of adrenaline and noradrenaline was negligible in the untreated lymph. In denatured lymph there was some destruction but in Tyrode solution there was almost complete destruction after 2–3 hours. Results were similar for concentrations of adrenaline and noradrenaline varying between 1 and 10 μg./ml. This suggests that the catechol amines in some way combine reversibly with the protein molecules or radicals connected with these molecules and that the

free oxidation of the amines under such circumstances is largely inhibited. Denaturing the proteins by heating decreases but does not completely abolish the preserving capacity of lymph. M. M.

CB1348 in the Treatment of Hodgkin's Disease. B. A. Bouroncle, C. A. Doan, B. K. Wiseman and W. J. Frajola. (*Arch. int. Med.*, 1956, **97**, 703.) CB1348 is a nitrogen mustard derivative, *p*-bis-(2-chloroethyl)amino-phenylbutyric acid, which has been shown capable of producing, when administered shortly after implantation, complete inhibition of the growth of the Walker rat tumour. It has also been found to inhibit particularly the lymphocytes of the peripheral blood of rats. This is a report on the treatment of 42 patients with CB1348; the patients included 24 with Hodgkin's disease, 10 with monocytic leukaemia, 3 with chronic lymphatic leukaemia, 1 with lymphosarcoma, 1 with acute lymphatic leukaemia, 1 with mycosis fungoides, and 1 with multiple myeloma. Of the 24 patients with Hodgkin's disease all were in an advanced stage of the disease with the exception of 4, and most of the patients had previously failed to respond to X-rays, nitrogen mustard or triethylene-melamine, or were unsuitable for the last two because of marked depression of the haemopoietic system. Excellent remissions were obtained in 6 patients with Hodgkin's disease, 1 with reticulum-cell sarcoma and 1 with monocytic leukaemia. Marked improvement was obtained in 9 patients with Hodgkin's disease. The initial dose of CB1348 in most patients was 0·3 mg./kg./day for a total period of 21 days, a second course being administered when symptoms of activity recurred. All patients after the second course, and most patients after the initial course were kept on a maintenance dosage of 0·05 mg./kg./day. Twenty patients received more than 0·5 g. of CB1348, the largest total dosage being 2·17 g. given over a period of 12 months. The compound was administered by mouth as 2 mg. tablets, given preferably before meals, 2 or 3 times daily. A few patients complained of nausea and anorexia, but insufficient to cause withdrawal of treatment. Several patients complained of nervousness. Most patients developed slight to moderate leucopenia, anaemia and thrombocytopenia, but at therapeutic doses the depressant effect on the bone marrow was only moderate and was rapidly reversible. The authors conclude that the compound is of value in the treatment of selected patients with Hodgkin's disease as a supplement to X-ray therapy. It has proved safer than TEM, and was preferred to nitrogen mustard in some cases, because it has few side-effects and is less damaging to the haemopoietic system. S. L. W.

Cortisone Acetate in Chronic Asthma, Controlled Trial of Effects of. Report to the Medical Research Council by the Subcommittee on Clinical Trials in Asthma. (*Lancet*, 1956, **271**, 798.) A trial of cortisone acetate was carried out at 6 centres on patients with chronic bronchial asthma who showed no evidence of severe broncho-pulmonary infection. One group of patients were given tablets of cortisone acetate and another group were given placebo tablets; the participating clinicians did not know which kind of tablets their patients were taking. Dosage of cortisone acetate was 300 mg. on the first day, 200 mg. on each of the next two days, and 100 mg. on each of the next four days. Thereafter dosage was adjusted to the patient's requirements. The daily dosage was given in 3 divided doses. 40 patients on cortisone and 37 on the placebo completed the course, which lasted 24 weeks. It was hoped to withdraw treatment during the subsequent follow-up period of 12 weeks but many patients in both treatment groups were still receiving tablets at the end of this period. There was unequivocal evidence of a slight advantage for the cortisone-treated patients as

regards physical signs and exercise tolerance from the 2nd to the 8th week of treatment. Thereafter in both respects the control group gained ground and at the end of the 24th week there was very little difference between the two groups. Cortisone showed no advantage in facilitating a return to work by those previously unable to work. Antispasmodic therapy was still needed. Two cortisone treated patients who did not complete the course withdrew from the trial because they developed status asthmaticus. Five of the placebo-treated developed status asthmaticus. 19 cortisone-treated and 15 placebo-treated patients wished to continue taking the tablets during the follow-up period. 36 cortisone-treated patients were followed for a further period of 6 to 12 months and, of these, 22 were still receiving cortisone; 12 of them were doing well. Of 14 patients not receiving cortisone, 7 were reported to be well. Side-effects during treatment were not serious. A psychoneurosis in one patient and a duodenal ulcer in two others may have been caused by or exacerbated by the treatment. In one patient the progress of tuberculosis of the kidney, discovered after 20 weeks treatment, may have accelerated. A few patients developed mild hypertension. In no case were the side-effects severe enough to cause any practical difficulties. H. T. B.

2 : 5-Dimethyl-1 : 4-piperidyl Benzoate, Local Anaesthetic and other Pharmacological Properties of. M. N. Gordin and G. I. Samarina. (*Izv. Akad. Nauk Kirg. SSR*, 136, *Ser. Fiziol. i Meditsinȳ*, 1954, **4**, 97–104; *Sovetskoe Med. Referat. Obozrenie*, 1956, No. 26, 129.) The effect of variations in the spatial configurations of the two methyl groups (*cis* and *trans*) and of the hydrogen atom and hydroxyl group on carbon (4) of 2 : 5-dimethyl-1 : 4-piperidyl benzoate is studied. Preparations A and B, differing only in the configuration of the hydroxyl group and the hydrogen atom in the 4-position, had about the same anaesthetic power as cocaine. Preparation C, in which the methyl groups of the piperidine ring are in the *cis* position, had a considerably greater anaesthetic effect and was more toxic than A or B. The following properties were common to all the preparations: the subcutaneous administration of aqueous solutions produced local anaesthesia; a fall in blood pressure followed subcutaneous or intravenous administration, except when B and C produced convulsions; a 1 : 1000 solution produced constriction of the posterior extremities of the frog; with more dilute solutions this effect was less marked and irregular; a 1 : 1000 solution lowered smooth-muscle tonus and caused temporary paralysis of the contractile activity of the isolated section of the small intestine of the rabbit. Because of their high toxicity, the preparations are unsuitable for injection. E. H.

Hydroxyaluminium magnesium Aminoacetate, a New Antacid. A. G. Zupko. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 208.) Hydroxyaluminium magnesium aminoacetate ($C_2H_5O_4Al_3Mg,15H_2O$) is a white powder with a slightly sweet taste. It is almost insoluble in water and moderately soluble in dilute acids and alkalis. Solutions in acid rapidly form a heavy stable gel. The substance is non-toxic when tested in rats, and, when examined by the method of Hammarlund and Rising (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 586) it exhibits a long-sustained buffering action, the pH being maintained within the range 3·0 to 4·5 for 4½ hours, and does not stimulate the production of gastric acidity and acid rebound. It has little effect on the toxicity of the newer atropine-like agents (methantheline, methylhyoscine, propantheline, diphemanil, oxyphenonium and tricyclamol) in rats and affords partial protection against gastrointestinal ulceration to guinea pigs treated with antihistamine/depot histamine injections. It appears to be a suitable antacid for use in hyperacidity or peptic ulcer therapy. G. B.

5-Hydroxytryptamine Creatinine Sulphate, Vasopressor Effect in Man. S. I. Magalini, M. Stefanini and F. Smith. (*Proc. Soc. exp. Biol. N.Y.*, 1956, **92**, 433.) The vasopressor effect of 5-hydroxytryptamine creatinine sulphate (5-HT) was studied in 52 individuals who were either healthy or who were suffering from various diseases which were not associated with a high venous pressure. Intravenous injection of 0·3 μg./min. of 5-HT caused a transitory elevation of local venous pressure. Higher doses caused a greater rise of local venous pressure and, when administered in a short period of time, of arterial pressure. Elevation of the systemic venous pressure was not obtained even with extremely high doses of 5-HT. Intra-arterial administration caused a rise of pressure in the homolateral veins for 30 minutes or longer. Thus it is suggested that injected 5-HT is quickly removed from the circulation, perhaps by platelets or by other cellular elements retained in capillary beds and released slowly at a later time. M. M.

Mecamylamine in the Treatment of Hypertension. E. D. Freis and I. M. Wilson. (*Arch. int. Med.*, 1956, **97**, 551.) Mecamylamine (3-methylamino-*iso*camphane hydrochloride) is a ganglion-blocking agent which is well absorbed from the gastrointestinal tract. The hypotensive action following ingestion begins after 1 hour, reaches its lowest values in 2 hours, and disappears in 6 to 12 hours. In equipotent hypotensive doses mecamylamine did not produce as marked an inhibition of sympathetic vasoconstrictor reflexes as had been observed with hexamethonium. In 36 patients with severe hypertension treatment with mecamylamine in an average dose of 29 mg./day was followed by a mean reduction in blood pressure of 21 per cent systolic and 16 per cent diastolic in the supine position, and 27 per cent systolic and 20 per cent diastolic in the erect position. Continuous treatment for 1 to 4 months frequently resulted in improvement in the optic fundi and occasionally in the electrocardiographic patterns. A decrease in blood urea nitrogen levels occurred in patients exhibiting slight elevations but not in those with marked nitrogen retention. The side-effects were typical of those experienced with other ganglion-blocking agents, and included constipation, impaired visual accommodation, postural faintness, impotence, difficulty in micturition, and dryness of the mouth. Development of tolerance was slight or non-existent. The addition of small doses of hydrallazine appeared to produce a slight additional hypotensive effect in 3 of 13 patients, and reserpine seemed to produce an additional hypotensive effect in 5 of 11. Mecamylamine appears to offer a slight advantage over other ganglion-blocking agents in that the effective dose is much smaller and with careful dosage a more uniform reduction in blood pressure can be obtained. S. L. W.

Mecamylamine, Pharmacology of. R. V. Ford, J. C. Madison and J. H. Moyer. (*Amer. J. med. Sci.*, 1956, **232**, 129.) Mecamylamine is a ganglion-blocking agent with an action similar to that of hexamethonium and pentolinium. This paper presents observations of the pharmacological effects of mecamylamine in the experimental animal (dog) and in human hypertensive patients. The most important aspects of the drug are its long duration of action (average 17 hours), its prompt onset (average 68 minutes), and its complete absorption when administered orally. The average dose of mecamylamine required to produce significant effects in hypertension was 28 mg./24 hours, while with hexamethonium it was 2307 mg./24 hours, and with pentolinium 341 mg./24 hours. The percentage of patients achieving a responsive level was 90, 76 and 79 respectively (out of 81, 75 and 75 patients treated with each of the three drugs). The preference is for a three-dose-a-day treatment schedule, with the largest dose

given at noon. Side-effects include constipation, dry mouth, weakness and fatigue. Sympathomimetic drugs are effective in reversing hypotension due to mecamylamine. There is suppression of renal haemodynamics when the drug is administered in doses that produce marked reduction of blood pressure. It is considered that mecamylamine is the drug of choice in the treatment of moderate to severe hypertensive patients. S. L. W.

Novobiocin, Clinical and Laboratory Studies of. W. M. M. Kirby, D. G. Hudson and W. D. Noyes. (*Arch. int. Med.*, 1956, **98**, 1.) Staphylococci were shown to be remarkably sensitive to novobiocin *in vitro*, and there was no cross-resistance with other antibiotics. Pneumococci and Group A streptococci were inhibited by relatively low concentrations but are less sensitive than staphylococci. Among the Gram-negative organisms members of the *Proteus* group were shown to be moderately sensitive. An *in vitro* observation which might limit the clinical usefulness of the drug is that resistance of staphylococci develops rapidly and that the antibiotic is less active when there is an increase in the size of the inoculum of the bacteria. Of even more importance is the marked decrease in activity in the presence of serum; more than 90 per cent of novobiocin appears to be bound by serum proteins. Results of treatment with novobiocin in 75 patients with a variety of clinical infections were in general favourable, and appeared comparable to those obtained with erythromycin. In staphylococcal infections where prompt healing did not occur the infecting organisms rapidly became resistant to novobiocin. It is recommended therefore that novobiocin should be used exclusively for the treatment of infections caused by staphylococci resistant to other antibiotics. It is also recommended that novobiocin should be given in conjunction with another antibiotic to which the organism is sensitive in an attempt to prevent or delay the emergence of antibiotic-resistant staphylococci. S. L. W.

Novobiocin Treatment of Pyodermas. J. F. Mullins and C. J. Wilson. (*Antibiotic Med.*, 1956, **2**, 201.) Thirty patients with pyogenic infections of the skin were treated with novobiocin. Excellent results were observed in 24 cases, good results in 3, and failure in 3. Among the conditions successfully treated were impetiginised dermatitis, acute paronychia, furunculosis, ecthyma, and sycosis vulgaris; the failures occurred in one case of hidradenitis, and 2 cases of pustular bacterid. Infections caused by *M. pyogenes* var *aureus* and *Str. pyogenes* responded extremely well to dosages of 1·5 to 2 g./day administered by mouth. In the majority of cases the improvement was evident within 48 hours and the total treatment period usually ranged from 4 to 8 days. Lesions complicated by a Gram-negative organism responded poorly or not at all. No side reactions to the drug nor complicating candidiasis were observed. The authors conclude that novobiocin is as effective as the broad spectrum antibiotics in combating pyodermas due to *M. pyogenes* var *aureus* and *Str. pyogenes*. S. L. W.

Phenoxymethylpenicillin Acid and Salt, Serum Concentrations of. W. J. Kaipainen and P. Härkönen. (*Scand. J. clin. lab. Invest.*, 1956, **8**, 18.) A study of the blood levels of penicillin following the oral administration of phenoxymethylpenicillin acid and its potassium salt have shown that absorption was rapid and a high blood concentration was obtained in half an hour. The maximum level was reached in one hour and declined to a minimum after 4 hours. Higher blood levels were obtained when the penicillin was given on a fasting stomach than when administered after meals. The experiments were carried out on male and female bedridden patients and the dose was 300,000 units in tablet form. G. F. S.

Whooping Cough, Vaccination Against: Relation Between Protection in Children and Results of Laboratory Tests. Report to Medical Research Council. (*Brit. med. J.*, 1956, **2**, 454.) Two series of trials are reported. In the first, British vaccines were used, while the second series was planned to give information on the value of laboratory tests of vaccines and also to see whether British manufacturers could successfully use the manufacturing methods used by Michigan Department of Health which were known from previous trials to give much more effective products than those hitherto used in this country. In the first series 9 different vaccines were used, either plain suspensions or alum precipitated, and 9794 children aged 6 to 18 months completed the course of 3 injections. At least one-third of the children visited 24 to 48 hours after each injection with the plain suspension vaccines had no reaction either local or general; 3 had malaise severe enough to be kept in bed and 6 had screaming attacks. One severe reaction occurred in the group receiving alum precipitated vaccine after the third dose. Four children had convulsions within 72 hours after an injection. A total of 1325 cases of pertussis were diagnosed during a follow-up period of about 2 years. No striking differences in the protective power of the different vaccines were noted. Since vaccines were known to be of some value there were no control unvaccinated children but evidence from the home exposure rate suggested that all the vaccines were poor; of 547 exposed to home infection 379 (69 per cent) developed the disease. In the second series Michigan vaccine made from a culture of freshly isolated strains of *Haemophilus pertussis* in Bordet-Gengou medium was used as standard, and 3 British vaccines made by Michigan methods were tried together with a vaccine made by the State of New York Department of Health from a liquid culture of the same strain of *H. pertussis* as used in the Michigan vaccine. Eleven separate trials were made and 31,557 children completed the course of injections consisting of 3 doses each of 1 ml. Children visited after the first injection were also seen after the second and third injection, the total number of visits being nearly 10,500. Redness was noted in 6 per cent of visits and the children were "obviously disturbed by the tenderness" on 0·4 per cent of visits. 99 children had vomited once or more and 72 had other mild reactions. 8 children were recorded as having had convulsions within a period of 4 to 28 days. 34 developed paralytic poliomyelitis during the study; 3 who developed poliomyelitis within a month after an injection had paralysis only in the injected limb. Whooping-cough was subsequently diagnosed in 231 of the children. In some cases of home exposure chloramphenicol was used to treat the affected sibling with a view to shortening the duration of exposure to infection. The attack rate among 128 vaccinated children in such cases was 20 per cent whereas in 673 vaccinated children exposed to infection from siblings not treated with chloramphenicol the attack rate was only 13 per cent. No outstanding differences in the protective effects of the various vaccines were noted. The over-all attack rate in home exposures was 14 per cent whereas in previous trials the attack rate from home exposures in unvaccinated children was 87 per cent. Considerable protection was therefore afforded and the vaccine from the liquid culture was as good as that from cultures in solid media. In the laboratory test groups of 15 mice were immunised by intraperitoneal injection of graded doses of vaccine and challenged 10 to 14 days later by intracerebral injection of a challenging dose of *H. pertussis*. Some vaccines were tested for specific agglutinin production in mice and also in children. A correlation was found between the degree of protection in children and each of the laboratory tests but the mouse intracerebral challenge test is considered the most satisfactory. A freeze-dried British standard pertussis vaccine has been established and is thought to be stable.

H. T. B.

BOOK REVIEWS

MEDICINAL CHEMISTRY (Vol. 2). A Series of Reviews prepared under the auspices of the Division of Medicinal Chemistry of the American Chemical Society. Edited by F. F. Blicke and C. M. Suter. Pp. vii + 311 (including Index). John Wiley & Sons, Inc., New York, and Chapman & Hall, Ltd., London, 1956. 80s.

This second volume of *Medicinal Chemistry* contains chapters on four topics, namely, the Cardiac Glycosides by A. Stoll, Synthetic Oestrogens by J. A. Hogg and J. Korman, Analgesics of the Arylpiperidine Series by C. M. Suter, and β-Haloethylamine Adrenergic Blocking Agents by G. E. Ullyot and J. F. Kerwin. As might be expected with such widely different subject matter and different authorship, the four chapters are uneven in both length and presentation.

The chapter on Some Chemical Aspects of the Cardiac Glycosides deals systematically with the constitution, configuration and synthesis of the aglycones, with the sugar components, and with the cleavage of the glycosides by enzyme action. A brief section is devoted to the pharmacology of these compounds. It is unfortunate that this chapter is already out of date, since it contains no references later than 1950. An attempt to make up for this deficiency by the inclusion of a six-page supplement by T. L. Johnson barely reaches the year 1953. The chapter on Synthetic Oestrogens which, largely as a result of numerous tables occupies more than half of the whole volume, contains little reading matter but will be valuable mainly as a documented record of published work. A few pages are devoted to historical matter and four members only, namely diethylstilboestrol, hexoestrol, doisynolic acid, and bisdehydrodoisynolic acid, are selected for discussion from the point of view of synthesis. Brief sections follow on the correlation of structure with oestrogenic activity, the metabolism of oestrogens, carcinogenesis, and methods of assay and biological tests. The Tables, which occupy 126 pages, record all compounds tested and give the method of testing used, the activity found, and the relevant references to the original literature. The usefulness of these Tables is limited by the absence of references dating later than about 1950. Again, a brief supplement records some more recent references up to 1953, but these do not refer directly to the Tables.

The chapter on Analgesics deals with the methods of synthesis of arylpiperidines and discusses the relationships which appear to hold in this series between structure and activity. This is a short and concise contribution with a few references up to 1953. The final chapter on β-Haloethylamine Adrenergic Blocking Agents provides a useful survey of structure-activity relationships in this field, and summarises methods of synthesis and testing. Numerous Tables are included which give the activity of compounds tested and appropriate references. The latter, however, do not extend beyond 1952.

There can be little doubt that this volume will prove to be of value to both pharmacologists and organic chemists. The general impression left with the reviewer, however, is that these chapters were written some years ago, and that for some undisclosed reason they were kept in cold storage until their publication in 1956. In three of the contributions half-hearted attempts have been made to make good these deficiences by the provision of supplements or addenda. In any rapidly advancing field such procedures must inevitably produce a pervading sense of staleness. It is much to be hoped that future volumes in this potentially most valuable series will be more up-to-date.

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MEDICINAL CHEMISTRY (Vol. 3). A Series of Reviews prepared under the auspices of the Division of Medicinal Chemistry of the American Chemical Society. Edited by F. F. Blicke and R. H. Cox. Pp. vi + 346 (including Index). John Wiley & Sons, Inc., New York, and Chapman & Hall, Ltd., London, 1956. 84s.

This volume, like its predecessors, is intended to serve the interests of chemists and pharmacologists by the provision of comprehensive and systematic summaries in chosen fields. The topics selected for inclusion in the third volume are Methadone and Related Analgesics by T. P. Carney, Quaternary Ammonium Germicides by P. L. de Benneville, Non-mercurial Diuretics by V. Papesch and E. F. Schroeder, and Synthetic Analogues of Physostigmine by A. Stempel and J. A. Aeschlimann. Each chapter contains brief discussions on methods of synthesis and biological tests, but the main emphasis is on the recording of the results of tests for a particular type of pharmacological activity with references to the original literature. These records are systematically tabulated, with the result that more than half of the volume consists of Tables.

The chapter on Methadone and related analgesics is concise and self-contained, in contrast to those on Quaternary Ammonium Germicides and Non-mercurial Diuretics. The quaternary germicides cover a very wide range of chemical types, and brief mention is also made of related quaternary derivatives of arsenic and phosphorus. Among the non-mercurial diuretics is to be found an even greater variety of compounds, among which can be found no common chemical feature at all. For this reason it becomes almost impossible to discuss the question of the relation between diuretic activity and chemical structure in any wide sense. For practical reasons this chapter is devoted mainly, but not exclusively, to the field of the xanthines and structurally related compounds. In contrast, the final chapter on analogues of physostigmine, as might be expected, contains a very full account of the correlation of structure with activity.

The subjects chosen for this volume are all of live and wide interest, and the combinations of chemical and pharmacological information here assembled will be of great value to workers in these and closely related fields. One has to report, however, that the references survey the literature only up to 1952 (with a few references to 1953). The gap between the completion of the manuscript and the date of publication is still too wide.

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