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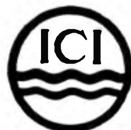

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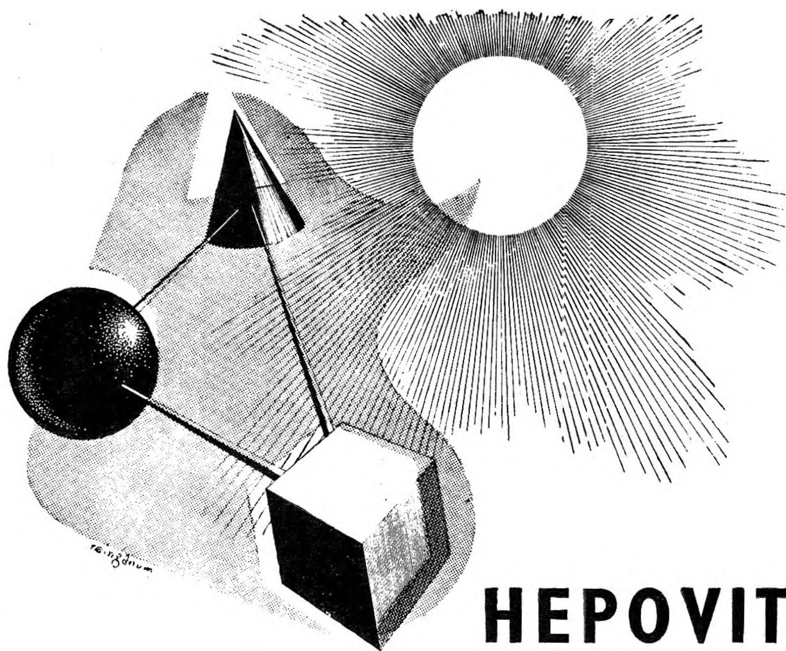

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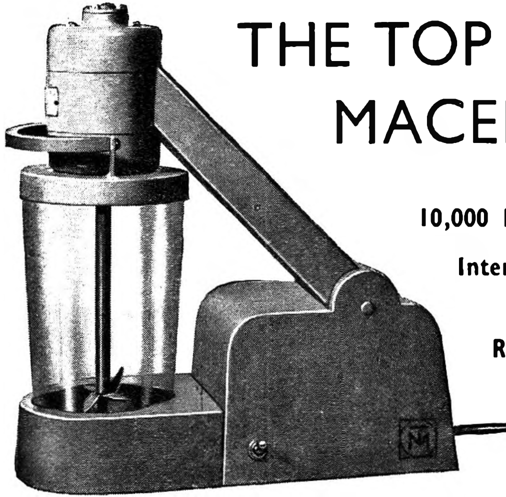

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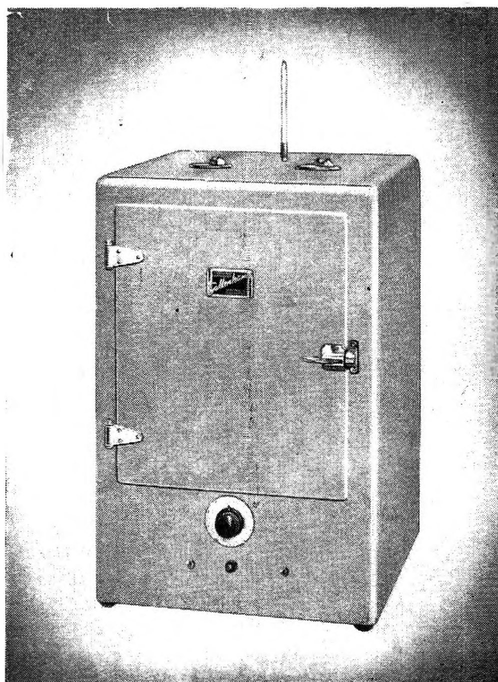

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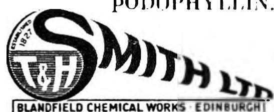

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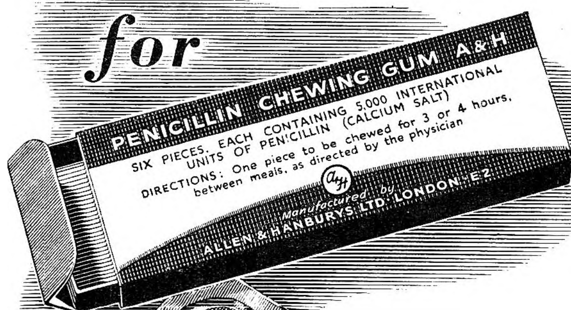

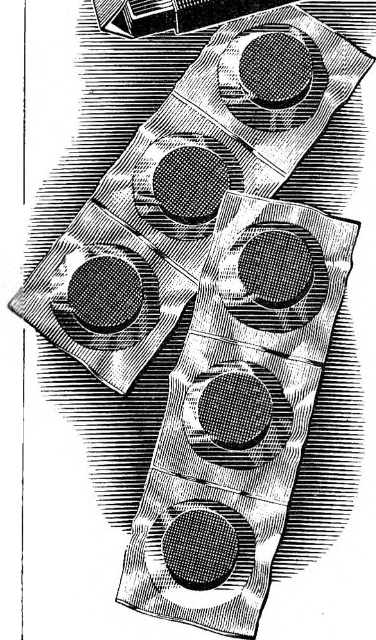

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REVIEW ARTICLE

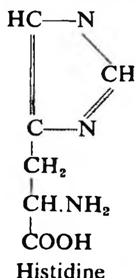
THE ANTIHISTAMINE DRUGS

By D. M. Dunlop

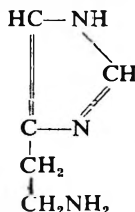
B.A.(Oxon.), M.D., F.R.C.P.(Edin.), F.R.C.P.

Professor of Therapeutics and Clinical Medicine in the University of Edinburgh
Chairman of the British Pharmacopœia Commission

Histamine is a base formed from the amino-acid histidine by the removal of the carboxyl group from the latter substance, which change can be brought about by some bacteria or by prolonged heating with acids. It is present in most cells in the body, but it only becomes



Histidine



Histamine

active when liberated from the cell by trauma or in some other way. It is normally present in large quantities in the intestine from which it is absorbed to some extent, and is destroyed by the enzyme histaminase which is present in particularly high concentration in the intestinal mucosa.

The pharmacological actions of histamine vary according to the experimental animal into which it is injected. Contraction of plain muscle occurs which may be followed by an inhibitory phase. This constricting effect has a striking species specificity. It is mainly to be observed in the bronchioles of the guinea-pig, the muscular tissue round the hepatic veins of the dog and in the pulmonary arteries of the rabbit. Capillary dilatation also takes place, causing a fall in blood pressure and shock, which is particularly obvious in the cat. This action can be demonstrated on the human skin by scratching it through a drop of histamine solution or by liberating histamine from the cells by moderate trauma as may be caused by drawing a blunt pointed instrument firmly across the skin. The characteristic triple response may then be seen: redness, due to dilated capillaries; a weal, due to exudation of plasma from the capillaries under the epidermis; a flare, due to an axon reflex. A higher concentration of histamine will cause itching in addition to the weal formation. Lastly, histamine is a strong stimulant of gastric secretion. This action is used as a clinical test of gastric function and is not antagonised by atropine.

Chemical methods of detecting histamine and of estimating it quantitatively are extremely laborious and difficult, and most of our know-

ledge of its behaviour in the body is based on biological assays, chiefly on isolated plain muscle. Human blood contains only a minute amount of histamine, most of which is present in the granulocyte cells. Attempts to estimate the release of histamine by following its concentration in the general circulation are likely to fail, since any histamine released into the blood will be absorbed before reaching the veins after circulating once round the body. Indeed, sufficient quantities of histamine can be injected intravenously in man to cause marked symptoms, without effecting any detectable increase in its concentration in the venous blood.

Anaphylaxis and Immunity

Anaphylaxis and immunity are probably two stages of the same reaction, since anaphylaxis can only be produced by proteins (antigens) which cause the production of antibodies. It is true that anaphylaxis may occasionally be caused by substances of low molecular weight, such as drugs, which have in themselves no antigenic activity. Landsteiner, however, has demonstrated that drugs can be converted into antigens by being attached to proteins. Like immunity, anaphylaxis is extremely specific. Thus a guinea-pig, sensitised to albumen from a hen's egg, will not respond with an anaphylactic reaction to albumen from a duck's egg.

If an antigen is injected into an animal and its serum is tested at intervals, it is found that the resulting antibodies are present in the blood for some days, during which time the animal is immune to the antigen, and the animal's blood will precipitate it *in vitro*. After a week or two, however, the antibodies are absorbed by the tissue cells. If a fairly large dose of the same antigen is now injected intravenously the serum is found to have lost its power to precipitate the antigen, which now combines with the antibodies inside the tissue cells and an anaphylactic shock results.

That anaphylaxis is due to some reaction in the tissues can be shown by the fact that it can be produced by adding antigen to the isolated plain muscle of an animal sensitised to it. Such a sensitised tissue will only react once to its specific antigen and thereafter becomes insensitive to it, owing to the precipitation of the antibodies which the tissue contains. In this way an animal surviving an anaphylactic shock may become desensitised, and therapeutic desensitisation consists in giving small, repeated doses of the antigen, insufficient to cause anaphylaxis, but sufficient to cause ultimate desensitisation. Apart from intensive desensitisation, which is a highly specialised and rather dangerous clinical technique, ordinary therapeutic desensitisation is a prolonged and laborious procedure, which, even if the correct antigen is found, is by no means invariably successful. The facile methods of desensitisation, so often employed clinically, of giving a few injections, say, of a mixed pollen vaccine a few weeks before the hay-fever season is a concession to psyche rather than a serious tribute to soma.

Fortunately true anaphylaxis is uncommon in man, if reasonable care

is exercised in the administration of serums, but allergy, which probably depends on a similar mechanism, is common. It shows itself in the form of skin rashes—particularly urticaria and angioneurotic œdema—hay fever, paroxysmal rhinorrhœa, drug fever, gastro-intestinal disturbances and many other clinical phenomena.

Histamine as a Cause of Anaphylaxis

There is little doubt that anaphylaxis is due to the damage produced by the combination of antigen with antibody inside the cells of the body. Some of the resulting symptoms may be directly due to the damage itself, but most are due to the release of toxic substances from the damaged tissues. In 1910 Dale and Laidlaw first pointed out that the symptoms of anaphylactic shock closely resembled those produced by an injection of histamine, even to the extent of exactly stimulating the varying manifestations resulting from an injection of histamine in different animals. Thus anaphylaxis in the guinea-pig causes death from bronchial spasm, in the dog from a fall in blood pressure consequent on arrest of blood in the liver, and in the rabbit from heart failure due to inability of the right ventricle to force blood through the constricted pulmonary arteries—all the same effects as are produced by histamine in these animals respectively. There is also much experimental evidence in animals to show that histamine is actually released during anaphylaxis. There is, for instance, during anaphylaxis a fall in the histamine content of the lungs of guinea-pigs and the livers of dogs, and a rise in the histamine content of the perfusate of sensitised guinea-pigs' lungs and dogs' livers when antigen is added. Much work has been done by such methods, and there is now no doubt that histamine plays a part in the manifestations of anaphylaxis in animals. There is no reason to suppose that man differs from the brute in this respect, though the evidence is not so conclusive, owing to the difficulty and danger of the experimental study of anaphylaxis in the human subject.

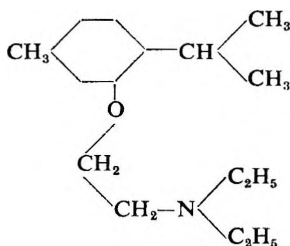
Histamine, though the most important, is not the only toxic substance released in anaphylaxis. There is, for example, an increase in the clotting time of the blood due to the release of heparin, and another substance is also produced, known as "the slow reacting substance" because of its slow action on plain muscle. The phenomenon of anaphylaxis is, therefore, a more complex one than can be accounted for by the simple release of histamine; damage to tissue, heparin, "the slow reacting substance" and possibly other products play a subsidiary role, but there is overwhelming evidence to suggest that a release of histamine is the dominating factor.

Antihistamines

Ever since it was established that histamine was intimately concerned in the production of anaphylaxis and allergy many attempts have been made to find a drug or form of treatment which would counteract these states. In recent years efforts have been made to desensitise patients to histamine by means of histamine injections, histaminase and histamine

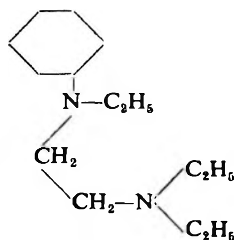
azoprotein. There may still be a future for work along these lines, but up to the present the benefits of such treatment have not been striking and no-one who has had considerable experience of the use of histamine azoprotein, which may produce some clinical benefit, can think it is the philosopher's therapeutic stone in dealing with allergic disorders.

From 1933 onwards French workers had been systematically searching for synthetic antihistamine drugs, and between 1937 and 1939 certain active compounds were actually discovered which would protect guinea-pigs against anaphylactic shock and lethal doses of histamine, but which were, however, too toxic for human use. The first of these substances, thymoxy-ethyl-diethylamine was discovered by Staub and Bovet and



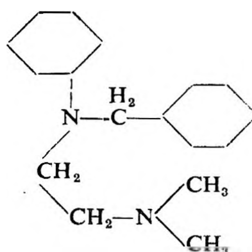
929 F.

labelled 929F. in their series, and the second discovered by Staub was another Fourneau compound containing an ethylenediamine radical labelled 1571F.



1571 F.

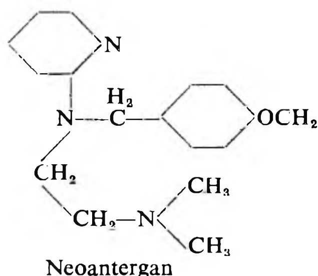
In 1942 antergan (2339 R.P.)—a phenyl-benzyl-dimethyl-ethylene-diamine compound—was introduced by Halpern, and soon the results of



Antergan

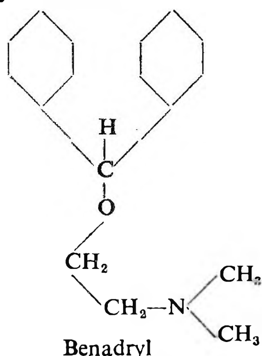
its experimental and clinical trial began to appear in the French literature. Such was the chaotic state of Europe at that time, however, that Hal-

pern's discoveries did not become generally known till after the liberation of France, by which time neoantergan (2786 R.P.) had also been introduced.



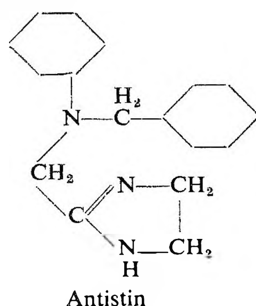
Neoantergan

It differed from antergan in the replacement of a benzene by a pyridine group and the addition of a methoxy group to the benzene ring, and was a more potent, specific and less toxic antihistamine. In 1945 pyribenzamine and benadryl were introduced in America, in which country both drugs began to be widely used therapeutically, though for some time benadryl was the only antihistamine to be generally employed in



Benadryl

Britain. Pyribenzamine differs from neoantergan in the absence of a methoxy group on the benzene ring, and benadryl is dimethylaminoethylbenzhydryl ether hydrochloride.



Antistin

In attempts to produce more potent and less toxic antihistamine drugs new derivatives of these compounds have been studied recently. Antistin is closely related to antergan, but the dimethylamino linkage is replaced

by an imidazole ring, while hetramine is the pyrimidine analogue of pyribenzamine. Two further compounds—α-naphthyl-methylethyl-β-chlorethylamine and β-2-biphenyloxyethyl-β-chlorethylamine are halogen derivatives of neoantergan. They are of interest because they antagonise the action of adrenaline, whereas all the earlier antihistamines, with the exception of the original one, 929F., potentiate the action of adrenaline. The search for more potent and less toxic antihistamines continues, and it may well be that we have as yet only touched the fringe of new developments.

Potency. The potency of antihistamine substances has been tested in a number of ways. The lethal dose of histamine injected intravenously is determined for a group of guinea-pigs, which usually varies from 0·4 to 0·8 mg./kg. of body weight. The antihistamine to be tested is then injected subcutaneously and increasing doses of histamine are thereafter given to determine the maximum dose which the animal survives, and therefore the protective effect of the antihistamine. The second test is designed to discover the protective action of the antihistamine against the lethal effect of histamine inhaled by a guinea-pig. The third test determines the power of the antihistamine to prevent the action of histamine in causing contraction of the guinea-pig's isolated intestine. The fourth test determines its effect in preventing the depressor action of histamine on a dog's blood pressure, and the fifth test measures its power to abolish or diminish the size of the weal caused by an intradermal injection of histamine.

The relative antihistamine activity of the drugs which have been commonly employed in clinical practice has been tested by these animal experiments. Using such tests it was found that benadryl and the old-fashioned French preparation, antergan, were less effective than pyribenzamine, and all of them very much less effective than neoantergan The relative clinical effectiveness of the various drugs is, however, not nearly so divergent in human beings as the experiments on laboratory animals would have led us to expect, though it does seem that neoantergan is at present the most potent and specific antihistamine which we possess.

Mode of Action. A knowledge of the mode of action of this group of drugs is necessary if they are to be used efficiently. It is apparent that they might act in a number of ways: they might prevent the release of histamine from the tissues; they might abolish its action by entering into some inert chemical combination with it; they might set up a directly antagonistic pharmacological action; or they might block the action of histamine by competing with it successfully for the tissue receptors. If they acted by preventing the formation of histamine they would have no effect on the production of the typical skin weal when histamine is injected subcutaneously, but they do have a striking effect in this respect. Further they would prevent the stimulating action of histamine on the gastric secretion, which, as we shall see, does not occur. There is no evidence whatever that they destroy histamine or render it inactive by

entering into chemical combination with it. The only type of pharmacological antagonistic action to histamine which is at all likely would be the potentiation of adrenaline, but as some potent antihistamines antagonise rather than potentiate adrenaline it is impossible to believe that there can be any relationship between the sympathomimetic and antihistamine properties of the others. Thus, by a process of *reductio ad absurdum* we come to the conclusion that antihistamines act by blocking the action of histamine by combining with its tissue receptors.

If this theory is correct it is apparent that the underlying allergic or anaphylactic tendency persists in spite of the use of antihistamines since the abnormal production of histamine is not interfered with, and, therefore, the administration of the antihistamine in clinical practice has to be continued either indefinitely in a few cases or at least till the allergic or anaphylactic tendency has subsided spontaneously, or as the result of artificial or natural desensitisation. It is thus important to realise that the use of these drugs does not absolve the physician from considering the advisability of specific desensitisation in certain cases, though such desensitisation may be frequently impossible, undesirable or unnecessary.

Other Effects. Few drugs have only one property. Most of them produce—perhaps to a lesser degree—effects additional to that for which they are principally prescribed in therapeutics. Antihistamines are no exception to this rule, for, besides abolishing the effects of histamine, they have many other actions, some of which are inconvenient when they are used in clinical practice. In addition to their antihistamine activity, they have to a varying extent anti-acetylcholine, local anæsthetic and sympathomimetic or sympatholytic properties. Antispasmodic, analgesic, and quinidine-like actions have also been demonstrated by some members of this group. Benadryl has, even in therapeutic doses, a pronounced atropine-like action, causing dryness of the mouth and some dilatation of the pupil, an analgesic action causing drowsiness and some slight spasmolytic effect. These properties are shared, but to a less extent, by neoantergan and pyribenzamine. As we have seen, however, some of the newer antihistamines and 929F. are sympatholytic drugs. Antihistamines are local anæsthetics. Neoantergan, benadryl and antistin, for instance, are 3·3, 2·5 and 1·5 times as potent as procaine. When taken by the mouth, however, they do not produce a demonstrable local anæsthetic effect on the skin. Their power as antihistamines has nothing to do with their local anæsthetic effect, since the latter wears off in about an hour's time, whereas their antihistamine action lasts for at least four hours. Neoantergan has been found to be twice as powerful as quinidine on the auricle of the rabbit, but this effect has not been demonstrated in the human subject. Thus, as Burn has pointed out, antihistamines "join the group of other substances which include spasmolytics like trasentin and syntropan, analgesics like pethidine and papaverine, local anæsthetics like procaine, and atropine-like substances. None of these can be sharply distinguished from one another. Probably each possesses

every property in some degree." The common properties of all these drugs suggest that their site of action must be a similar one.

Dosage and Administration. Antihistamine drugs are usually given by the mouth in tablets or capsules. Benadryl and pyribenzamine are prescribed in doses of 50 to 100 mg., with a maximum daily dose in the case of benadryl of 400 mg. and in the case of pyribenzamine of 600 mg. Neoantergan and antistin, being less toxic, can be given in bigger doses of 100 to 200 mg. with a maximum daily dose of 800 mg. Children tolerate the drugs well, and over the age of twelve can be given the adult dose, with appropriately smaller doses under that age. The drugs are quickly absorbed and fairly quickly excreted in the urine. The effect of a single dose does not last for more than six hours, so that in order to maintain a satisfactory concentration, dosage should be well spaced, the drug being given at least three times a day. In severe cases four doses should be administered—the last one as late as possible at night so as to "cover" the hours of sleep. The tablets or cachets should be swallowed whole and not chewed as they have an unpleasant taste, and if brought in contact with the mucous membrane of the mouth or pharynx will have a marked local anæsthetic effect. Neoantergan and antistin are not spasmolytics like benadryl, and their use may, indeed, produce increased motility of the alimentary tract in experimental animals. In consequence they may occasionally cause nausea if given on an empty stomach, and should, therefore, be taken after food. Tolerance to antihistamines does not seem to take place.

Benadryl is procurable in a purified solution containing 10 mg. of the drug per ml. for intravenous use and its administration in this way has been recommended for anaphylactic emergencies, but otherwise the parenteral use of antihistamines is unnecessary and may, indeed, cause on occasion rather alarming symptoms of collapse. As might be expected from the mode of action of antihistamines, no effect, apart from diminishing skin irritation, is produced on established lesions, which will subside spontaneously, though new lesions are prevented from occurring. Thus antihistamines, even if given intravenously, will have little effect on an established allergic emergency such as swelling of the tongue or œdema glottidis, for which adrenaline is the drug of choice.

Antihistamines may be used in a suitable base for local application in some of the itching dermatoses, and may be applied locally to the nose in cases of allergic rhinorrhœa. For this latter purpose antistin is the most suitable preparation.

Side Effects.—No deaths or toxic effects leading to organic change have occurred as the result of the administration of antihistamines, even though they have been administered to some patients for years. Side effects are, however, very common, and in about 5 per cent. of cases may be sufficiently distressing to necessitate discontinuing their use. A full dose of benadryl or antistin will cause effects in about 50 per cent. of cases. Pyribenzamine and neoantergan are less toxic, but cause side

effects in about 30 per cent. of cases. The practical superiority of neoantergan, and to a less extent pyribenzamine, over benadryl lies in the fact that they are not only more active antihistamines milligramme for milligramme, but can be tolerated in larger doses and may thus benefit some patients for whom the necessarily smaller dose of benadryl had proved ineffective. The great majority of the side effects of antihistamines only occur when the drugs are first administered and tend to wear off in a few days.

The principal side effect of all antihistamines, but particularly benadryl, is sleepiness, fatigue or dizziness. In some patients on first taking the drug this hypnotic effect may be very marked indeed, and may in a few cases persist even after they have been taking the drug for a long time. Patients should be warned about this effect before they are given antihistamines, and, until their reaction to the drug in this respect has been ascertained, should not take their first few doses before undertaking work requiring skilled judgment. It is wise also to start treatment with a small daily dose and gradually to increase it till the optimum effect has been obtained, as in this way the patient usually becomes quickly tolerant to any hypnotic effect which may be present. Alternatively, 5 mg. of amphetamine may be administered coincidently in the morning and at mid-day for the first few days of treatment till the hypnotic effect of the antihistamine wears off. Owing to the fact that antihistamines often produce soporific effects when they are first taken, the coincident use of hypnotics and sedatives should be prescribed with care. On the other hand, a curious sensation of tension, nervousness and unreality is occasionally produced by antihistamines, and these sensations may lead to insomnia rather than to sleepiness.

As benadryl has a strong atropine-like action, it is not surprising that patients frequently complain of dryness of the mouth as the result of its use. Pyribenzamine, neoantergan and antistin may also produce this side effect, but less commonly and to a less extent. Atropine and its congeners should not, therefore, be prescribed along with these drugs, though there is no contra-indication to the coincident use of sympathomimetic preparations.

Other side effects have occasionally been noted as the result of the therapeutic use of antihistamines, but they are rare and unimportant.

Therapeutic Uses. It might be anticipated that drugs which antagonise histamine would have a wide range of therapeutic application. Their value in treatment, however, is actually somewhat limited to allergic conditions characterised by vascular reactions in the skin and mucous membranes resembling the effects produced by the local application of histamine. Thus they may be claimed almost as specifics in cases of acute and chronic urticaria or angioneurotic œdema, and in many of the urticarias encountered when a patient becomes sensitive to drugs such as penicillin, liver extract and insulin. The itching of pruritus

vulvæ and ani is often greatly ameliorated by the use of antihistamines, while they either cure or very much alleviate some 75 per cent. of cases of hay fever and a somewhat smaller proportion of cases of perennial vasomotor rhinitis.

The administration of histamine does not cause bronchospasm in healthy persons, but it has this effect in asthmatic subjects, just as it has in guinea-pigs, and this artificially produced bronchospasm can be prevented by antihistamine drugs. These observations do not *prove* that naturally occurring asthma is due to a simple release of histamine or that it can be prevented by antihistamine drugs, but they encouraged the hope that such drugs might be of some value in the prevention and treatment of the condition. The results of their clinical trial in asthma are, however, extremely conflicting, and much of the work claiming antihistamines to be of value in the disorder is based on poorly controlled evidence. It is at any rate certain that the benefits to be derived from the use of antihistamines in asthma, if they exist, are in no way comparable to their value in the allergic reactions in the skin and mucous membranes mentioned above.

It is almost certain that histamine is the natural stimulant of gastric secretion. It might be expected, therefore, that antihistamine drugs would be of value in the treatment of hyperchlorhydric dyspepsia and peptic ulcer. All clinical and experimental evidence unfortunately goes to show that they are of no practical use in these conditions and have no significant effect in modifying gastric secretion. The drugs have also been tried in a great variety of other allergic states with negative results.

In summary, then, antihistamine drugs are of great value in superficial allergies, in the treatment and prevention of which they constitute a major therapeutic advance, but they are of little or no value in the treatment of the more deep-seated visceral allergies in the human subject. It may be that histamine does not play a part, or a predominant part, in the production of some of these visceral disorders and that this may account for the failure of antihistamines to influence their course favourably. We do know, however, that histamine does stimulate gastric secretion, and that in spite of this antihistamine drugs have no influence on this action of histamine. It may be, therefore, that in some visceral allergies histamine is released in such intimate contact with the effector cell that antihistamines are impotent to block its action.

RESEARCH PAPERS

HYDROLYTIC CHANGES IN SOLUTIONS OF STILBAMIDINE

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Henry[1], working in the Sudan, suggested that solutions of 4:4′ diamidinostilbene dihydrochloride (stilbamidine dihydrochloride), underwent a number of changes, including hydrolysis of the amidine groups to the corresponding mono- and di-amides, when kept in diffuse daylight. He[2] isolated a substance shown by analysis to be 4-carbamyl-4′-amidinostilbene hydrochloride. From kinetic considerations he deduced that a dimer of stilbamidine was also formed. The same author[3] gave further experimental details when this investigation had been completed. Fulton[4] found by chemical methods that, in this country, 1:2:3:4-tetra-(4′-amidinophenyl)-*cyclo*butane was the only product formed on exposure of stilbamidine solutions to light. On account of our interest in this subject Drs. Henry and Kirk in 1947 kindly sent us some bottles of the old solutions of stilbamidine dihydrochloride prepared in the Sudan in 1941 and 1942, in which we have confirmed the presence of 4-carbamyl-4′-amidinostilbene and have also obtained 4:4′-dicarbamylstilbene in pure form. The same substances have now been obtained by us from stilbamidine solutions kept at 37°C. for long periods or on autoclaving the solutions for a few hours. Oastler and Fidler[5] described cerebral lesions in dogs following intravenous administration of stilbamidine solutions which had been autoclaved for a short period. Sen Gupta[6] suggested that the drug had been affected by this treatment. From toxicity experiments with mice we have not obtained any evidence in support of the latter view.

Experimental

Six bottles of 1 per cent. solution of stilbamidine dihydrochloride of approximately 100 ml. volume were received by us from the Sudan in September, 1947, with the information that they had been prepared in 1941-42 and kept in the dark or in diffused daylight. The contents (see Table I) were yellowish in colour and large crystals as well as some microcrystalline material were present, the latter being sometimes very adherent to glass. The two substances were readily separated by their different solubilities in water, in which the larger crystals dissolved. The soluble material after several recrystallisations were shown by analysis to have the composition of 4-carbamyl-4′-amidinostilbene monohydrochloride with two molecules of water of crystallisation. (Found: in solid dried at 90°C.; C, 63·33; H, 5·30; N, 13·85, 14·1; Cl, 21·1; loss at 90°C. 10·45, 10·64 per cent. $C_{16}H_{16}ON_3Cl$, requires C, 63·66; H, 5·34; N, 13·93; Cl, 11·76; H_2O, in hydrated material 10·66 per cent.) The substance

crystallised in thin laths from water, in which it is less soluble than stilbamidine dihydrochloride and had no m.pt. up to 320°C. Its aqueous solution contained Cl′ ions and rapidly decolorised aqueous bromine or

TABLE I

PRODUCTS PRESENT IN OLD SOLUTIONS OF STILBAMIDINE FROM THE SUDAN

Number of sample	*p*H of solution	Total solid	Water-insoluble portion
		mg.	mg.
1	6·8	590	200
2	6·4	505	57
3	6·2	460	30
4	6·4	525	33
5	6·2	395	40
6	6·4	180	37
TOTAL		2655	397

permanganate solutions. When mixed with ammonium nitrate in excess a yellowish somewhat insoluble nitrate was formed which crystallised in fine rods from water m.pt. around 290°C. as given by Henry[2]. The aqueous solutions of 4-carbamyl-4′-amidinostilbene hydrochloride and those of stilbamidine dihydrochloride showed a similar blue fluorescence and their absorption spectra were identical. The microcrystalline material, which proved to be the diamide, was insoluble in common organic solvents but soluble to a limited extent in acetic acid and was somewhat more soluble in ethylene glycol, from which plates and fine rods were respectively obtained with no m.pt. up to 320°C. (Found: N, 10·7 per cent. $C_{16}H_{14}O_2N_2$ requires N, 10·53 per cent.)

The solutions fluoresced blue except number 6, which had a greenish tinge. Spectrophotometric analysis showed that samples 1 and 2 contained only saturated material, formed from the parent substance by the action of light, while sample 3 contained only the original material. In samples 4, 5 and 6 both substances were present. Deposits had never been observed by us in this country from solutions of stilbamidine dihydrochloride kept for more than a year at laboratory temperatures, which did not exceed 20°C., in light or dark. A series of solutions was therefore subjected, in stoppered bottles, to different conditions of light and temperature and observed over a period of 6 months; the initial *p*H of the solutions was approximately 6·8 and did not alter appreciably during the experiment. The results obtained are shown in Table II.

The product was practically all monoamide, with only negligible traces of diamide, and formed crystals in some cases 2 cm. long. Temperature is apparently of importance in the reaction as no amides were formed at that of the laboratory. It also seems as if light exerted some influence since solutions kept in complete darkness yielded relatively very small amounts of amide. Good yields of both amides were obtained by autoclaving stilbamidine dihydrochloride solutions; this proved a rapid and convenient method of obtaining both products as shown in Table III.

It appears from the data recorded that hydrolysis of the amidino groups in stilbamidine is readily accomplished by heating solutions of the dihydrochloride under pressure. The amides are much less soluble

TABLE II

RESULTS OF KEEPING STILBAMIDINE SOLUTIONS AT 37° C. FOR A PERIOD OF 6 MONTHS

Number of sample	Solutions of dihydrochloride	Conditions of keeping	First appearance of deposit	Percentage yield
1	100 ml. of 1 per cent.	At 37° C. exposed to electric light for short intervals	8 weeks	31
2	50 ml. of 1 ,,		6 weeks	22
3	100 ml. of 1 ,,	Complete darkness at 37° C.	—	None
4	100 ml. of 1 ,,		16 weeks	5
5	100 ml. of 0·2 ,,		17 weeks	2
6	100 ml. of 1 ,,		8 weeks	2·5
7	100 ml. of 1 ,,	Complete darkness at room temperature 5° to 20° C.	—	None
8	100 ml. of 1 ,,		—	None

than the parent substance and the yields recorded in the table represent the solid obtained on cooling the treated solutions. Because of the much greater solubility of the parent di-isethionate and resulting monoamide salt it is more satisfactory to start with the dihydrochloride. When a

TABLE III

RESULTS OF AUTOCLAVING SOLUTIONS OF STILBAMIDINE UNDER DIFFERENT CONDITIONS

100 ml. of solution		Treatment in autoclave		Percentage yield	
Nature	Strength	Atmospheres	Hours	Monoamide	Diamide
4 : 4′ diamidinostilbene dihydrochloride	1·0 per cent.	½	½	None	None
	1·0 ,,	1	½	Trace	—
	1·0 ,,	1½	4	40	7
	0·5 ,,	1½	4	24	5
	1·0 ,,	1½	7	52	16
	1·0 ,,	2	4	45	11
4 : 4′ diamidinostilbene di-isethionate	1·0 ,,	1½	4	7	Trace
	0·5 ,,	1½	4	10	Trace
	10·0 ,,	1½	2	2·5	Trace
4-carbamyl-4′-amidinostilbene ...	1·0 ,,	1½	2	—	Trace

solution of the monoamide was treated under the above conditions, conversion to the corresponding diamide took place only to a slight extent. It was found by analysis and spectrophotometric measurements that the mono- and di- amides prepared by us in different ways are identical with the products formed in the Sudan. The absorption spectra of these two substances are indistinguishable from that of the parent *trans*-stilbamidine. The values obtained for the latter and the mono-

amide in aqueous solution and of the diamide in acetic acid were as follows:

	λ max	ε max
4:4′-diamidinostilbene dihydrochloride	328 mμ	37,800
4-carbamyl-4′-amidinostilbene hydrochloride ...	328 mμ	38,200
4:4′-dicarbamylstilbene	328 mμ	40,000 (approx.)

The value ε for the diamide is only approximate on account of its extreme insolubility. The fact that its spectrum was observed in acetic acid does not invalidate comparison with the monoamide since the latter's spectrum was unchanged in this solvent. The two groups $> C = O$ and $> C = NH$ thus appear to be chromophorically identical.

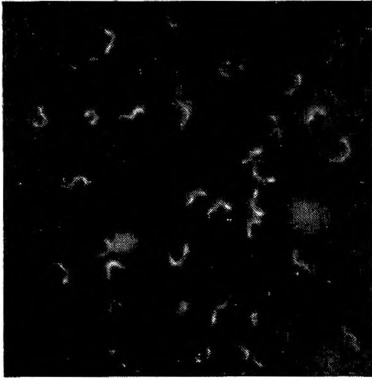
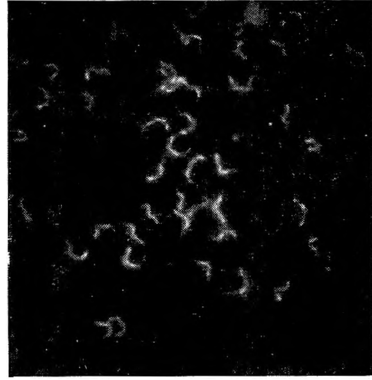
Various methods have been reported for the estimation of stilbamidine, for example fluorescence (Henry and Grindley[7]); colour reaction with glyoxal (Devine[8]), (Fuller[9]); spectrophotometric (Fulton and Goodwin[10]); fluorophotometric (Saltzman[11]). None of these methods is entirely satisfactory. Wien [12] and Hampton[13], using the fluorimetric and colorimetric methods to estimate excretion of stilbamidine in the urine of laboratory animals, found that the values obtained by the latter method were much higher than those obtained by measurement of fluorescence. In view of these results and the possession of similar optical properties by the monoamide and parent substance it seems unlikely that the former is a metabolic product of stilbamidine. Their toxicities as well as those of solutions of stilbamidine autoclaved at 5lb. pressure for 20 minutes, as used by Oastler and Fidler[5], have been compared in mice. The results are shown in Table IV.

TABLE IV

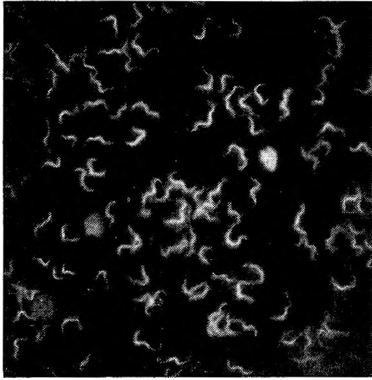
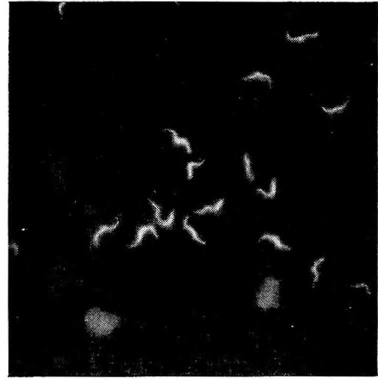
TOXICITY OF VARIOUS SUBSTANCES FOR MICE
D—Died in less than 1 hour after injection
P—Died within a few days of injection
S—Survived observation period of 1 week

Drug	Nature of solution	Effect of doses (mg./20 g. mouse intraperitoneally)		
		2·0	1·0	0·5
4 : 4′ diamidinostilbene dihydrochloride	Fresh	6D 2P 2S /10	2P 18S /20	5S/5
	Autoclaved at 5 lb. pressure for 20 minutes	5D/5	1P 15S /16	5S/5
	Autoclaved at atmospheric pressure for 20 minutes	5P/5	2P 18S /20	5S/5
4-carbamyl-4′-amidino-stilbene	Fresh	6D/6	9D 3S /12	6S/6

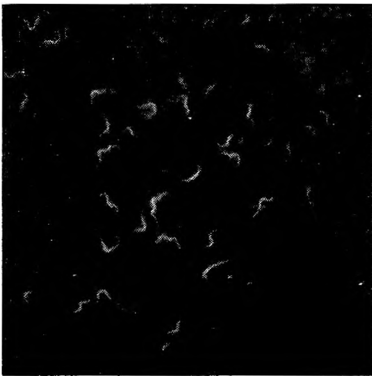
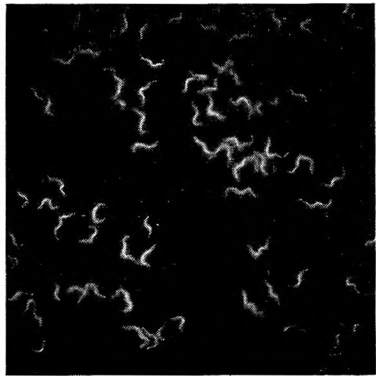
They indicate that 4-carbamyl-4′-amidinostilbene is more toxic than stilbamidine for mice and also that autoclaving of the latter solutions even

Figs. 1 and 4. Appearance under ultra-violet illumination of *T. congolense* and *T. rhodesiense* respectively. Exposed *in vivo* to 4 : 4′-diamicinostilbene.

Figs. 2 and 5. The same parasites respectively exposed under the same conditions to 4′-carbamyl-4-amidinostilbene.

Figs. 3 and 6. *T. congolense* and *T. rhodesiense* respectively not exposed to drug.

at a pressure of 1 atmosphere has not led to significant increase in toxicity.

Therapeutic tests on mice infected with *T. rhodesiense* and *T. congolense* were carried out with 4-carbamyl-4'amidinostilbene hydrochloride as shown in Table V. It was not possible to test 4:4' di-carbamylstilbene in the same way on account of its insolubility.

TABLE V

RESULTS OF TREATMENT OF TRYPANOSOME INFECTED MICE.
R Blood free from trypanosomes, but relapse occurred.
N Blood never free from trypanosomes.

Mice infected with :	Drug	Effect of doses (mg./20 g. mouse intraperitoneally)	
		0·5	0·25
T. rhodesiense	4-carbamyl-4'-amidino stilbene hydrochloride	1R 15N/16	6N/6
T. congolense		2R 14N/16	1R 5N/6

It is evident that 4-carbamyl-4'-amidinostilbene is inactive therapeutically in the above infections and the life of each mouse was prolonged for only a short period. On the other hand 4:4'-diamidinostilbene is curative at high dilutions in the former infection, but requires a dose approaching the maximum tolerated (1 mg./20 g. mouse) to eradicate *T. congolense* infections. In order to find out the distribution of these drugs in trypanosomes exposed *in vivo* to their action, use was made of their similar fluorescent properties. For this purpose mice heavily infected with *T. rhodesiense* and *T. congolense* were treated with a solution of 0·5 mg. of each drug intraperitoneally and 1 hour later when the trypanosomes were still actively motile, blood smears of treated and untreated animals were made on a quartz slide. The slide was mounted dry without cover glass on the Beck-Barnard ultra-violet microscope and the object was illuminated by means of a quartz dark ground illuminator using the group of lines of the magnesium spark spectrum at 2830Å. Micrographs were taken using a Zeiss 4 mm. apochromat (N.A. 0·95) and a Zeiss No. 2 projection ocular. The length of exposure was 2 minutes in each case. The correction collar on the objective was adjusted to give the best image at a magnification of X 150. The appearances produced are those shown in Figures 1 to 6.

In the case of both trypanosomes exposed to stilbamidine selective absorption of the drug has occurred, as shown by the presence in them of two bright granules. The position of one granule corresponds to that of the blepharoplast, but the nature of the other at the anterior end has not been determined. The remainder of the cytoplasm does not fluoresce more brightly than that of untreated trypanosomes. There is absence of fluorescence in accompanying red cells which are not readily visible. The

inactive 4-carbamyl-4′-amidinostilbene appears to have been generally absorbed throughout the bodies of the trypanosomes as indicated by the increased brightness compared with that of parasites not exposed to the drug.

Summary and Conclusions

The formation of 4-carbamyl-4′-amidinostilbene and 4:4′-dicarbamylstilbene from solutions of stilbamidine had been shown to occur when the latter were maintained for a number of weeks at 37°C., in diffuse light, and to a lesser extent when kept at the same temperature completely in the dark. When the same solutions were maintained at temperatures which varied from 5° to 20°C. the formation of amides did not take place. Henry's observations made in the Sudan have been confirmed. Good yields of the amides were obtained by autoclaving solutions of the parent substance at 1 to 2 atmospheres pressure for several hours. The monoamide was inactive against *T. rhodesiense* or *T. congolense* infections of mice and does not appear to be selectively absorbed by the trypanosomes like the active stilbamidine. The fact that solutions of stilbamidine autoclaved under the conditions employed by Oastler and Fidler undergo no demonstrable change and are not more toxic for mice than similar solutions freshly prepared, suggests that the lesions encountered by these authors in dogs were due to unchanged stilbamidine.

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THE INHIBITION OF ADRENALINE BY SPASMOLYTIC AGENTS

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It has long been known that[1] certain fibres in the autonomic nervous system which are classified on anatomical grounds as post-ganglionic sympathetic fibres are functionally cholinergic in nature. The effects of stimulation of such fibres are not reproduced by injection of adrenaline, but are potentiated by previous administration of eserine and are abolished by atropine sulphate. Bussel[2] has collected much evidence, from a review of earlier literature on the subject and from new experimentation, which supports the view that atropine has an inhibitory action upon the effects of stimulating post-ganglionic adrenergic sympathetic fibres and upon the actions of adrenaline itself. These investigations were carried out upon various preparations of portions of the vascular bed of animals and upon the contraction of the nictitating membrane in cats. It was found that in contrast to the behaviour of atropine, hyoscine hydrobromide did not modify the action of adrenaline on the vessels of the dog leg or on the nictitating membrane of the cat, or depress the level of the blood pressure in etherised cats in the doses administered.

The present paper reports upon an action of adrenaline which is inhibited by relatively small doses of atropine sulphate. A comparison was made between the effects of atropine sulphate (*r*), *l*-hyoscine, *l*-hyoscyamine, *l*-homatropine, trasentin 6H and 2786 R.P. (neoantergan) upon adrenaline activity in the preparation described below. The effect of neoantergan on the action of adrenaline on the isolated uterus of pregnant rabbit, and on the isolated gut segment of rabbit and guinea-pig was investigated. The effect of neo-antergan, atropine and trasentin 6H on adrenaline action on the blood pressure of spinal cats was also tested. In some cases comparison was made between the effects of the spasmolytic agents on the actions of *l*-adrenaline (B.P.) and *l*-*nor*-adrenaline.[3]

Graham[4] showed that the isolated duodenum of the duck or drake contracts on addition of adrenaline to the fluid in which the strip of gut is suspended. The tissue is stiff and shows little spontaneous movement. The ileum of the drake and all parts of the intestine of fowls and pigeons show more activity and relax on addition of adrenaline. As Barsoum and Gaddum[5] have shown, the rectal cæcum of the fowl is sensitive to adrenaline in a concentration of 10^{-9}. The bath in which the duck gut was suspended was kept at 38°C. and was of 100 ml. volume. Drugs were added in solution in 0·5 ml. of saline solution.

The Action of Adrenaline on the Isolated Duodenum of the Drake and Interference with it by Spasmolytic Agents

Adrenaline in a concentration of 2×10^{-8} or more causes a transient but powerful contraction of the isolated duodenum of ducks. This contraction is usually but not always followed by a period of relaxation which may or may not be accompanied by inhibition of spontaneous movement, so that the response to adrenaline may be purely motor[4] or biphasic (see Figure 2). In the three preparations tested the response to *l-nor*-adrenaline was purely motor. In the same concentration as *l*-adrenaline it produced a longer though less vigorous contraction of the gut. Acetylcholine also causes a contraction in this preparation, but is less potent in this respect than adrenaline. The effect of acetylcholine 10^{-7} is roughly equal to that of adrenaline 2×10^{-8}. Atropine sulphate in a concentration of 10^{-8} abolishes or prevents the effect of the acetylcholine while leaving the action of adrenaline but little modified. If, however, the concentration is raised 100 fold to 10^{-6} the motor effect of adrenaline 2×10^{-8} on the gut is prevented. This effect is shown in Figure 1.

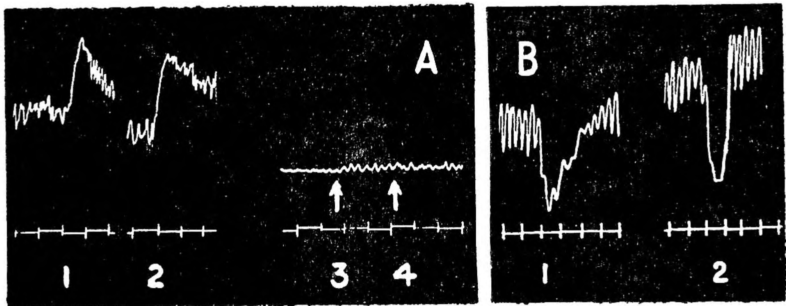


Fig. 1.—A. Isolated duodenum of duck. At 1. and 3. adrenaline 2×10^{-8}, at 2. and 4. acetylcholine 10^{-7}. Between 2. and 3. atropine sulphate 10^{-6}. B. Isolated rectal cæcum of fowl. At 1. and 2. adrenaline 10^{-9}. Between 1. and 2. atropine sulphate 2×10^{-6}. Time in 10 secs.

The motor effect of adrenaline 10^{-7} is not inhibited by *l*-hyoscine or *l*-hyoscyamine 10^{-5}, but is reduced to about one half by *l*-homatropine 10^{-5} and is abolished by atropine and trasentin 6H in the same concentration. The latter two compounds have a profoundly inhibitor effect on the tone and spontaneous movement of the gut in these high concentrations, the former have not. In the rectal cæcum of the fowl the spontaneous movements are not inhibited by atropine (2×10^{-6}) nor is the inhibitor action of adrenaline (10^{-8}) modified by atropine in this concentration (see Figure 1).

The antihistamine compounds have been shown to have varying potencies as spasmolytic agents against contraction of smooth muscle caused by acetylcholine, histamine and barium (Graham[6]), and in the course of that work some inhibition of the effect of adrenaline on the

blood pressure of the chloralosed cat was noted after addition of these compounds. Accordingly the effect of neoantergan (Dews and Graham[7]) was tested upon the action of adrenaline and *l-nor*-adrenaline on duck duodenum. As already stated the effect of adrenaline was to produce

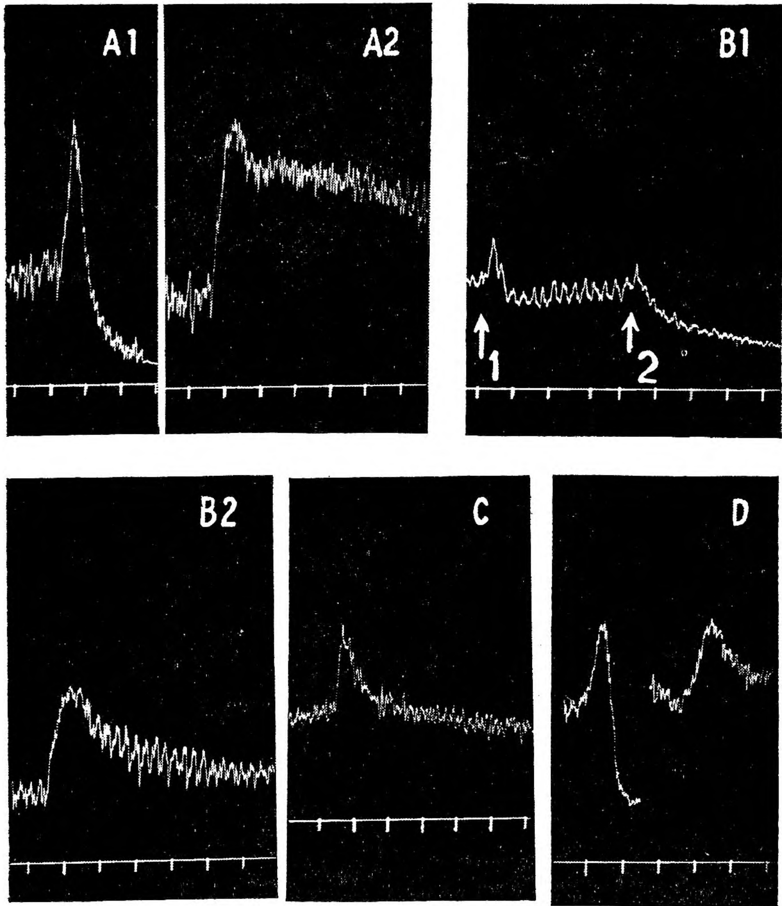


FIG. 2.—Isolated duodenum of duck. A1, biphasic response to *l*-adrenaline 10^{-7}. A2, motor response to *l-nor*-adrenaline 10^{-7}. B_1, neoantergan 10^{-6} injected at arrow 1, inhibits the motor part of the biphasic response to *l*-adrenaline 10^{-7} injected at arrow 2. B2, as in A2 but in the presence of neoantergan 10^{-7}. C, effect of *l*-adrenaline 10^{-7} 5 minutes after neoantergan 10^{-6} had been washed out twice. Motor part of biphasic response only restored. D, as in A1 and A2 after 10 minutes washing out of neoantergan 10^{-6}. Time in 10 secs.

a biphasic response, that of *l-nor*-adrenaline to produce a motor response. Neoantergan in a concentration of 10^{-6} abolished the motor part of the response to adrenaline and reduced the inhibitor part of the response; the response to *l-nor*-adrenaline was partially inhibited (see Figure 2B). A concentration of 10^{-5} of neoantergan abolished the re-

sponse to either compound. The characteristic effect of both compounds could be restored after repeated washings, but the motor part of the response to adrenaline returned before the inhibitor portion of the response (see Figure 2C). These reactions are illustrated in Figure 2.

The Effect of Neoantergan (2768 r.p.) on the Response to Adrenaline of Isolated Gut of Rabbit and Guinea-pig, and Isolated Pregnant Uterus of Rabbit

In view of the inhibition by neoantergan of the action of adrenaline on the duodenum of ducks a similar trial of its effect on the action of adrenaline on isolated rabbit duodenum and guinea-pig duodenum was made. As is well known, the movement of such specimens is regular and is inhibited by adrenaline (10^{-8}). Preparations vary in the degree and duration of inhibition caused by this concentration of adrenaline. Neoantergan in a concentration of 10^{-5} usually caused the gut to relax and inhibited spontaneous movements; during this inhibition adrenaline caused no relaxation, but as the inhibition was already maximal no further effect could be expected. After repeated washings of the preparation the spontaneous contractions were small and frequent and the tone was high (Fig. 3F and 3H). Under these conditions in some preparations the action of adrenaline (10^{-8}) was abolished or reversed (3F) for a variable time, but ultimately returned. In other preparations (3H) the action of adrenaline was not abolished. These variations were related to the amount of neoantergan administered to the bath, the duration of its action, and to the period allowed for recovery and the number of wash-outs given. Inhibition of adrenaline action was better seen with specimens of duodenum than of ileum.

Neoantergan in a concentration of 10^{-5} occasionally caused immediate spasm of the muscle preparation which lasted for some 2 minutes and was followed by increased spontaneous activity (3B). Under these circumstances the action of adrenaline could be tested in the presence of neoantergan (3B), when it was found to be less effective. After repeated washing the action of adrenaline was abolished (3C) and only partially restored after 10 washes at minute intervals (3D). Atropine, homatropine and trasentin had no effect on adrenaline inhibition of rabbit gut in low concentrations (10^{-8}); higher concentrations diminished gut movement so that further action by adrenaline could not be tested.

The isolated strip of pregnant uterus of rabbit responds to the addition of adrenaline (10^{-8}) with a transient spasm (Fig. 4A). Neoantergan in a concentration of 10^{-5} produces a prolonged increase in tone with frequent strong contractions superimposed upon it (4B). If adrenaline is added at the beginning or during the course of this period of increased tone caused by neoantergan the muscle is inhibited (4C and 4D). The action of adrenaline on this preparation is thus reversed by neoantergan in the concentration stated. Backman and Lundberg[8] have shown that atropine has a similar effect on rabbit uterus.

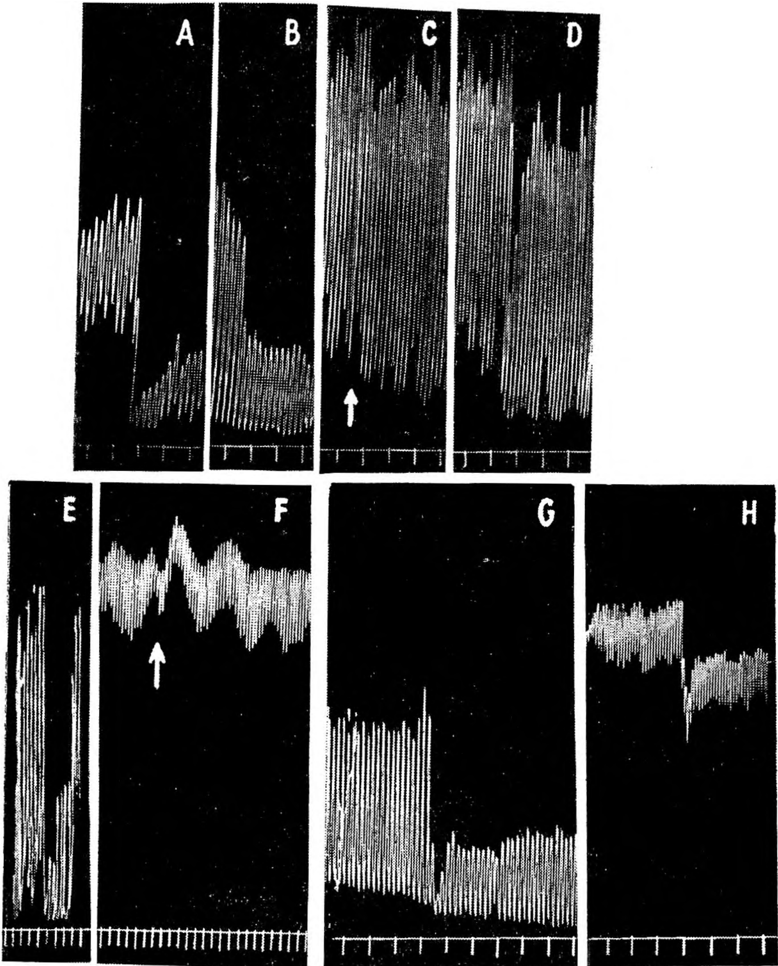


FIG. 3.—Isolated strips of rabbit duodenum. A. effect of adrenaline 10^{-8}. B. effect of A. in presence of neoantergan 10^{-5} C. effect of A. after 5 changes of bath fluid at 1 minute interval. D. as in C. after 10 minutes interval. E. effect of adrenaline 10^{-8} on a fresh strip of duodenum. F. reversal of adrenaline effect 5 minutes after washing out neoantergan 10^{-5}. G. effect of adrenaline 10^{-8} on a fresh strip of duodenum. H. failure to modify action of adrenaline 5 minutes after neoantergan 10^{-5}. Contrast with E. and F. Time in 10 secs.

THE EFFECT OF ATROPINE, HYOSCINE, TRASENTIN AND NEOANTERGAN ON THE ACTION OF ADRENALINE ON THE BLOOD PRESSURE OF SPINAL CATS

Bussel[2] illustrates the fall in blood pressure in etherised cats which results from injection of atropine, and the inhibition of the response of the blood pressure to stimulation of the thoracic sympathetic chain and of the contraction of the nictitating membrane after administration of adrenaline to spinal cats, following upon injection of atropine. Hyoscine did not have these actions.

In a series of spinal cats adrenaline and *l-nor*-adrenaline were injected intravenously and the similar effects recorded. A comparison of the pressor effects of the two compounds on spinal cats indicated that *l-nor*-adrenaline has 165 per cent. of the pressor activity of *l*-adrenaline (B.P.) which agrees closely with the assay carried out by Tainter *et al*[3] on dogs. It was found that injection of neoantergan 0·5 mg./kg. caused a transient small rise in blood pressure and slightly potentiated the

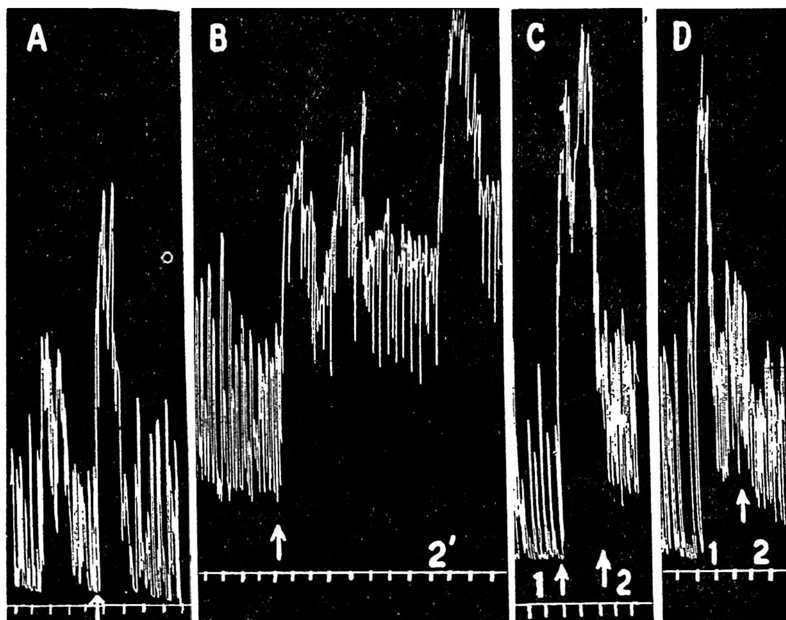


FIG. 4.—Isolated uterus of pregnant rabbit. A. motor effect of adrenaline 10^{-8}. B. prolonged motor effect of neoantergan 10^{-5}. C. adrenaline 10^{-8} added at arrow 2 inhibits the spasm caused by neoantergan 10^{-8} added at arrow 1. D. the effect of adrenaline 10^{-8} added at arrow 2 is reversed by previous addition of neoantergan 10^{-5} at point 1. Time in 2 minutes intervals.

action of these compounds (5B), while a dose of 4·0 mg./kg. caused a transient fall in blood pressure and inhibited the action of these compounds. This effect was brief. 8·0 mg./kg. caused a profound fall in blood pressure and further blocked the action of adrenaline and arterenol. Higher doses were too toxic to allow of further tests; benadryl and antistine[6] cause a sharp fall in the blood pressure of chloralosed cats into which they are injected intravenously in doses of 1·0 mg./kg. Similar action has been cited by Bussel[2] as evidence of anti-adrenaline activity by atropine sulphate.

Trasentin 6H in a dose of 1·0 mg./kg. slightly potentiates the pressor effect of adrenaline or *l*-arterenol, while 4·0 mg./kg. inhibits the response. Larger doses of trasentin 6H (8·0 mg./kg.) completely suppress the response to adrenaline and *l*-arterenol, but are toxic, and under such

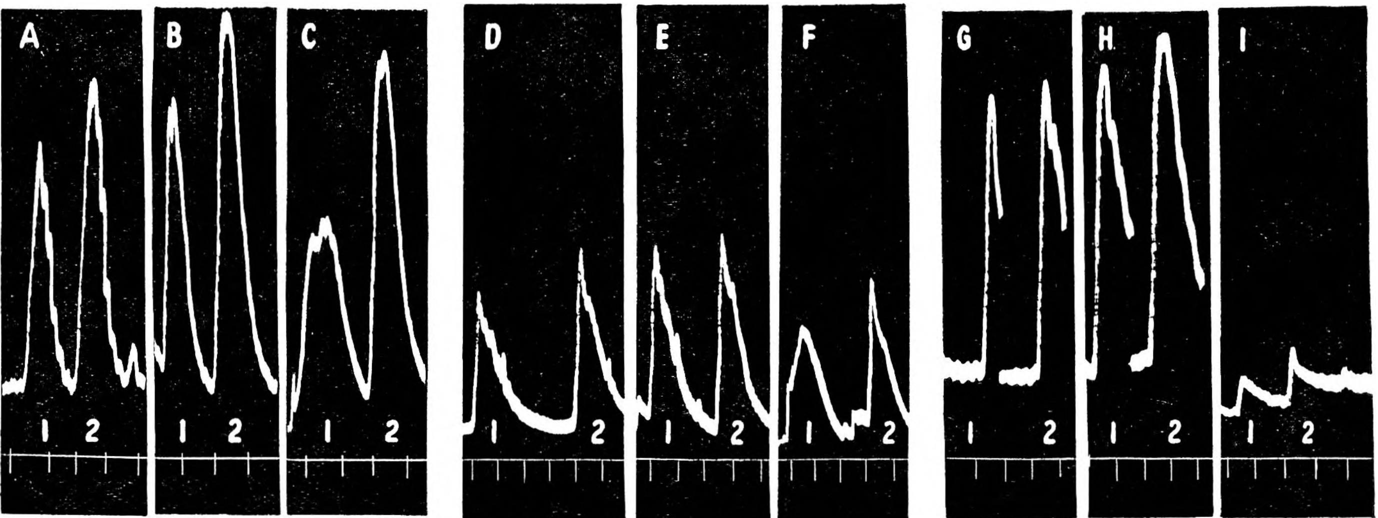


FIG. 5.—Carotid blood pressure taken from spinal cats of 2·5 to 3·5 kg. wt. Time in 30 sec. A1. pressor effect of *l-nor*-adrenaline 1·0 μg./kg. A2. pressor effect of *l*-adrenaline 2·0 μg./kg. B1. and 2. as above after neoantergan 0·5 mg./kg. showing potentiation of effect of A1. and A2. C1. effect as in A1. inhibited after neoantergan 4·0 mg./kg. C2. effect as in A2. still potentiated. Reversal of the order of administration of C1. and C2. reverses this picture which is due to the transient action of 4·0 mg./kg. of neoantergan in blocking adrenaline activity. D1. and D2. as in A1. and A2. but different cat. E1. and E2. as in D1. and D2. but after trasentin 1 0 mg./kg., effect potentiated F1 and F2. as in D1. and D2. but after trasentin 4·0 mg./kg., effect inhibited. G1. and G2. *l-nor*-adrenaline 1·0 μg./kg. and *l*-adrenaline 1·6 μg./kg. respectively. H1. and H2. as in G1. and G2. but after atropine sulphate 1·0 mg./kg., effect potentiated. I1. and I2. as in G1. and G2. but after atropine sulphate 20 mg./kg., effect inhibited.

conditions the failing heart and low blood pressure are incapable of responding to any stimulus.

l-Hyoscine (1·0 mg./kg.) slightly potentiates the effect of adrenaline and *l-nor*-adrenaline, but has no further effect in doses up to 8·0 mg./kg. Atropine sulphate 1·0 mg./kg. slightly potentiates the effect of *l*-adrenaline and *l-nor*-adrenaline, 4·0 mg./kg. has a stronger action, and 20·0 mg./kg. almost abolishes the effect of both compounds without undue toxic actions on the cardio-vascular system of the cats (5I).

In drakes anæsthetised with pentobarbitone solution injected into the breast muscles followed by inhalation of ether, the administration of atropine sulphate 1·0 mg./kg. intravenously causes a sharp transient fall in blood pressure of about 20 mm. Hg. This is similar to the effect seen in etherised cats and rabbits. Doses of 10·0 mg./kg. of atropine do not inhibit the pressor effect of adrenaline 10 μg./kg. intravenously in this preparation.

Discussion

Much recent work has increased our knowledge of the close and complex relationships between the functions of the sympathetic and parasympathetic nervous systems and the actions of acetylcholine and adrenaline and other compounds which modify their activity. The effect of such a substance on any organ is closely related to the dose administered. McDowall[9] and Elio[10] have shown that small doses of acetylcholine may stimulate the heart; larger doses inhibit it. Acetylcholine may potentiate the effect of adrenaline on the heart and blood vessels, and the opposite may occur in the central nervous system (Burn[12]). Small doses of adrenaline lower the blood pressure, larger doses raise it. Many sympathomimetic compounds such as ephedrine, amphetamine, tyramine, cocaine and other local anæsthetics have a different effect on the activity of adrenaline according to the concentration in which they are administered (Jang[13], Graham and Gurd[14]).

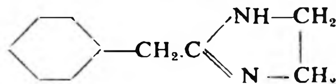
Sherif[15] has shown that the hypogastric nerve to the uterus of the bitch is cholinergic in nature but is not paralysed by atropine, while Secker[16] has shown that salivary secretion in the dog following upon injection of adrenaline and sympathetic stimulation is inhibited by atropine. The inhibitory action of atropine on the effect of adrenaline on duck duodenum is moderately potent (atropine 10^{-6} is effective), while the inhibitory action of transentin 6H and homatropine is less effective (10^{-5}) and *l*-hyoscine and *l*-hyoscyamine appear to be relatively ineffective. There is no apparent relation between the activity of these compounds in inhibiting the action of adrenaline on this preparation and their relative potencies in inhibiting the action of acetylcholine (Graham and Gunn[17]). No action could be shown of these spasmolytic compounds in preventing the inhibitor effects of adrenaline in isolated rabbit gut since in effective doses they are themselves powerful inhibitors of spontaneous movement in this preparation. While atropine and trasentin 6H in doses of 1·0 mg./kg. produce a transient fall in blood pressure

in cats which have a good vascular tone and a high blood pressure (ether or chloralose anæsthesia) it takes much greater quantities of these drugs to inhibit the pressor effect of adrenaline in the spinal cat. It may be therefore that the transient fall in blood pressure seen in etherised cats after atropine, trasentin, etc., 1·0 mg./kg. is a non-specific spasmolytic effect on the arteriolar muscle in high tone rather than a specific anti-adrenaline effect as Bussel[2] suggests, but the clear diminution in the pressor response to stimulation of the thoracic sympathetic chain in spinal cats shown by that author and the great inhibition of the pressor response to injected adrenaline and *nor*-adrenaline illustrated in Figure 5 of this report show that atropine exerts a blocking effect on the motor actions of adrenaline, as does trasentin 6H. Bussel[2] attributes this blocking effect of atropine to its structural resemblance to cocaine which when present in low concentration competes with adrenaline for receptors on the enzymes which inactivate adrenaline and thus potentiates the effect of the latter, and in high concentrations competes with adrenaline for cell receptors and thus inhibits its action (MacGregor[18]). In support of Bussel's explanation of atropine in high concentration inhibiting adrenaline activity is the finding that doses of 1·0 mg./kg. (1/20 of the adrenaline-inhibiting dose) increased the pressor response to adrenaline, as did *l*-hyoscine, trasentin 6H and neoantergan.

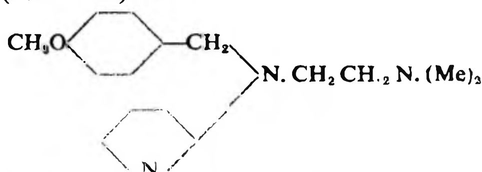
Neoantergan[6,7] inhibits spasm of smooth muscle caused by histamine, acetylcholine and barium. It also blocks the relaxation of some but not all smooth muscle caused by histamine. It appears from the experiments described that it not only inhibits the pressor effect of adrenaline on the vascular bed but reverses the motor effect on the uterus, and inhibits the relaxor effect of adrenaline on some preparations of gut. Its action in this respect differs from that of dibenamine[19] which can reverse the motor response of rabbit uterus and the pressor response of the cat to administration of adrenaline, but does not affect the inhibitory action of adrenaline on gut. The properties of adrenolysis and sympatholysis are shown in varying degree by ergot[20], yohimbine[21], 2-diethylaminoethyl-1 : 4-benzdioxan (833F) and 2-piperidinomethyl-1 : 4-benzdioxan (933F), ephedrine, cocaine, atropine, trasentin 6H, neoantergan, priscol[22], etc. Of these compounds only priscol has been stated to show the property described for neoantergan of blocking the inhibitory action of adrenaline on some segments of rabbit gut. Ahlquist *et al*[22] do not consider this a specific effect of priscol because of the irregularity of appearance of the phenomenon.

Adrenaline may be written as R_1.CHOH.CH.NH.R_2. A similar ethylamine chain may be visualised with varying degrees of ease in all the adrenolytic compounds mentioned.

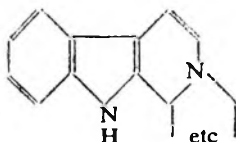
In dibenamine it appears thus:—Cl.CH_2.CH_2.N(CH_2.Ph)$_2$.
in priscol:—



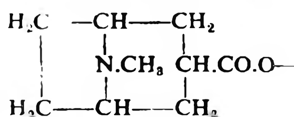
in neoantergan (2786 R.P.):—



-in yohimbine[23]:—

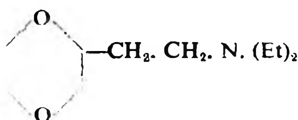


in atropine:—

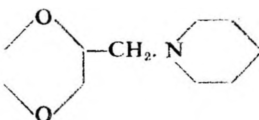


in trasentin 6H:—

in 833 F:—



and 933 F:—



The possession of a common element of structure would account for common properties on the basis of such a theory as that propounded by MacGregor[18] for the effect of cocaine on adrenaline action.

That the effect of spasmolytic compounds in blocking adrenaline action is essentially similar to their effect in blocking *l-nor*-adrenaline is of interest in view of the suggestion of Euler[24], Bacq and Fischer[25] and Gaddum and Goodwin[26] that *nor*-adrenaline is sympathin. West[27] compared *l*-adrenaline and *dl-nor*-adrenaline, and found that both had a powerful inhibitory effect on isolated segments of rabbit gut. The same effects were found above with *l-nor*-adrenaline but in duck gut, in which *l*-adrenaline produces a biphasic response, *l-nor*-adrenaline is purely motor.

Summary

1. Isolated segments of duodenum from the duck react to the addition of adrenaline by a biphasic response, contracting and relaxing. Atropine, trasentin 6H, homatropine and neoantergan (2786 R.P.)

abolish this contractile response. Hyoscine and hyoscyamine have no effect in concentrations of 10^{-5}.

2. Rabbit gut is inhibited by adrenaline and *l-nor*-adrenaline. This action is abolished after neoantergan has been in contact with some segments of gut.

3. Rabbit uterus contracts with adrenaline or neoantergan. In the presence of the latter the former inhibits movement of the uterus.

3. The pressor response to adrenaline and *l-nor*-adrenaline in spinal cats is inhibited by atropine, trasentin and neoantergan in high doses (atropine sulphate 20·0 mg./kg.).

4. Certain differences between *l*-adrenaline and *l-nor*-adrenaline are discussed.

5. A tentative hypothesis is offered to account for the adrenolytic activity found in a wide variety of compounds. This is an extension of MacGregor's well-known explanation for the reactions of cccaine and adrenaline, based on certain common structural features in the adrenolytic agents discussed.

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THE PREPARATION AND RESOLUTION OF ACRIDYL(5)-N.ALANINE ETHYL ESTER

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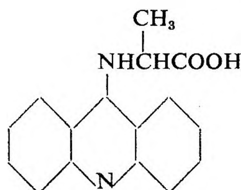
IT HAS BEEN REPORTED that D amino-acids are present in the molecules of various antibiotic polypeptides such as gramicidin[1,2], tyrocidin[3], gramicidin S[4], aerosporin[5] and bacitracin[6]. The penicillins on degradation give D-penicillamine (β:β-dimethylcysteine) and du Vigneaud and his collaborators[7] have shown that an antibiotic penicillin may be synthesised from D-penicillamine, whereas the isomer from L-penicillamine is biologically inactive.

Several workers have prepared substances containing D amino-acids for study as antibacterial agents. These substances either represented possible fragments of the antibiotic molecules, or were of closely related structure. Harris and Work[8] prepared two open chain pentapeptides containing the five amino-acids of gramicidin S in the sequence suggested for the amino-acids in the antibiotic molecule[9]. In one of the pentapeptides the phenylalanine possessed the L configuration; in the other it had the D configuration as in the antibiotic. No significant difference in antibacterial action was found between the two pentapeptides. Fruton[10] synthesised the diketopiperazine from D-leucyl-L-tryptophane because gramicidin had an unusually high content of these two amino-acids; the compound possessed no antibacterial activity. D-leucine and D-valine have been isolated from gramicidin hydrolysates and Fling, Minard and Fox[11] synthesised the prolyl derivatives of both D- and L-valine and D- and L-leucine. Proline was chosen as the second component of the dipeptides because gramicidin contained no free amino groups. No antipodal specificity was observed in the growth inhibitory actions of the dipeptides and a similar result was observed for the corresponding phthalyl derivatives. The four diastereoisomeric leucyl-leucines were prepared by Fox, Kobayashi, Melvin and Minard[12], who considered D-leucyl-D-leucine of especial interest because of its relationship to D-valyl-D-valine, which had been isolated from partial hydrolysates of gramicidin. No appreciable activity was found in any of the dipeptides. Linnell and Smith[13] synthesised DL-Nβ-hydroxyethylalanine which combined the essential structural features of both alanine, the simplest amino-acid exhibiting optical isomerism, and also ethanolamine which had been isolated from gramicidin hydrolysates. The racemic compound possessed no growth inhibitory properties.

These results indicate that growth inhibitory properties are not concomitant with the presence of D amino-acids in a molecule. The available evidence supports the opinion of Work[14] that antibiotics containing D amino-acids are active, not because they have this character in common, but rather in virtue of their individual structures, of which the D amino-

acid must be regarded as an integral structural part. The toxicity of these antibiotics may be due to the possession of cyclic structures, such as have been suggested for gramicidin S and tyrocidin[14], or to some hitherto undiscovered features of the molecules. The mere presence of a D amino-acid in a molecule does not seem to be sufficient for the production of antibacterial properties in that molecule and some other structural feature or features appear to be necessary. An illustration of this consideration is the contrast between the thiazolidine-β-lactam system of the penicillins and the structure of D-penicillamine which has no antibacterial properties.

It therefore appeared of interest to prepare the optical isomers of a substance which combined the structures of an amino-acid and an established antibacterial nucleus. Comparison of the antibacterial activities of the two isomers would afford information relating to the importance of configuration of the amino-acid residue in such compounds. 5-Aminoacridine was chosen as the antibacterial nucleus and the compound DL-acridyl(5)-N.alanine was prepared in 96 per cent. yield, as a yellow powder, m.pt., 214°C., by the condensation of 5-chloracridine with DL-alanine in phenol solution.



Bacteriological testing of this compound was not possible because of its insolubility and the instability of its salts in aqueous media. A similar result had been reported for acridyl(5)-N.glycine by Dupré and Robinson[15]. Esterification with ethyl alcohol in the presence of dry hydrochloric acid gas gave an 80 per cent. yield of the racemic ethyl ester as small yellow prisms, m.pt. 75°C. The ester gave well defined crystalline compounds with picric and picrolonic acids and its diacetate and monohydrochloride were quite stable in aqueous solutions. A 0·1 per cent. aqueous solution of the diacetate possessed growth inhibitory activity against *Staphylococcus aureus* and *Streptococcus pyogenes*.

The racemic ester was resolved by the use of (+) tartaric acid. Hot absolute alcoholic solutions, containing equivalent quantities of the ester and (+) tartaric acid, on mixing gave an 87 per cent. yield of the racemic ester (+) tartrate. This salt was a deep yellow powder, m.pt., 165° C. $[\alpha]_D^{20°C.}$ + 18° (water, c = 4·0). Fractional crystallisation from alcohol (80 per cent.) gave the (+) ester (+) tartrate as long yellow needles, m.pt., 118° C. $[\alpha]_D^{20°C.}$ + 54·5° (water, c = 4·0). Evaporation of the mother liquors and repeated crystallisation of the residue from absolute alcohol gave the (−) ester (+) tartrate as a yellow powder, $[\alpha]_D^{20°C.}$ − 19·5° (water, c = 4·0).

The ester isomers were isolated by making aqueous solutions of the respective (+) tartrates alkaline with ammonia and extracting with benzene. They formed viscous yellow oils, (+) acridyl(5)-N.alanine theyl ester, $[\alpha]_D^{20°C.}$ + 128° (alcohol (96 per cent.), c = 2); (–) acridyl (5)-N.alanine ethyl ester, $[\alpha]_D^{20°C.}$ – 122° (alcohol (96 per cent.), c = 2).

Preliminary bacteriological tests on aqueous solutions of the (+) tartrates showed that these salts caused inhibition of growth in the dilutions shown in Table I.

TABLE I

ANTIBACTERIAL ACTIVITIES OF ISOMERS OF ACRIDYL(5)-N.ALANINE ETHYL ESTER

Organism	Racemic ester (+) tartrate	(+) ester (+) tartrate	(–) ester (+) tartrate
(1) *Staphylococcus aureus* ...	1/6000	1/6000	1/3000
(2) *Streptococcus pyogenes* ...	1/4000	1/4000	< 1/2000

Two conclusions may be drawn from these results. In the first instance, a small but significant difference does exist between the antibacterial activities of the two isomers and secondly, the introduction of the amino-acid residue has considerably reduced the activity of the parent amino-acridine. In order to provide a more definite answer to the query involved in this research, it will be necessary to find a compound in which no reduction of activity is caused by the introduction of the amino-acid residue. In fact, it would be an advantage if an enhancement of activity could be achieved. To this end it is intended to prepare β-acridyl-(5)-alanine and, if promising results are obtained, it is intended to extend the work to other amino-acids such as leucine, phenylalanine, etc.

EXPERIMENTAL

(1) *5-Chloroacridine.*—N.phenylanthranilic acid, prepared in 80 per cent. yield by the method of Allen and McKee,[16] was converted to 5-chloroacridine in 75 per cent. yield by heating with phosphorus oxychloride according to the directions of Magidson and Grigorowski.[17] Purification of the crude reaction product was effected by Soxhlet extraction with light petroleum (boiling-range 80° to 100° C.), the 5-chloracridine being obtained as flat yellow plates m.pt., 118° C.

(2) DL-*Acridyl*(5)-*N.alanine.*—10·7 g. of 5-chloracridine (1/20 mole) was mixed with 40 g. of phenol in a 250-ml. flask and the mixture heated in an oil bath to 80° C., when a yellow solution was formed. 8·9 g. (1/10 mole) of finely powdered alanine was added and the mechanically stirred mixture heated at 120° to 125° C. for 2 hours. It was then allowed to cool and poured, with stirring, into 500-ml. of ether. A brownish yellow oil was formed which quickly solidified to a hard yellow solid. This was filtered, powdered and triturated with successive quantities of 10 per cent. aqueous ammonia until the washings gave a negative test for chloride ion. It was insoluble in most organic solvents, with the except of glacial acetic acid, but could be recrystallised from

a large volume of alcohol (96 per cent.), being soluble about 1 in 500 in the boiling alcohol. The recrystallised material formed a yellow granular powder, m.pt., 214°C. (decomposes with effervescence). Yield 12·7 g. (95 per cent.). Found: C, 70·7 ; H, 5·48 ; N, 10·7 per cent. $C_{16}H_{14}N_2O_2$ requires C, 72·1 ; H, 5·26 ; N, 10·52 per cent.

(*a*) *Monoacetate.*—0·5 g. was dissolved in 5 ml. of cold glacial acetic acid and 20 ml. of ether was added. A yellow precipitate was obtained which on filtration and drying formed a light yellow powder. Yield: 0·42 g., m.pt., 226°C. (decomposes with effervescence). Found: C. 66·8 ; N, 8·7 per cent. $C_{18}H_{18}N_2O_4$ requires C, 66·3 ; N, 8·6 per cent.

(*b*) *Monohydrochloride.*—0·5 g. was dissolved in 10 ml. of hot absolute alcoholic hydrochloric acid and the solution filtered. Addition of 30 ml. of dry ether to the filtrate gave a yellow precipitate. This on drying formed a yellow powder which was easily soluble in water and gave a positive test for chloride ion. Yield: 0·3 g.; m.pt., 170°C. (decomposes with effervescence). Found: C, 62·8 ; N, 9·5 ; Cl, 12·0 per cent. $C_{16}H_{15}N_2O_2Cl$ requires C, 63·6 ; N, 9·24 ; Cl, 11·73 per cent.

Both the acetate and hydrochloride were easily water-soluble, but deposition of the free acid, which was practically insoluble in water, commenced after 24 hours and was almost complete after 96 hours.

3. DL-*Acridyl(5)-N.alanine ethyl ester.*—15 g. of DL-acridyl(5)-N. alanine were mixed with 300 ml. of absolute alcohol and the mixture refluxed for 90 minutes, while a stream of dry hydrogen chloride gas was passed. It was then poured into a mixture of 100 ml. of 10 per cent. aqueous ammonia and 100 g. of ice, when a yellowish brown oil was precipitated. This was extracted with successive quantities of chloroform, which were separated and dried over anhydrous sodium sulphate. Removal of the solvent gave a brown oily residue which crystallised from 70 per cent. aqueous alcohol as small light yellow prisms, m.pt., 75°C. Yield: 13·1 g. (80 per cent). Found: C, 71·4 ; H, 6·26 ; N, 9·25 per cent. $C_{18}H_{18}N_2O_2$ requires C, 73·4 ; H, 6·12 ; N, 9·5 per cent.

The substance was practically insoluble in water, but easily soluble in organic solvents and a dilute aqueous alcoholic solution gave a strong greenish fluorescence in visible and ultra-violet light.

(*a*) *Diacetate.*—1 g. of the ester was heated with 10 ml. of glacial acetic acid for 5 minutes on a boiling water-bath. Addition of 200 ml. of ether to the cold solution gave an almost immediate deposition of clusters of yellow needle crystals which were recrystallised from benzene. Yield: 1·1 g. m.pt., 117°C. Found: C, 63·75; H, 6·32; N, 6·97 per cent.: $C_{22}H_{26}N_2O_6$ requires C, 63·77 ; H, 6·28 ; N, 6·76 per cent.

(*b*) *Monohydrochloride.*—Cooling of the esterification reaction mixture caused the appearance of yellow needles of the ester hydrochloride which contained one molecule of alcohol crystallisation, m.pt., 193°C. Found: C, 61·3 ; H, 6·5 ; N, 7·85 per cent.; $C_{18}H_{19}N_2O_2Cl$, C_2H_5OH requires C, 62·1 ; H, 6·46 ; N, 7·6 per cent.

(*c*) *Picrate.*—This, and the two succeeding derivatives, were made by dissolving 0·2 g. of the ester in 10 ml. of alcohol (90 per cent.) and mixing the boiling solution with a solution of 0·3 g. of the respective nitro

compound in 10 ml. of alcohol. Yellow powder. Yield: 0·25 g. m.pt., 194°C. Found: C, 55·0; H, 4·07; N, 13·0 per cent. $C_{24}H_{21}N_5O_9$ requires C, 55·1; H, 4·0; N, 13·37 per cent.

(*d*) *Styphnate.*—Light yellow powder. Yield: 0·2 g. m.pt., 178°C. Found C, 53·1; H, 3·8; N, 13·2 per cent. $C_{24}H_{21}N_5O_{10}$ requires C, 53·4; H, 3·9; N, 13·0 per cent.

(*e*) *Picrolonate.*—Yellow powder. Yield: 0·25 g. m.pt., 227°C. (decomposed). Found: C, 59·4; H, 4·5; N, 14·2 per cent. $C_{27}H_{26}N_6O_8$ requires C, 57·5; H, 4·6; N, 14·9 per cent.

4. *Resolution of* DL-*Acridyl*(5)-*N.alanine ethyl ester.*

(*a*) DL-*Acridyl*(5)-*N.alanine ethyl ester* (+) *tartrate.*—8 g. of the racemic ester and 4 g. of (+) tartaric acid were dissolved in 100 ml. of hot absolute alcohol. On cooling small yellow crystals appeared. Yield: 10·5 g. (87 per cent.), m.pt., 165°C. (decomposes with effervescence), $[\alpha]_D^{20°C.}$ + 18° (4 per cent. solution in distilled water). Found: C, 57·7; H, 5·65; N, 6·2 per cent.; $C_{22}H_{24}N_2O_8$ requires C, 59·4; H, 5·4; N, 6·3 per cent. The optical rotation and melting-point remained unchanged after several recrystallisations from alcohol, but as the substance was easily soluble in water, fractional recrystallisation was attempted from a series of aqueous alcohols. It was found that alcohol (80 per cent.) caused the preferential separation of the (+) ester (+) tartrate.

(*b*) (+) *Acridyl*(5)-*N.alanine ethyl ester* (+) *tartrate.*—20 g. of the racemic ester (+) tartrate were dissolved in 400 ml. of hot aqueous alcohol (80 per cent.). Long yellow needle crystals slowly formed and these were filtered after 48 hours' standing. Yield: 7·1 g. m.pt., 118°C. $[\alpha]_D^{20°C.}$ + 54·5° (4 per cent. solution in distilled water). The melting-point and optical rotation remained constant after several recrystallisations from the same solvent and it was considered that this material was the (+) ester (+) tartrate.

(*c*) (+) *Acridyl*(5)-*N.alanine ethyl ester.*—2 g. of the (+) tartrate salt were dissolved in 50 ml. of distilled water. 30 ml. of benzene was added and then 1 per cent. aqueous ammonia drop by drop. After each addition of the ammonia the yellow precipitate formed was shaken into the benzene. When no further precipitation occurred the benzene layer was separated and dried for 24 hours over anhydrous sodium sulphate. Removal of the solvent under reduced pressure left a yellow viscous oily residue. Yield: 1·05 $[\alpha]_D^{20°C.}$ + 128° (2 per cent. solution in alcohol (96 per cent.). Found: C, 74·4; H, 6·5; N, 9·02 per cent. $C_{18}H_{18}N_2O_2$ requires C, 73·4; H, 6·12; N, 9·5 per cent. The (+) ester gave a crystalline picrate, m.pt., 175°C. (m.pt. of racemic ester picrate, 194°C.) Found: C, 54·5; H, 3·65; N, 13·3; $C_{24}H_{21}N_5O_9$ requires C, 55·1; H, 4·0; N, 13·37 per cent.

The use of chloroform instead of benzene in the preparation caused complete racemisation of the ester.

(*d*) (−) *Acridyl*(5)-*N.alanine ethyl ester* (+) *tartrate.*—The mother liquid from the crystallisation of the (+) ester (+) tartrate was allowed

to remain at room temperature for a further 48 hours and 1·5 g. of a yellow powder, m.pt., 111° to 113°C., $[\alpha]_{D}^{2\ °C.}$ + 30·5°, was obtained by filtration. The solvent was removed from the filtrate and the yellow viscous residue repeatedly crystallised from absolute alcohol until a constant value for the optical rotation was obtained. The material formed a yellow powder which did not possess a sharp melting-point, the substance softened gradually from 80°C. onwards. $[\alpha]_{D}^{20°C.}$ – 19·5° (4 per cent. solution in distilled water.)

(*e*) (–) *Acridyl*(5)-*N.alanine ethyl ester.*—This was isolated from the (–) ester (+) tartrate by a similar procedure to that used for the (+) ester. It was a yellow viscous oil, $[\alpha]_{D}^{20°C.}$ – 122° (2 per cent. solution in alcohol (96 per cent.)).

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PALTHÉ SENNA AS AN ADULTERANT OF INDIAN SENNA LEAVES

By J. L. Forsdike

Received September 27, 1948

During recent months, a considerable quantity of Indian senna leaves adulterated with a foreign leaf has been offered for sale in this country. This adulteration has ranged from 5 to 10 per cent. in better quality leaves to upwards of 90 per cent. in some cheaper samples. The adulterant has been identified, by comparison with authentic specimens from the Herbarium of the Pharmaceutical Society of Great Britain, as the leaf of *Cassia auriculata* Linn., commonly known as Palthé Senna. The substitution of this leaf for senna has been previously reported[1], a colour reaction for its detection has been suggested by Vamossy[2] and some description of the leaves is given in Thoms' Handbuch[3] and by Wasicky[4]. However, since this adulteration appears to be occurring on a somewhat extensive scale, it was thought that some more detailed investigation, particularly of the anatomy of Palthé senna might be of value.

Material

The following description is based on leaflets taken from two sheets in the Herbarium of the Pharmaceutical Society of Great Britain marked:—

(1) Cassia auriculata, Deccan. Dr. T. Cooke

(2) Cassia auriculata, January, 1888. Rajputana. Coll. J. G. Prebble,

and on leaflets picked out from nine commercial samples, offered for sale as Tinnevelly senna.

Macroscopical Characters

The leaflets are oblong or obovate and generally smaller than those of genuine senna, being 8 to 30 mm. long and 4 to 15 mm. wide, yellowish to greyish-green, sometimes with a purple tinge; they are thin and brittle; the margin is entire; the apex blunt and mucronate and the base unequal, with a very short stalk (Fig. 1, A.). Both surfaces of the leaf bear numerous hairs, visible under a hand lens; the veins are more prominent on the under surface.

Microscopical Characters

Upper Epidermis. Polygonal prisms, with nearly straight anticlinal walls and a thin cuticle; many cells containing mucilage attached to the inner periclinal wall; this mucilage staining with ruthenium red. (Fig. 1, B, ep_1; C.) Stomata, 100 to 200 per sq. mm., sunk below the general epidermal level, rubiaceous, commonly having one subsidiary cell much larger than the other. (Fig. 1, B, C, st.) Trichomes 130 to **240** to **650** to 830 microns long and 14 to 22 microns wide, conical, unicellular, thick-walled, with a pointed apex and only very slightly warty cuticle. (Fig. 1, B, D, t.)

Lower Epidermis. Similar to the upper, except that the cells are generally rather smaller, the anticlinal walls are sometimes slightly wavy and the stomata are more numerous, 130 to 260 per sq. mm. (Fig. 1, B, ep_2 : D.).

Mesophyll. Dorsiventral, the palisade on the upper surface consists of two layers of cells, the cells of the upper layer being very elongated, up to ten times as long as they are wide. (Fig. 1, B, p_1, p_2.) The spongy mesophyll consists of 3 to 4 layers of cells, the lowermost layer being often slightly elongated at right angles to the epidermis. (Fig. 1, B, s.)

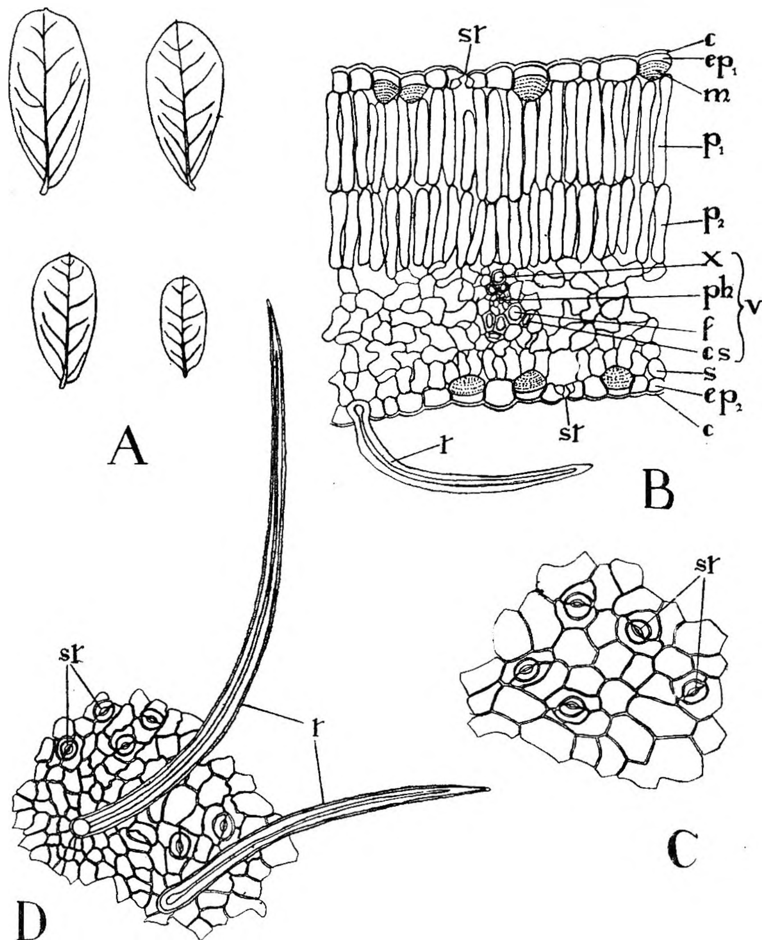

FIG. 1. *Cassia auriculata.*—A, whole leaflets, natural size. B, transverse section of lamina; C, upper epidermis, surface view; D, lower epidermis, surface view; c, cuticle; ep_1, upper epidermis; ep_2, lower epidermis; m, mucilage; p, upper layer of palisade; p_2, lower layer of palisade; x, xylem; ph, phloem; f, fibres; cs, crystal sheath; s, spongy mesophyll; st, stomata; t, trichomes; v, veinlet. All x 150, except A.

Cluster crystals of calcium oxalate, 6 to **8** to **15** to 20 microns in diameter occur, but are not common, being usually found along the main veins and most frequent in the neighbourhood of the midrib. Both layers of palisade are continuous over the midrib.

Stelar Tissues. The midrib consists of a radiate xylem, containing

annular, reticulate and pitted vessels, below which are groups of sieve tissue. There is an arc of pericyclic fibres below the phloem and a group of fibres above the xylem. The fibres are surrounded by a sheath of cells, each containing a single prism of calcium oxalate, 7 to **10** to **20** to 24 microns long by 4 to **5** to **8** to 10 microns wide. Both xylem vessels and fibres have lignified walls. The midrib projects slightly on the under side of the leaf, the projection being filled with collenchyma.

The large veins form a network and are accompanied by a complete crystal sheath, similar to that described for the midrib. In the smaller veinlets the complete sheath is lacking, but occasional prisms of calcium oxalate are found along these veinlets. (Fig. 1, B, v.)

Quantitative Data

Stomatal Index. The stomatal indices of Palthé senna and of the official sennas are:—

Cassia auriculata 7·1 to **9·0** to **13·0** to 14·5
Cassia acutifolia 8·1 to **10·0** to **14·0** to 15·5 (Rowson[5])
Cassia angustifolia 15·6 to **16·0** to **21·5** to 22·7 (Rowson[5]).

The stomatal index will thus serve to distinguish Palthé senna from Indian senna, but not from Alexandrian senna.

Vein-islet Number. The vein-islets numbers of the three sennas are:—
Cassia auriculata 18 to 26.
Cassia acutofolia 25 to 20 (Wallis[6])
Cassia angustifolia 20 to 23 (Wallis[6]).

This feature is thus available to distinguish *C. auriculata* from Alexandrian senna, but will not give a distinction from Tinnevelly senna.

Palisade Ratio. The palisade ratio of *Cassia auriculata* was determined under the same conditions as those used by George[7] for genuine senna. The results are:—
Cassia auriculata 2·5 to **4·5** to 8·0
Cassia acutifolia 3·5 to **8·0** to 18·0 (George[7])
Cassia angustifolia 2·5 to **5·6** to 12·0 (George[7]).

George reports that an average of 20 determinations on Alexandrian senna will give a result of over 7·5. On Palthé senna an average of 20 determinations may be expected to give a result of less than 6·0, so that palisade ratio determinations serve as a ready means of distinguishing these two species. The values obtained for *C. auriculata* and *C. angustifolia* are, however, too close for palisade ratios to be of much value in differentiating between these species.

Colour Reactions

Sulphuric Acid (*80 per cent. v/v*). As first reported by Vamossy[2], Palthé senna leaves give a crimson colour with 80 per cent. v/v sulphuric acid. This test may be carried out by sprinkling the broken leaves, or powder, on to the surface of the acid in a test-tube. When examining powdered senna for possible adulteration with Palthé senna, the test is best carried

out by mounting the powder in 80 per cent. v/v sulphuric acid and examining microscopically. Treated in this way, particles of Palthé senna show a brilliant crimson colour and 2 per cent., or less, of this leaf may be detected in admixture with genuine senna.

Chloral Hydrate. Heating Palthé senna leaves, whole or in powder, with solution of chloral hydrate (5 in 2), in a boiling water-bath also results in a crimson colour, changing to brownish-red on longer heating.

Bornträger's Reaction. Palthé senna leaves give no rose-red colour with Bornträger's reaction, either direct or after hydrolysis by boiling with dilute sulphuric acid. It was concluded from this that the leaves contain no anthraquinone derivatives; a fact which is confirmed by Wasicky[4]. It should be noted, however, that Maurin[8] reported finding 0·7 per cent. of oxymethylanthraquinones in the leaves of *C. auriculata* and 1·9 per cent. in the stem bark.

Summary

The principal characters which serve to differentiate Palthé senna from the official sennas, particularly in the broken or powdered condition, are:—

1. The trichomes, which are about three times as long as those of genuine senna and lack a conspicuously warty cuticle.
2. The presence of two layers of palisade cells on the upper side of the leaf and none on the lower.
3. The cluster crystals being usually along the main veins, whereas the clusters of genuine senna are not found in the neighbourhood of the veins.
4. The presence of occasional prisms of calcium oxalate on the smaller veinlets, where they are lacking in the official sennas.
5. The palisade ratio, stomatal index and vein-islet number.
6. The colours produced by 80 per cent. v/v sulphuric acid and chloral hydrate.
7. The absence of anthraquinone derivatives, resulting in the leaf giving a negative result with Bornträger's reaction.

I wish to thank Dr. T. E. Wallis, the Curator of the Pharmaceutical Society's Museum, for the supply of authentic material and also for calling my attention to some of the literature cited. I am indebted to the Directors of Boots Pure Drug Company, Ltd., in whose Analytical Laboratories the work was carried out, for permission to publish this paper.

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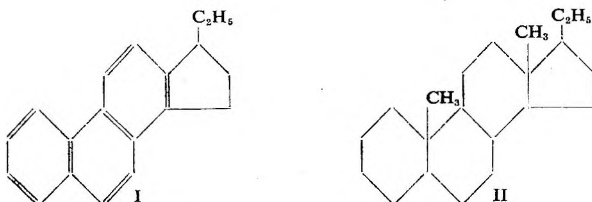
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Conessine, Constitution of. R. D. Hayworth, J. McKenna and N. Singh. (Nature, 1948, **162**, 22.) Careful fractionation of the selenium dehydrogenation products of the hydrocarbon mixture $C_{21}H_{30}$, from pyrolysis of conessine dihydriodide, yielded a crystalline hydrocarbon $C_{19}H_{18}$, m.pt. 78° to 79°C., which showed many similarities with synthetic 3-ethyl*cyclo*-pentenophenanthrene (I), m.pt. 84·5° to 85·5°C., although the identity was not convincingly established.



Degradation of conessine by the Hofmann and Emde processes gave a hydrocarbon $C_{21}H_{30}$ which on reduction yielded approximately equal amounts of two isomeric hydrocarbons $C_{21}H_{36}$, m.pt. 56° to 58°C. and 83° to 84°C. The latter isomer is identical chemically and physically with *allo*pregnane (II) prepared from progesterone. The carbon atoms of conessine are therefore accounted for by the *allo*pregnane structure (II) and the three N-methyl groups, but the positions of the ethylenic linkage and the points of attachment of the basic centres are still uncertain. R. E. S.

Iodosalicylates of Alkaloids. M. Covello and A. Capone. (*Ann. Chim. appl., Roma*, 1948, **38**, 123.) 3-Iodosalicylic acid and 5-iodosalicylic acid were compared with picric acid and styphnic acid as reagents for the detection of alkaloids. In general the solubilities of the salts are greater than those of the corresponding picrates and styphnates, that is, the reaction is less delicate. Pilocarpine did not precipitate in 10 per cent. solution and codeine only in solutions stronger than 0·7 per cent; these salts may be useful for hypodermic use. The 3-iodosalicylates of brucine (m.pt. 224°C. decomp.), quinine (m.pt. neutral 164° to 164·5°C., basic 156°C. decomp.) cinchonine (m.pt. 167·5° to 168°C.) morphine (m.pt. 194° to 194·5°C. decomp.) and strychnine (decomposes at about 192°C.) were prepared and illustrations of the microscopic appearance of the crystals, which may be useful for identification of the alkaloids, are given.

H. D.

ANALYTICAL

Adrenaline, Determination of, with Iodine. J. Ehrlen. (*Farm. Revy*, 1948, **47**, 321.) The photometric determination is usually carried out at *p*H 4 to 7. At a higher *p*H the colour rapidly fades owing to further oxidation; at a lower *p*H the colour development is very slow. By using oxidising agents such as potassium ferricyanide, adrenochrome is produced with an absorption maximum at 485 mμ. With iodine, however, a mixture of adrenochrome and iodoadrenochrome is obtained and the absorption maximum may be anywhere

between 485 and 525 mμ. The more acid the solution, the greater the proportion of iodoadrenochrome. The mixed colour has sometimes been considered incorrectly as a variation of colour with the pH. In the method of Thorvik, carried out at pH 4·8, the adrenaline is converted almost quantitatively into iodoadrenochrome. Other oxidising agents simplify the course of the reaction; potassium ferricyanide is very suitable. At pH 6 the oxidation velocity is fairly high and nearly quantitative, corresponding to a calculated molar extinction coefficient of 4250 at 485 mμ. The reaction of the solution is then adjusted to pH 3 to 4, when the adrenochrome is very stable. An advantage of this method is that, in presence of procaine, no precipitate is formed. When iodine is used, the extinction curves for adrenochrome and iodoadrenochrome cut one another at 525 mμ and at this point the result is independent of the proportions of the two compounds formed. G. M.

Adrenaline, Fluorimetric Determination of. J. Ehrlen. (*Farm Revy.*, 1948, **47**, 242.) The method, which is applied particularly to procaine solutions, is a development of that recently published by the author, and the fluorescence is now measured photometrically, using a filter (maximum transmission at 405 mμ: cut-off at 480 mμ) in the incident light, and another filter (not transmitting below 500 mμ) between the sample and the photo cell. The fluorescence results from atmospheric oxidation in alkaline solution, but is rapidly destroyed by further oxidation. It is suggested that the fluorescent compound is 1-methyl-3 : 5 : 6-trihydroxyindol, formed directly from adrenochrome. Under the conditions given by the author, the oxidation of adrenaline to adrenochrome is quantitative, and in the next stage the addition of a reducing agent prevents the further oxidation of the fluorescent compound during the assay. Details are as follows. A sample containing 3 to 30 μg. of adrenaline and 10 to 50 mg. of procaine is diluted with water to 3 ml. and mixed with 0·1 ml. of 0.1M hydrochloric acid and 0·5 ml. of 0·15M (2 per cent.) sodium acetate solution. To this is added 1·0 ml. of a 0·5 per cent. solution of potassium ferricyanide. After two minutes the mixture is made up to 25 ml. with a mixture of 5 ml. of 5M sodium hydroxide, 5 ml. of alcohol (95 per cent.), 10 ml. of water, and 0·5 ml. of a 5 per cent. solution of ascorbic acid. The solution is transferred to a sample holder, and the fluorescence is read off after 15 minutes. A blank on the reagents is also done. The standardisation should be carried out on solutions and under conditions similar to those of the assay. G. M.

Alkyl Nitrates, Determination of, in Pharmaceutical Preparations. P. Lundgren and T. Canbäck. (*Svensk farm. Tidskr.*, 1948, **52**, 298, 313, 333.) Although the phenoldisulphonic acid method of determination of nitrates is in general satisfactory, it has certain limitations. In particular it is not suited for the determination of alkyl nitrates in oil solutions or in ointments. These limitations do not apply to *m*-xylenol-(4-hydroxy-1 : 3-dimethylbenzene), which is nitrated to *o*-nitroxylenol(5-nitro-4-hydroxy-1 : 3-dimethylbenzene). A photometric determination of the dissociation constant of the nitro compound showed that it had a value for pk_A of 7·98 ±0·04. This indicates that to develop the full colour the pH of the solution must be not less than 11. The absorption curve has two peaks, at 268·5 and 396 mμ. A detailed examination of the reaction, as applied to the determination of glyceryl trinitrate, mannityl hexanitrate, sorbityl dinitrate, and pentaerythrityl tetranitrate showed that these compounds were quantitatively hydrolysed under the conditions chosen. The nitration of the *m*-xylenol gave an 82 per cent. yield of the nitro compound, and the recovery of the latter by steam distillation was 95 per cent. Thus

an accurate analysis may be obtained by using a standard curve prepared from potassium nitrate. For tablets it is necessary to adjust the quantities according to the following table.

Substance	Wt. per tablet	*a* mg.	*b* ml.	*c* ml.	*d* ml.	*e* mg.	Solvent
Glyceryl trinitrate	0·00025—0·001	1·5—4	10	50·0	5·00	0·15—0·40	ether
Mannityl hexanitrate	0·005 —0·015	7·5—20	20	100·0	2·00	0·15—0·40	ether
Pentaerythrityl tetranitrate	0·030	15—40	20	100·0	1·00	0·15—0·40	acetone
Sorbityl dinitrate	0·002—0·010	6—15	10	50·0	2·00	0·25—0·60	ether

A weight of powdered tablets corresponding to *a* mg. of alkyl nitrate is extracted with *b* ml. of solvent for some minutes, and the solution is decanted through a filter paper, the extraction being continued with three more quantities of solvent, and the combined solution is made up to *c* ml. An aliquot of *d* ml. (corresponding to *e* mg. of alkyl nitrate) is transferred to a 250-ml. beaker, evaporated in a current of cold air, and treated with 1·0 ml. of a 2 per cent. solution of *m*-xylenol in acetone and 10·0 ml. of 72 per cent. sulphuric acid. The beaker is covered and left for 30 minutes, after which time the reaction is stopped by adding 50 ml. of water. The mixture is then distilled into a cylinder containing 5 ml. of N/1 sodium hydroxide, the distillation being carried out slowly, taking in all about 5 to 8 minutes, and stopped when 15 ml. have distilled over. The product is made up to 50 ml. and the extinction determined at 447 mμ. The percentage of nitrate is determined from a standard curve obtained with potassium nitrate. If necessary, the ether solution may be washed with sodium sulphate solution before the hydrolysis to remove interfering substances. In the case of sorbityl dinitrate, white flecks may appear in the receiver and it is necessary to filter the solution before making up to volume. For ointments the method is similar, but it is necessary to agitate the mixture from time to time during the hydrolysis. Ointment bases such as soft paraffin or lanoline do not interfere. G. M.

Bismuth, A New Reaction of. M. Jean. (*C.R. Acad. Sci., Paris,* 1948, **226,** 85.) To 1 ml. of a slightly acid solution of bismuth nitrate (containing about 0·2 mg. of bismuth) is added 10 ml. of a solution containing 0·32 g. of rubianic acid and 6·5 g. of silicotungstic acid per l. A white turbidity is produced. On heating for 10 minutes in a water-bath a brown colour appears, proportional to the amount of bismuth. The optimum acidity for the reaction corresponds to 0·1N nitric acid ; the limits being from 2N to *p*H3. A distinct reaction is obtained with 20 μg. of bismuth at a dilution of 1 in 100,000. Interference is produced by metals which react with rubianic acid (copper, nickel, cobalt, ruthenium and platinum) or with silicotungstic acid (caesium), also by silver, mercury, zinc, cadmium and antimony. Chlorides weaken the reaction; acetates and tartrates prevent it. The reaction may be used for colorimetric determination. G. M.

Calcium and Magnesium, in Solutions for Hypodermic and Intravenous Use, Determination of. R. Vigni. (*Ann. Chim. appl., Roma,* 1948, **38,** 133.) Solutions of calcium and magnesium thiosulphates are used parenterally. To determine the amounts of the bases present in these solutions, the calcium is precipitated by adding a large excess of ammonium chloride, heating to boiling, making alkaline with ammonia and adding ammonium oxalate; the precipitate

is washed 8 or 10 times with ammoniacal water, heated to 40°C. for 2 hours to remove free ammonia, dissolved in dilute sulphuric acid and titrated with permanganate. The precipitate is free from magnesium, which will all be in the filtrate and washings. These are mixed, heated to 80°C. and a slight excess of the reagent recommended by Autenrieth added, which precipitates the magnesium as ammonio-magnesium phosphate. After 3 or 4 hours, the precipitate is washed by decantation with 2·5 per cent. ammonia, dried at 40°C. for 2 hours to remove excess of ammonia, and titrated with N/1 hydrochloric acid, using methyl orange as indicator, until an orange colour is obtained; 1 ml. of N/1 hydrochloric acid equals 0·01216 g. of magnesium. H. D.

Diamidines, Amperometric Microtitration of. J. B. Conn. (*Anal. Chem.*, 1948, **20,** 585.) A number of diamidines were observed to give highly insoluble red alizarinsulphonates in neutral solutions, but attempts to use the reaction colorimetrically or gravimetrically failed; an amperometric titration procedure was successful. Alizarinsulphonic acid is polarographically reducible, the half-wave potential being −0·67 volt (against the saturated calomel electrode) and at −0·90 volt a steady diffusion current is reached. When a solution of diamidine salt in neutral buffer was titrated with a solution of sodium alizarinsulphonate a plot of the diffusion current at −0·90 volt against reagent volume could be resolved into two straight lines intersecting at a 1 to 1 equivalence point. The diamidines studied were (1) 4:4′-stilbenedicarboxyamidine diisethionate (stilbamidine), (2) 4:4′-oxydibenzamidine dihydrochloride (phenamidine), (3) *p,p*-(trimethylenedioxy) dibenzamidine dihydrochloride (propamidine), and (4) 4:4′-(pentamethylenedioxy) dibenzamidine dihydrochloride (pentamidine). Graphs are given for the titration of these compounds, and also for their decomposition under sterilisation conditions. The overall reproducibility found was ±0·5 per cent., the greatest spread of results being 1·5 per cent. for phenamidine.

R. E. S.

Digitoxin, Colorimetric Assay. A. T. Warren, F. O. Howland and L. W. Green (*J. Amer, pharm, Ass., Sci. Ed.*, 1948, **37,** 186.) In alkaline solution digitoxin gives a purple colour with sodium-β-naphthaquinone-4-sulphonate which changes to yellow on acidification. The yellow colour is stable and can be measured photometrically. Procedures are described for detecting digitoxin and for estimating it in powder and tablets. Interfering substances include lanatoside A and lanatoside C, which give strongly positive tests and lanatoside B and gitoxin, which give weakly positive tests; digitonin gives a negative result. The intensity of the colour is increased by aldehydes, necessitating the use of aldehyde-free alcohol. The effect of lactose in inhibiting the formation of the purple colour was avoided in the assay of tablets by extracting with chloroform; no other excipient interfered. Results of the assay of 5 samples of digitoxin powder and 8 samples of tablets, indicate the repeatability of the method, which should be useful for rapid routine analysis. G. R. K.

Fatty Acids of Intermediate Chain Length, Estimation by Partition Chromatography. M. H. Peterson and M. J. Johnson. (*J. biol. Chem.*, 1948, **174,** 775.) The partition chromatograms used consisted of tubes packed with a coarse diatomaceous earth moistened with water or aqueous sulphuric acid as the non-mobile phase. As the developing solvent, thiophene-free benzene alone and mixed with Skellysolve B, and butanol-

chloroform mixtures were used. Sulphuric acid (27 to 35N) was found to be a better solvent and a better non-mobile phase than water; using both these solvents quantitative separation of formic, acetic, propionic, *n*-butyric, caproic, caprylic, and capric acids is possible. Detailed procedure is given for the quantitative analysis of fatty acids in biological materials. Fatty acids, in known mixtures or fatty acids added to butter fat, were recovered with a maximum error of 8 per cent. R. E. S.

Frangula Bark, Assay of. K. Erne. (*Svensk farm. Tidskr.* 1948, **52**, 345, 377.) The method adopted for determining the biological activity of frangula bark was as follows: White mice were starved for 15 hours, and varying doses of the bark mixed with soft cheese, malt extract and water (8 + 8 + 1) were given. Observations were made after 4 hours. A positive reaction was shown by diarrhœa with copious yellowish-brown excretions. Each sample was tested on 3 groups of about 20 animals, each group receiving a different dose. The results were compared with those obtained with a sample which was taken as standard. Chemical tests were as follows. Total anthraquinones: 0·1 g. of the bark was refluxed with 30 ml. of M/2 sulphuric acid for half an hour. The mixture was then extracted in a separating funnel with successive 25-ml. quantities of ether until all the colour was extracted. The ether solution was filtered, and shaken out with 20-ml. quantities of M/2 sodium hydroxide. The alkaline solution was acidified with sulphuric acid and re-extracted with ether. The filtered ethereal extract was shaken with M/4 lead acetate until no more dark precipitate was formed, then extracted with M/2 sodium hydroxide, and the extract was made up to 50 ml. with the alkali. Ten ml. of this solution was diluted to 50 ml. with M/2 sodium hydroxide and, after 20 minutes, the extinction was determined at 530 mμ. The extinction coefficient for emodin is 3·88. Free anthraquinones: These were determined as above with the omission of the acid hydrolysis. Anthranols: 0·1 g. of the bark was refluxed with 10 ml. of M/2 sulphuric acid for 15 minutes, and the mixture extracted with 10-ml. quantities of ether until all the colour was extracted. The ether solution was dried with sodium sulphate, filtered, and passed through an alumina column (Brockmann) containing 5 g. of alumina. After developing the chromatogram with 50 ml. of dry ether, the yellow fluorescent zone was separated and treated with a few drops of a solution of 0·5 g. of selenious acid in concentrated sulphuric acid. A greenish black colour showed the presence of anthranols. The approximate percentage was determined by acid hydrolysis of the glycosides, extraction with benzene and petroleum ether, and chromatographing on calcium and magnesium carbonates, the anthranols being finally isolated and oxidised to anthraquinones which were determined photometrically. The results of the chemical and biological tests were not closely parallel, but it is considered that the chemical test is able to estimate the clinical value of the bark within about 20 per cent. The results obtained do not show any definite relation between bark thickness and physiological activity, although for a thickness below 0·7 mm. the activity was proportional to the thickness, and above this value the activity appeared to be constant, although for one sample of bark there was a marked peak at 2·5 mm. Thus the view that there is a maximum activity in bark from stems of 3 to 4 years old is not confirmed. There was in general no inequality between barks of the official thickness (1·5 mm.) and thicker ones. Anthranols may be present even in samples which have been heated at 100°C and then stored for one year. An official

test for anthranols would appear to be desirable. Heating at 100°C. for one hour had no deleterious effect on the potency, but stabilised the anthraquinone content. Such a treatment might well be substituted for that of storage for one year. G. M.

Morphine, Electrophotometric Determination of. Laura Nicolini. (*Ann. Pharm., Fr.*, 1947, **5**, 528.) The official colorimetric method of the British Pharmacopœia for the determination of morphine is criticised. In the search for a reaction specific for morphine, which allows determination of the alkaloid without previous extraction, the author adapted the colorimetric method of Guarino. A solution, containing from 5 to 10 mg. of morphine, was placed in a 50 ml. flask and 10 ml. of N/10 hydrochloric acid added. The mixture was shaken, while 10 ml. of a 1 per cent. solution of iodic acid was added, followed after exactly 30 seconds, by 15 ml. of a saturated solution of ammonium carbonate. The mixture was allowed to stand for a short while with occasional shaking. The volume was adjusted with the solution of ammonium carbonate and several drops of a 0·2 per cent. solution of ferric chloride, acidified with N/5 hydrochloric acid, were carefully added drop by drop, till no further colour change took place. Excess of ferric chloride must not be added, since it causes precipitation of a hydrate or the iodide, which colours the violet solution yellow and would interfere with the electrophotometer reading. The assay was continued by Guarino's method, and colour intensity was estimated photometrically. Opium had first to be extracted by triturating with N/10 hydrochloric acid, and shaking for 20 minutes, before using this method. The photometer readings plotted against the content of morphine in mg., gave a straight line and obeyed Lambert-Beer's law. L. H. P.

Senna Glycosides, Colorimetric Estimation of. By W. Kussmaul and B. Becker. (*Helv. chim. Acta.*, 1947, **30**, 59.) A satisfactory and reproducible colour for the estimation of the glycosides sennoside A and B can be produced as follows: the aglycone is obtained by heating 10 ml. of a 0·5 to 1·0 per cent. alkaline solution of glycoside with 5 ml. of concentrated hydrochloric acid on a steam bath for about 15 minutes, when precipitation should be complete. After cooling, the precipitate is redissolved in concentrated sodium hydroxide, and the solution is extracted with 80 ml. of ether in a separating funnel, and then acidified with 50 per cent. sulphuric acid. The yellow ethereal layer is run off, any further precipitate in the aqueous layer is dissolved with more sodium hydroxide and the solution is shaken with 40 ml. of ether. The ether-fraction is next shaken with 3 or 4 quantities, each of 5 ml., of sodium bicarbonate solution. The mixed sodium bicarbonate solution is then extracted with 60 ml. of ether and acidified with 50 per cent. sulphuric acid. The aqueous layer is twice extracted with 20 ml. quantities of ether. Any deposit should be redissolved by the addition of alkali before shaking with ether. The mixed ether extracts are then filtered and the filtrate should be used within 10 hours. 5 ml. of the filtered solution is then extracted with 10 ml. of N/1 caustic soda. It is then treated with 0·2 ml. of 3 per cent. hydrogen peroxide and gently heated for about 4 minutes. A standard colorimeter curve is prepared by dissolving 30 mg. of pure sennoside B (dried in vacuo at 80°C.; equivalent to 18·75 of aglycone) in about 50 ml. of water with aid of a few drops of alkali. 10 ml. of this solution is hydrolysed with hydrochloric acid as above and 5 ml. of the resulting solution is oxidised as above. This solution is estimated in either a Zeiss-Pulfrich Photometer or a " Weka " Havemann photoelectric

colorimeter and the readings are transferred to a graph which for both sennoside A and B is a straight line passing through zero. A. D. O.

Sodium Diethyl- and Phenylethylbarbiturates, Acidimetric Titration of. H. Baggesgaard-Rasmussen and F. Reimers. (*Dansk Tidsskr. Farm.*, 1948, **22**, 166.) Determination of the acidity exponents for diethylbarbituric acid and phenylethylbarbituric acid in aqueous alcohol gave the following figures. Diethylbarbituric acid, *p*k8·96 (alcohol 50 per cent.) ; 9·54 (alcohol 75 per cent.) ; phenylethylbarbituric acid *p*k8·61 (alcohol 50 per cent.), 9·20 (alcohol 75 per cent.). Since the barbituric acids are weaker in dilute alcohol than in water, the end point is sharper and there is no precipitation of the free acid during titration. The titration of the sodium salts is carried out as follows. 0·100 g. of sodium diethylbarbiturate or 0·1250 g. of sodium phenylethylbarbiturate is dissolved in a mixture of 10 ml. of alcohol (86 per cent. by weight) and 5 ml. of water, and titrated with aqueous N/10 hydrochloric acid to a green colour, using bromophenol blue as indicator. G. M.

Sulphonamides, Microscopic Identification of. G. L. Keenan. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 202.) Optical crystallographic data and microchemical tests are described for sulphanilamide, sulphadiazine, sulphapyridine, sulphathiazole, sulphaguanidine and sulphamerazine, together with optical crystallographic data of certain of the complexes obtained in the microchemical tests. The tests described are based upon complexes formed with the following reagents: for sulphanilamide, aromatic aldehydes and silver nitrate; for sulphadiazine, gold bromide and hydrochloric acid; for sulphapyridine, gold chloride; for sulphathiazole, picrolonic acid; for sulphaguanidine, nitric acid; and for sulphamerazine, gold chloride and sodium bromide, and picrolonic acid. G. R. K.

Sulphur in Organic Compounds, Microdetermination of. A. Steyermark, E. Bass and B. Littman. (*Anal. Chem.*, 1948, **20**, 587.) Sulphur-containing organic compounds which gave low results by some micro-analytical methods were analysed satisfactorily by burning, following the Carius method, after which the resulting sulphate was titrated with barium chloride by the tetrahydroxyquinone indicator technique. R. E. S.

Tragacanth, Powdered, Evaluation of. Report No. 1 of the Tragacanth Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts. (*Analyst*, 1948, **73**, 368.) This report deals with the measurement of the viscosity of mucilages made from the powdered gum. The falling sphere method and the U-tube method were rejected as being unsuitable or difficult to apply, and a method is recommended using a Redwood No. 1 viscometer. The principle of determining the concentration of the dry gum that would be required to produce a mucilage with a corrected efflux time of 100 sec. for 50 ml. of mucilage at 20°C. was adopted. Details of preparation of the mucilage, determination of viscosity and method of calculation are given. The moisture content is determined by drying about 1 g. of the powder in an open dish to constant weight in a steam-heated oven. R. E. S.

Water, Detection of. P. Boymond. (*Pharm. Acta Helvet.*, 1948, **23**, 207.) A powder which may be used for the detection of water is composed as follows: bromophenol blue, 5 parts; sodium carbonate, anhydrous, 15 parts; starch, 40

parts; tragacanth, 40 parts. This is dusted on the concave face of a watch-glass which has previously been smeared with a thin layer of soft paraffin. The watch-glass is placed on a crucible containing the material to be tested. On heating the bottom of the crucible with a small flame, the presence of water is shown by a violet colour appearing on the watch-glass. The method will detect 2 mg. of water. The reaction is not given by organic liquids, and it may be used to detect water in pomades and other galenical preparations. G. M.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Potato Starch, Fractionation of. Part IV. Absorption Spectra and Colour Intensities of the Starch-Iodine Complexes. L. H. Lampitt, C. H. F. Fuller and N. Goldenberg. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 97.) Over the range 2400 to 7500 Å, the absorption spectra of the starch-iodine complexes of potato starch and its cold-water-soluble and hot-water-soluble fractions are very similar qualitatively to those of wheat starch fractions, the visible part of the spectrum (4300 to 7500 Å) being most sensitive to any changes in the starch. Depolymerisation by grinding lowers absorption only at the longer visible wave-lengths; at 5700 Å the effect, if any, is very small and disappears over the range 4300 to 5300 Å. Depolymerisation by grinding appears to consist in disruption of the unbranched parts of the amylopectin molecules and of the unbranched parts of the amylose molecules. The cold-water-soluble fractions of potato starch are richer in amylose and poorer in amylopectin than the hot-water-soluble fractions; in wheat starch fractions, the reverse is the case. The greater solubility of potato starch amylose in cold water is attributed to its lower molecular weight. The different behaviour of wheat starch is due either to the absorption by the amylose of the lipids present in the starch or to increased association between the amylose and amylopectin by means of hydrogen bonding, or to a combination of these factors. G. R. K.

INORGANIC

Clays, Effect of the Absorption of Sulphur on the Colloidal Properties of. A. Malquori. (*Ann. Chim. appl., Roma*, 1948, **38**, 137.) The thixotropic index of clays, measured by Winkler's method, is increased by heating them with sulphur. The clay is thoroughly mixed with flowers of sulphur and heated in a tube for one hour at 130° to 140°C. For comparison the original clay is similarly treated without sulphur. The index increases up to a point with increased quantities of sulphur and then falls off, but the behaviour varies greatly with different clays. A sample of bentonite, which consisted of montmorillonite, increased up to 30 per cent. of sulphur, one of kaolin, consisting of kaolinite, up to 5 per cent., and one of Gavi clay (sericite) up to 15 per cent. The author discusses the relation of these different behaviours with the crystalline structures of the different types of clay, and connects it with the content of hygroscopic moisture. H. D.

Hydrogen, Evolution from Ferrous Hydroxide. U. R. Evand and J. N. Wanklyn. (*Nature*, 1948, **162**, 27.) Ferrous hydroxide, pure enough to be almost colourless, was precipitated by mixing ferrous chloride and potassium hydroxide solutions in an atmosphere of hydrogen. After precipitation the ferrous hydroxide and supernatant liquid were left in a flask connected to a manometer, but no hydrogen evolution was detected under these conditions even with ferrous chloride in excess. Further experiments

following those of Schikorr also failed to yield hydrogen even when the reactants were heated to 100°C. Hydrogen was, however, evolved at room temperature with excess of ferrous sulphate if platinum chloride, colloidal platinum, nickel sulphate, nickel powder, copper powder or sodium sulphide was added. Manganese sulphate yielded no hydrogen under these conditions. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Antimalarial Agents and Their Action on the Glucose Metabolism of Plasmodia. P. B. Marshall. (*Brit. J. Pharmacol.*, 1948, **3,** 1.) Using blood from chicks heavily infected with *Plasmodium gallinaceum*, the authors show that quinine and mepacrine exert inhibitory activity at several points in the glucose metabolism of plasmodia. Quinine inhibits hexokinase and phosphoglyceraldehyde dehydrogenase moderately, and possibly lactic dehydrogenase and pyruvate oxidation. Mepacrine inhibits hexokinase strongly, phosphoglyceraldehyde dehydrogenase moderately, and probably pyruvate oxidation. Further studies are necessary to find what proportion of the total inhibitory action takes place at the different points in the carbohydrate metabolism, and, indeed, whether the greater part of the inhibition does take place against the carbohydrate metabolism, or against other metabolic functions. S. L. W.

Benzimidazole, The Folic Acid Activity and Antagonism of Two Structurally Related Derivatives of. P. C. Edwards, Dorothy Starling, A. M. Mattocks and H. E. Skipper. (*Science*, 1948, **107,** 119.) Substances having an action like folic acid have previously been reported, and so have compounds which are antagonistic to folic acid. The authors have studied two benzimidazole derivatives for their folic acid activity or antagonism, because the benzimidazole nucleus closely resembles the purine nucleus and shows competitive action with amino-purines. The first compound examined was N-(4-[{(2-benzimidazoyl)-methyl} -amino]-benzoyl)-glutamic acid in which the pterin nucleus (pyrimido-4 : 5-pyrazine) of pteroylglutamic acid had been substituted by the benzimidazole nucleus; it retained a certain degree of folic acid activity, as measured by the growth stimulating effects on *Streptococcus fæcalis*. The other compound investigated was N-(4-[(2-benzimidazolyl)-methyl-amino]-benzenesulphonyl) glutamic acid, which had a sulphonyl group in place of the ketonic group of the first compound; the substance reversed the biological activity of folic acid on *S. fæcalis* and became a metabolite antagonist. This reversal of activity is reminiscent of the essential metabolite antagonist theory for the sulphonamides, and throws some doubt on the specificity of the pteridin nucleus for the folic acid system. L. H. P.

Heparin, a New Extraction Procedure for. O. Snellman, R. Jensen and B. Sylven. (*Nature*, 1948, **161,** 639.) Solutions of potassium thiocyanate exert a pronounced power of extracting the mast cell granular substance from liver tissue fairly quickly and completely. Using M/1 potassium thiocyanate solution for 24 hours, the authors obtained about 90 per cent. extraction of the metachromatic material in ox liver. Histochemical examination of the tissue residues showed only insignificant amounts of metachromatic substances left after such extraction. After extraction the potassium thiocyanate was readily removed by dialysis, leaving the following fractions to be analysed: tissue residue, precipitate, supernatant and dialysate. These fractions were

treated by tryptic digestion, and the heparin contents assayed by estimating the anticoagulating effects by means of the thrombin method of Jaques and Charles. The yield of heparin, expressed in mg. of "standard heparin," was 61·8 mg./kg. of liver, the tissue residue yielding 5·5 mg./kg., the precipitate 56·3 mg./kg., and the supernatant and dialysate nil. S. L. W.

Penicillin Standards. C. R. Bond. (*Analyst*, 1948, **73**, 254.) The development and history of British standard penicillins is described, with details of a new working standard consisting of a crystalline sodium salt with an appreciably higher potency and containing only 0·4 per cent. of penicillin F in addition to penicillin G. A comparison of the composition of various penicillin standards is given, the determinations being made by Goodall and Levi's micro-chromatographic method. R. E. S.

BIOCHEMICAL ANALYSIS

Mercury in Organic Material, Determination of, by Polarographic Methods. G. Costa. (*Ann. Chim. appl., Roma*, 1948, **38**, 157.) By the destruction of the organic matter by suitable means and conversion of the mercury to iodide the metal may be determined polarographically. For urine, pass a strong current of chlorine through 250 ml. at 80°C. for 1 hour; then pass a strong current of air in the cold for 1 hour, filter and concentrate on the water-bath to about 100 ml. Pass hydrogen sulphide through the liquid and, whether mercury is present or not, a brown precipitate is obtained. Set aside for 24 hours. Decant the clear liquid and centrifuge the remainder, washing the precipitate with a little water saturated with hydrogen sulphide. Then dissolve the precipitate in chlorine water, passing chlorine gas if necessary for a few minutes. The liquid remains turbid owing to the separation of sulphur. Filter into a graduated 20 ml. flask and make up to volume. This may be added to Schwartz's solution (potassium iodide, 4 g.; sodium acetate, 4·22 g.; gelatin (or, better, tylose) 0·2 g. in 100 ml. of water). Curves are given showing the results with different quantities of mercury and also the results of the presence of other heavy metals. The results are accurate within about 10 per cent. and 2 mg. of mercury per litre can be determined. H. D.

Œstrone, Equilin and Equilenin, Determination of, by Infra-red Spectrophotometry. J. Carol, J. C. Molitor and E. O. Haenni. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 173.) Mixtures of these ketosteroids were analysed by measuring the optical densities of solutions of their benzenesulphonyl esters in carbon disulphide at selected wave-lengths for each component in the infra-red region. The concentration of each component was calculated from graphs prepared by plotting optical density against concentration for each substance at the selected wave-lengths. The selected wave-lengths were 10·45 μ, at which equilenin shows maximum absorption, 10·88 μ, at which œstrone shows the maximum, and 10·96 μ, at which equilin shows the maximum. The benzenesulphonyl esters were chosen because they are readily prepared, show the greatest differences in absorption at the selected wave-lengths, and are sufficiently soluble in carbon disulphide. The method gave satisfactory results with 20 prepared samples, and with mixtures recovered from commercial oily solutions of natural œstrogens. G. R. K.

Penicillin, Assay by the Dilution Method. C. G. Pope. (*Analyst*, 1948, **73**, 247.) A full description of this assay has already been published (Pope

and Stevens, *Bull. Health Org., L. of N.*, 1946, **12,** 274.) and reference is made to essential details only. Growth curves are given from the practical results obtained, the purpose being to indicate the reason for the sharp end-point shown in this assay procedure. Figures are given of results obtained in comparative determinations of various standard penicillins and the advantages and disadvantages of the assay are tabulated. A method is outlined for the assay of penicillins G and K in commercial samples, depending on the fact that penicillins G and K give different values in terms of I.U./mg. when assayed against *Staphylococcus aureus*, while more nearly equal weights of each are adsorbed on charcoal. R. E. S.

Penicillin, Investigation on the Iodimetric Method of Estimation of. A. M. Wild, (*J. Soc. chem. Ind., Lond.*, 1948, **67,** 90.) The method depends upon measuring the amount of iodine absorbed by the penicilloates produced by alkaline hydrolysis (Alicino, *Industr. Engng. Chem., Anal. Edit.*, 1946, **18,** 619; *Quart. J. Pharm. Pharmacol.*, 1947, **20,** 59.) The results are shown to vary with temperature, concentration of potassium iodide in the iodine solution, *p*H, and the purity of the penicillin. The original suggestion that the effects of the impurities could be overcome by performing the blank titration immediately after addition of the iodine supposed that the impurities absorbed iodine immediately and that they were unaffected by alkali. Both suppositions are shown to be in error, and it seems doubtful whether the method can be used for the estimation of low-grade penicillin. For reasonably pure samples, the errors introduced are not large, and by strict control of conditions results are obtained which are more consistent and reliable than those from the usual bioassay. Using the modified procedure described, results have compared favourably with triplicate bioassays on the same samples. For samples of high penicillin G content, with potencies over 1000 units/mg., differences greater than 3 per cent. have been rare and the error progressively decreased as the samples tended to absolute purity. The repeatability of the method has been found to be of the order of $\pm$ 1·5 per cent. G. R. K.

Penicillin, Microbiological Assay by the Turbidimetric Method. C. R. Bond and O. L. Davies. (*Analyst*, 1948, **73,** 251.) The various factors affecting this assay method are discussed and a nutrient broth formula for *Staphylococcus aureus*, with details of the inoculum, is given. The following results of experimental work are quoted: small deviations in optimum temperature (37°C.) caused appreciable depreciation in growth; measurements over a period of 2½ to 4½ hours incubation showed a continuous increase in growth; the greatest rate of growth occurred at *p*H 7·5 while at *p*H 6·0 to *p*H 6·5 the growth rate was markedly retarded; the best bacteriostatic used to stop the growth of the test organism at the end of the incubation period was formalin. The advantages and disadvantages of the method are compared with those of the serial dilution and cylinder-plate methods. The standard errors found were: serial dilution, 10 per cent.; cylinder-plate, 4 per cent.; turbidimetric, 4 per cent. R. E. S.

CHEMOTHERAPY

Curare-like Action of Polymethylene *bis*-Quaternary Ammonium Salts. R. B. Barlow and H. R. Ing. (*Nature*, 1948, **161,** 718.) Tubocurarine chloride is a *bis*-tetrahydro*iso*quinoline alkaloid containing 2 quaternary nitrogen atoms, and its potency in blocking neuromuscular transmissions compared with that

of simple quaternary ammonium salts might be partly due to the presence of 2 such cationic groups at some optimum distance apart. The authors therefore prepared and tested for curare-like activity a number of simple *bis*-quaternary ammonium salts in which the nitrogen atoms were separated by polymethylene chains of different lengths. Among the salts prepared were polymethylene *bis*-trimethyl- and *bis*-triethyl-ammonium bromides, with chain-lengths of from 2 to 5 and 7 to 13 carbon atoms, and a smaller group of polymethylene *bis*-quinolinium and *bis*-strychninium dibromides; the drugs were tested on the phrenic nerve-diaphragm preparation of the rat. In the *bis*-trimethyl-ammonium series none of the compounds was more than 2/5 as active as tubocurarine chloride; on the other hand, using the rabbit head-drop test, the C_{10} member of this series was found to be about 3 times as potent as tubocurarine chloride, 0·08 mg./kg. being required to produce head-drop compared with 0·26 mg./kg. of *d*-tubocurarine chloride. Thus, the relative activities, and their variation within a homologous series, may be quite different in the rat diaphragm and the rabbit head-drop tests. As the augmentation of the contractions of the rat diaphragm produced by nearly all the *bis*-quaternary ammonium salts resembled superficially the effect of anticholinesterase drugs, the authors tested representative members on the cholinesterase of caudate nucleus (dog), with acetylcholine as substrate, and found that they all showed some inhibition of the enzyme at concentrations slightly lower than those needed to reduce the contractions of the rat diaphragm. S. L. W.

Curare-like Action of Polymethylene *bis*-Quaternary Ammonium Salts. S. D. M. Paton and E. J. Zaimis. (*Nature*, 1948, **161**, 718.) In a pharmacological study of a series of straight-chain aliphatic ω-*bis*-trimethyl-ammonium iodides, it was found that in the rabbit head-drop test for curare-like activity, the potency increased from the ethylene derivative to the octamethylene derivative, and the decamethylene derivative (C_{10}) was more potent still, 0·11 mg./kg. of the iodide being required to produce head-drop compared with 0·25 mg./kg. of *d*-tubocurarine chloride. The potency in relation to *d*-tubocurarine chloride varied however with the test object; thus, the approximate ratio of an effective dose of the C_8 derivative to an equipotent dose of *d*-tubocurarine chloride was, on the cat's tibialis, 1/3; on rabbit's head-drop, 3; on frog-nerve-sartorius preparation, 3; on rat's diaphragm preparation, 50 to 100. The curarine-like action was not antagonised by neostigmine in doses adequate to antagonise the effect of *d*-tubocurarine chloride either in the cat's tibialis or in the rabbit's head-drop test. Comparison of potency with *d*-tubocurarine chloride was complicated by the finding that while *d*-tubocurarine chloride is unaltered in potency when given after these *bis*-quaternary salts, the converse is not true; thus, following the injection of two-thirds head-drop dose, approximately twice as much of the C_{10} derivative was required to produce head-drop as was normally needed. The authors stress the importance of pharmacological testing of possible substitutes for *d*-tubocurarine chloride on more than one test object and suggest that before clinical application can be considered it is desirable to find some satisfactory antagonist to their effects. S. L. W.

Streptomycin, Sulphetrone and Promin; Chemotherapeutic Action in Experimental Tuberculosis. G. Brownlee and C. R. Kennedy. (*Brit. J. Pharmacol.*, 1948, **3**, 37.) This report describes a comparison between the chemotherapeutic antituberculous activity of streptomycin, sulphetrone, promin, and combined streptomycin and sulphetrone. Four groups of 18 guinea-pigs, and one control group of 11, infected with a human virulent strain of *M.*

tuberculosis, were treated 22 days after infection with drugs for 168 days. One group received 0·5 per cent. of promin in the diet, one 2 per cent. of sulphetrone in the diet, one 10 mg. of streptomycin parenterally daily, and a fourth 2 per cent. of sulphetrone in the diet and 10 mg. of streptomycin parenterally daily; the control group of 11 animals remained untreated. On the basis of survival time, change in weight, response to tuberculin tests, macroscopic evidence of gross tuberculosis *post mortem* or microscopic examination, all presented a uniform picture of degrees of protection. The order of efficiency of the drugs was streptomycin with sulphetrone, streptomycin, sulphetrone, and promin. The protection given by the combined streptomycin and sulphetrone treatment was so marked as to be clearly synergistic. The authors express the opinion that the disease was progressive in all groups, though at a much suppressed rate in those groups where protection was greatest. Nevertheless, the experimental effects produced by the combined streptomycin and sulphetrone therapy are considered to justify a careful clinical evaluation in selected cases. S. L. W.

PHARMACY

GALENICAL PHARMACY

Strophanthus, Preliminary Report on the Extraction of. C. L. Huyck. (*J. Amer. pharm, Ass., Sci. Ed.*, 1948, **37,** 191.) Tinctures were prepared by percolation with alcohol (95 per cent.) and alcohol (65 per cent.) and assayed colorimetrically by a modification of the picric acid method of Knudson and Dresbach. The results given show there is little to choose between the solvents or the methods, except that the tinctures prepared with the weaker alcohol showed a somewhat greater loss of activity after 6 months and formed a precipitate. G. R. K.

NOTES AND FORMULÆ

Polyvinyl Alcohol as an Emulsifying Agent. G. F. Biehn and M. L. Ernsberger. (*Ind. Engn. Chem.*, 1948, **40,** 1449.) Commercial polyvinyl alcohols (partially or completely hydrolysed polyvinyl acetates) were examined for their interfacial tension and emulsifying properties. The most effective polyvinyl alcohols were found to be high-viscosity products hydrolysed to the extent of 75 to 80 per cent. At a concentration of 0·5 per cent. or higher (based on the total emulsion) these emulsifying agents compared favourably with other agents in giving emulsions of small droplet size, foaming was less, and where present the foam was unstable. Results on the stability of emulsions showed that the effectiveness of emulsifying agents generally varied greatly with different water-immiscible liquids. Comparisons with the polyvinyl alcohols showed that these agents and sodium dodecyl sulphate gave the most stable emulsions of trichloroethylene; for dibutyl phthalate, polyvinyl alcohols, sodium oleate and methyl cellulose were equally effective; for linseed oil, sodium alginate and sodium oleate were good, while two polyvinyl alcohols tested were fairly effective. The emulsion stabilities were measured by a method involving the measurement of the rate of separation of the internal phase under a constant centrifugal force. For emulsions containing sodium chloride, magnesium chloride or calcium chloride, the latter in concentrations corresponding to hard waters, a high viscosity 76 to 79 per cent. hydrolysed polyvinyl acetate gave stable emulsions, but other polyvinyl alcohols unexpectedly proved

less effective even than some of the common ionic agents. Certain of the polyvinyl alcohols were effective over a wide *p*H range. Methods of preparation of emulsions using dry powdered polyvinyl alcohol as well as viscous aqueous solutions are given. R. E. S.

PHARMACOGNOSY

***Datura metel*, Effect of Colchicine Treatment on the Alkaloidal Content of.** A. E. Beesley and G. E. Foster. (*Nature*, 1948, **161**, 561.) In contrast to the results reported by J. M. Rowson (*Quart. J. Pharm. Pharmacol.*, 1945, **18**, 175) that the treatment of seeds of *D. metel* and other solanaceous plants with a 0·4 per cent. aqueous solution of colchicine produced polyploid plants with a higher alkaloidal content than untreated seeds, a batch of *D. metel* seeds, similarly treated, produced plants which showed no evidence of polyploidy and no significant increase in alkaloidal content when compared with plants grown from a control group of seeds under conditions as far as practicable identical. G. R. K.

Ergot and Preparations, Alkaloidal Content of. S. A. Schou, P. F. Jorgensen and V. G. Jensen. (*Dansk Tidsskr. Farm.*, 1948, **22**, 161.) An examination was made of the alkaloidal content of 35 samples of ergot of varying geographical origin and harvests from 1938 to 1947. The content of ergometrine alkaloids was nil in 14 cases, while in 15 samples it was over 0·025 per cent., the maximum being 0·060 per cent. Five samples contained no ergotoxine alkaloids, 21 contained 0·100 per cent. or over, and the maximum was 0·217 per cent. For the new Danish Pharmacopœia it is recommended that the minimum limit for ergot should be 0·025 per cent. of ergometrine alkaloids and 0·100 per cent. of ergotoxine alkaloids: for the liquid extract the corresponding figures are 0·020 and 0·060 per cent. respectively. If 1 per cent. of ascorbic acid is added to the extract, and it is made from a drug containing the minimum proportion of alkaloids, the extract will still meet the requirements after storage for 6 months. G. M.

Morphine Content of Poppy Capsules. H. Baggesgaard-Rasmussen and O. Lanng. (*Dansk Tidsskr. Farm.*, 1948, **22**, 203.) A number of methods of assay were tried, the most satisfactory being a polarographic one, as follows. 2 g. of powdered capsules is moistened with 1 ml. of 1·2 M sodium carbonate solution and heated at 70°C. in a flask provided with a reflux condenser for 1 hour with 20 ml. of a mixture of butyl alcohol (7 volumes) and benzene (3 volumes). The mixture is then filtered through a sintered glass filter, the residue being pressed down and then washed with about 50 ml. of the mixed solvent. The extract is shaken out with 10 ml. of N/1 hydrochloric acid, and washed 3 times with 10 ml. of water. The aqueous extracts, which are practially colourless, are evaporated to 10 ml., treated with hydrochloric acid and potassium nitrate, then with excess of potassium hydroxide and the morphine is determined polarographically. The method was applied to experimental crops from seed of capsules tested the preceding year. The morphine content of the capsules of the second year (1947) was much higher than that of the preceding year, but this is probably due to the very different weather conditions. The distribution of morphine in the capsule was investigated by cutting the capsules into three equal parts by horizontal cuts,

the placenta and stigma being examined separately. Typical results were as follows:—

	Weight per cent. of total	Morphine (anhydrous) per cent. of total	Morphine per cent.
Upper part	16·5	0·31	11·8
Middle part	26·4	0·43	26·5
Lower part	34·1	0·52	41·0
Placenta	14·0	0·50	16·0
Stigma	9·0	0·24	4·7

G. M.

Opium from Greece. P. G. Kritikos. (*Pharm. Acta. Helvet.*, 1948, **23,** 196.) A certain amount of opium is produced in Greece. Examination of a number of samples from different districts gave the following figures :—

	Sample						
	1	2	3	4	5	6	7
Moisture (100°C.) ...	6·38	3·48	4·03	3·68	6·01	3·54	5·78
Ash	2·90	3·35	2·97	2·65	2·08	4·98	3·60
Meconic acid ...	4·81	5·81	4·92	5·29	5·56	5·18	5·05
Narcotine	4·18	4·02	4·03	3·92	5·17	3·79	4·25
Codeine	1·50	1·27	1·27	1·17	1·22	1·25	1·57
Morphine	15·61	10·25	14·50	14·43	15·40	15·74	15·59

All the above percentages, other than the moisture content, are calculated on the dry material. G. M.

PHARMACOLOGY AND THERAPEUTICS

Amidone, Pethidine and Morphine; Analgesic Effects in Human Subjects. E. N. Christensen and E. G. Gross. (*J. Amer. med. Ass.*, 1948, **137,** 594.) A comparison in 11 volunteer subjects, employing the Wolff, Hardy and Goodell technique, showed amidone to be about three times as potent as morphine and many times more potent than pethidine when given subcutaneously, but that it lacked the sedative action of either of these drugs. Nausea and vomiting were not experienced by any of the subjects. With all three drugs the duration of analgesia following subcutaneous injection is much longer than that following intravenous injection. The simultaneous injection of a dose of 0·3 mg. of atropine decreased both the intensity and duration of the analgesic effects of both amidone and morphine, and the duration but not the intensity of pethidine. When atropine was given with any of the drugs administered intravenously the only consistent change was a shortening of duration of effect of amidone. The undesirable side-effects of morphine and pethidine, such as nausea and vomiting, were absent when atropine was given simultaneously. Sedation was more marked when atropine was injected with each analgesic. Simultaneous injection of 0·5 mg. of neostigmine increased both the intensity and duration of the analgesic action of all the drugs. From these studies, combined with the study of the clinical results in 69 patients to whom amidone combined with atropine was given as a pre-anæsthetic agent, the authors conclude that amidone is an unsatisfactory pre-medication agent, but is most useful as a post-operative analgesic and in pain from many other causes. S. L. W.

***para*-Aminosalicylic Acid in Experimental Tuberculosis.** W. T. McClosky, M. I. Smith and J. E. G. Frias. (*J. Pharmacol.*, 1948, **92,** 447.) Tests in rats, guinea-pigs and rabbits showed a relatively low toxicity in all these animals, but chronic toxicity tests on guinea-pigs indicated a cumulative action. The compound is well absorbed from the gastro-intestinal tract and is well retained for several hours. Analysis of the urine of rabbits showed that during the 24 hours following ingestion, from 10 to 20 per cent. of the dose administered was excreted as the free compound and about 80 to 90 per cent. as the conjugated compound. It was found to have little therapeutic activity in rabbits infected with a bovine strain and in guinea-pigs infected with a human strain of tubercle bacilli. When given in combination with streptomycin the chemotherapeutic efficacy was no greater than the sum of effects from the two drugs: there was no evidence of potentiation as with the sulphones and streptomycin.

S. L. W.

Diisopropyl Fluorophosphate; Effect on Anoxic Survival. A. F. Freedman and H. E. Himwich. (*Science*, 1948, **108,** 41.) It has been found that the use of diisopropyl fluorophosphate (D.F.P.) prolongs the survival period of medullary centres subjected to a complete arrest of circulation. If this increased resistance to anoxia observed in the isolated head should be found to apply also to the intact organism then it might be valuable in minimising the effects of anoxia. For this investigation the authors undertook a series of experiments under a variety of conditions, which included a comparison of the survival periods of animals previously injected with the drug with untreated controls, using (1) rats subjected to hypoxia, (2) the decapitated heads of new-born rats (length of gasping-time of head), (3) rats receiving excessive doses of pentobarbitone or of morphine. Only with morphine was there any suggestion of a beneficial effect from the previous use of the drug and on statistical analysis even this might be imputed to chance variation. S. L. W.

Dimercaprol (B.A.L.), Influence of, on the Toxicity and Therapeutic Activity of Mapharsen. N. Ercoli and W. Wilson. (*J. Pharmacol.*, 1948, **92,** 121.) The influence of dimercaprol on the therapeutic activity of mapharsen was studied in relation to its toxicity in mice infected with *Trypanosoma equiperdum*. It was found that the curative or sterilising action of mapharsen is influenced much more readily by dimercaprol than the trypanocidal action, while the toxicity is the least affected. Thus, while the curative effect of mapharsen disappears with doses of dimercaprol as low as one-eighth to one-half the mapharsen dose, from 1·0 to 2·7 times more dimercaprol than mapharsen is required to interfere with toxic mapharsen doses. In other words the " therapeutic index " for the combined treatment is lower than for mapharsen alone, since the curative activity is more reduced than the toxicity by the same proportional dose of dimercaprol. In general, the higher the dose of the arsenical the more dimercaprol proportionately is required for inhibition. These findings preclude the possibility of combined chemotherapy with dimercaprol and mapharsen. S. L. W.

Rutin, Effect on Anaphylactic and Histamine Shock. R. H. Wilson and F. De Eds. (*Science*, 1948, **107,** 369.) The conclusions drawn by Raiman, Later and Necheles (*Science*, 1947, **106,** 368) from their observation that rutin protects guinea-pigs from anaphylactic shock but not from histamine shock were that either histamine is not the direct cause of anaphylactic shock or rutin prevents the liberation of histamine. These conclusions are untenable in the light of results by other workers that rutin has a slight protective action

against histamine, that death from histamine can be prevented by compounds closely related to flavonols and that scorbutic guinea-pigs have an increased sensitivity to histamine which is counteracted by a mixture of *d*-catechin isomers. Rutin affords protection against histamine shock in an indirect way and is not a true antihistaminic. The evidence that it protects against anaphylactic shock supports the theory that symptoms of anaphylaxis are produced by histamine. G. R. K.

Thiouracil-treated Rats, Diffuse and Nodular Hyperplasia of the Thyroid Gland in. W. C. Kuzell, H. B. Tripi, G. M. Gardner and G. L. Laqueur. (*Science*, 1948, **107,** 374.) 58 albino rats were fed on a basic diet containing 0·1 per cent. of thiouracil for periods of 34, 51, 120, 142 and 233 days. Comparison of the thyroid glands at the end of these periods with those from a control group of rats showed extreme hypertrophy in the glands from those animals fed on the thiouracil diet for a long time (the glands from male rats which had received thiouracil for 233 days averaged an increase in weight above normal of 489·3 per cent.). Histologically, the glands from animals removed from the thiouracil diet after 34 and 51 days showed no hyperplasia, whereas those from rats fed on the diet for 120 days or more were distinctly hyperplastic with areas of nodular hyperplasia; the number of nodules was related to the amount of thiouracil ingested. Since this experiment was part of a study designed to show the effect of thiouracil-induced hypothyroidism on experimental polyarthritis, all animals were inoculated at varying times with a broth culture of pleuropneumonia-like organisms, but there was no evidence that this had any effect on the thyroid glands. G. R. K.

BACTERIOLOGY AND CLINICAL TESTS

Sodium *p*-Aminobenzoate, Bacteriostatic Properties of. R. Lecoq and and J. Solomides. (*C.R. Acad. Sci., Paris*, 1948, **226,** 846.) At dilutions of 1/130 to 1/250, sodium *p*-hydroxybenzoate has a bacteriostatic action, not only towards *Bact. coli* and the bacillus of Eberth, but also towards cultures of Gram-negative organisms—*B. dysenteriæ* Shiga and *Vibrio Choleræ*. On the other hand it has no action on Staphylococci, Streptococci, *B. subtilis* and *B. diphtheriæ*, even at 1/100. The development of *B. tuberculosis* (human and bovine) is inhibited even at a dilution of 1/1000. The action on moulds is variable: the growth of *Actinomyces griseus* is stopped at 1/250 while *Aspergillus niger* is resistant. G. M.

Vitamin K, Antibacterial Analogues of, Effect on *Mycobacterium tuberculosis.* C. N. Iland (*Nature*, 1948, **161,** 1010.) During a search for chemically defined growth-factors for *M. tuberculosis*, it was decided to investigate vitamin K, which is present in many organisms and is probably of nutritional importance. A pigment, 'phthiocol' (2-methyl-3-hydroxy-1:4-naphthoquinone), isolated from a laboratory strain of tubercle bacillus, has vitamin K-like activity, and it has been suggested that phthiocol is derived from vitamin K during the extraction of the bacteria. It has long been known that *M. paratuberculosis*, when freshly isolated, will grow only on media containing extracts of other acid-fast bacteria, notably *M. phlei*, and it has been claimed that phthiocol and 2-methyl-1:4-naphthoquinone can replace *M. phlei*, but the stimulant action is not so marked, hence it does not follow that the *M. phlei* growth-factor and these compounds are the same. The author concludes that the original hypothesis that vitamin K-like compounds are necessary for the nutrition of *M. tuberculosis* has not been proved, but that there are certain indications that similar substances play some part in the metabolism of the organisms. S. L. W.

PHARMACOPŒIAS AND FORMULARIES

THE BRITISH PHARMACOPŒIA, 1948

The Assay of Alkaloidal Salts

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In the control of the purity of alkaloidal salts and similar compounds the question arises whether it is necessary to estimate the physiologically active base, or if it is sufficient to determine the other part of the compound molecule. In general, the estimation of the active base gives the best control of purity, but the methods are often laborious and troublesome. It is often difficult to find a good method for the estimation of the active substance, whereas a simple and quick method for the estimation of the other part of the molecule is readily available.

The British Pharmacopœia, 1948, selects the estimation of the active base, and it must be said that this standpoint is scientifically unassailable. For many substances the Pharmacopœia describes assays on this principle, including Amethocainæ Hydrochloridum, Amphetaminæ Sulphas, Cinchocainæ Hydrochloridum, Codeinæ Phosphas, Emetinæ Hydrochloridum, Homatropinæ Hydrochloridum, Mepacrinæ Hydrochloridum, Mepacrinæ Methanosulphonas, Morphinæ Hydrochloridum, Morphinæ Sulphas, Quinidinæ Sulphas, Quininæ Bisulphas, Quininæ Dihydrochloridum Quininæ Hydrochloridum, Quininæ et Æthylis Carbonas, Quininæ Sulphas and Strychninæ Hydrochloridum. The principle of estimation of the active part of the molecule, however, is not introduced in the monographs on Apomorphinæ Hydrochloridum, Atropinæ Sulphas, Butacainæ Sulphas, Cocainæ Hydrochloridum, Diamorphinæ Hydrochloridum, Ephedrinæ Hydrochloridum, Hyoscinæ Hydrobromidum, Papaverinæ Hydrochloridum, Physostigminæ Salicylas, Pilocarpinæ Nitras and Procainæ Hydrochloridum. Possibly the assay is not introduced in these monographs, because some of the alkaloidal salts have a sharp melting-point or a sufficiently narrow melting-range to guarantee the purity of the drug.

Four methods for the determination of the purity of such compounds may be discussed.

1. *The Method of the British Pharmacopœia*, 1948. In this the base is liberated by alkali from a solution of the salt and extracted by shaking several times with a suitable solvent, the solvent is evaporated, and the residue is dried and weighed or titrated. Such assays involve much work, and cannot be indicated as simple pharmacopœia methods.

2. *Chromatographic determination.* This method was described by Reimers, Gottlieb and Christensen.[1]. I have used it for the salts of cinchocaine, cocaine, emetine, diethylmorphine physostigmine, pilocarpine, procaine, scopolamine and tetracaine, and find that it gives very good results. If the apparatus is kept ready for use and the worker has some experience, the chromatographic method requires less time and material than the extraction of the active base by a solvent. It is a condition for good results, that the aluminium oxide must be completely free from alkali and must give a good adsorption test.

3. *Titration of the acid.* This gives the correct percentage of the alkaloid

if it is correctly neutralised by its equivalent quantity of acid, and it is possible, by determining the acid part of the molecule, to get good control of the purity. The method is simply and quickly performed by titrating the alkaloidal salt solution with 0·1N sodium hydroxide, using phenolphthalein as indicator. If the alkaloid precipitates on adding sodium hydroxide solution, alcohol or chloroform is added to dissolve it. The method is used in the Netherlands Pharmacopœia for all the alkaloidal salts. If the alkaloid is not correctly neutralised in the salt, the titration will show a high percentage of alkaloid if there is too much acid, and a low percentage if there is too much base. It is possible to show that the alkaloidal salt has the correct composition by determination of the *p*H of an 0·1N solution. This can be calculated from the dissociation exponent of the base or estimated colorimetrically or electrometrically. Kolthoff[2] and Schoorl[3] have recorded the *p*H values of several alkaloidal salt solutions. Table I gives figures, determined in my laboratory, for the *p*H values of alkaloidal solutions of various concentrations.

TABLE I

	0·1 N	0·01 N	1 per cent.
	*p*H	*p*H	*p*H
Cinchocainæ Hydrochloridum	4·75	—	—
Pilocarpinæ Hydrochloridum	4·58	4·73	—
Procaine Hydrochloridum	5·72	—	5·82
Ethylmorphinæ Hydrochloridum	4·58	—	5·10
Emetinæ Hydrochloridum	5·23	—	5·25
Hyoscinæ Hydrochloridum	—	—	4·93
Homatropinæ Hydrochloridum	5·79	—	—
Codeinæ Phosphas	4·24	—	—
Dihydrocodeinonæ Bitartras	3·32	—	—
Dihydro-oxycodeinonæ Hydrochloridum	—	6·12	—
Acetyldimethyldihydrothebainæ Hydrochloridum	—	5·85	—

The following experiment proves that the determination of the *p*H of an alkaloidal salt solution is a very good method for the control of purity and correct composition.

To a sample of atropine sulphate a small quantity of acid or of alkaloidal base was added to ascertain if the *p*H change of the solution was practically measurable. Both substances caused a considerable change in the *p*H of the solution. Figures are given in Tables II and III.

TABLE II

THE EFFECT ON *p*H OF THE ADDITION OF ACID TO A SOLUTION OF ATROPINE SULPHATE

Concentration	*p*H	*p*H after addition of 0·05 ml. of N/1 acid.	Difference	*p*H after addition of 0·1 ml. of N/1 acid.	Difference
712 mg. of atropine sulphate in 20 ml. of water	5·10	4·20	−0·90	3·78	−1·32
	5·09	4·18	−0·91	3·76	−1·33
	5·10	4·18	−0·92	3·76	−1·35

The conclusion is that the *p*H determination is a very good test for the correct neutralisation of the base by the acid.

4. *Double Titration.* This method[4] consists of a titration of the acid and basic parts of the molecule. A milli-equivalent of the alkaloidal salt is dissolved in 5 ml. of water, this solution is mixed with 15 ml. of alcohol (96 per cent.) and titrated with 0·1 N sodium hydroxide with phenolphthalein

as indicator. Theoretically 10 ml. should be required. Then bromothymol blue is added and the liberated base is titrated by 0·1 N acid, of which also 10 ml. should be required. In the first titration, the first pink colour gives the end of the titration. Strong bases (atropine, emetine, etc.) which are

TABLE III

THE EFFECT ON *p*H OF THE ADDITION OF ATROPINE BASE TO A SOLUTION OF ATROPINE SULPHATE

Concentration	*p*H	*p*H after addition of 10 mg. of base	Difference
712 mg. atropine sulphate in 20 ml. of water	4·90	7·90	+3·00
	4·91	7·94	+3·03
	4·93	7·94	+3·03

alkaline to phenolphthalein must be extracted by means of an organic solvent such as chloroform. For the second titration, the alkaloidal base must be dissolved in as little alcohol as possible. If the alkaloidal base is extracted and dissolved in chloroform, it is possible to : (a) add an excess of acid and extract the base in the aqueous layer, after which the free acid is titrated with sodium hydroxide: (b) evaporate the solution and dry the alkaloid (if not volatile) dissolve it in an excess of 0·1N acid and titrate with 0·1N sodium hydroxide. The double titration can also be done with microburettes, and only a small quantity of material is then required. The method gives good control of the correct neutralisation of the alkaloid by the acid and also of the correct composition of the drug and directly the percentage of the active principle.

The conclusions of my experience are: 1. The direct determination of the active principle is scientifically unassailable but often requires complicated assays; 2. The direct determination by extraction with a solvent can be replaced by a chromatographic determination if a pure aluminium oxide is available; 3. The active principle can be determined indirectly by a simple titration of the acid part of the molecule and the determination of the *p*H of a dilute solution of the alkaloidal salt; 4. The same result can be obtained by the double titration of the acid part and alkaloid.

Methods (3) and (4) can be recommended for the control of purity and correct composition for all alkaloidal salts mentioned in the Pharmacopœia where there is no assay prescribed.

The author wishes to express his sincere thanks to Mr. J. S. Faber, Conservator of the Pharmaceutical Laboratory, University of Groningen, for his help in this work.

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BOOK REVIEWS

THE CHEMISTRY OF THE POLYSACCHARIDES by R. J. McIlroy. Pp. 116 and Index. E. Arnold & Co., London, 1948, 10s. 6d.

Though the nature of the simple sugar units present in polysaccharides had previously been recognised from examination of hydrolytic products, it is only in the last sixteen years that techniques have been developed which have led to our present conceptions of the chemistry and structure of a wide range of polysaccharides and their derivatives, of both animal and vegetable origin. It was in 1932 that Haworth and Machemer showed that with methyl alcoholic hydrogen chloride, the terminal unit of fully methylated polysaccharides was obtained as a methylated methyl glycoside and that by refined distillation methods it was then possible to separate and determine the proportion of the terminal unit. Haworth's " end group assay method " has since been widely applied. It has led to our present knowledge of the chemistry of starch, including both the water-soluble component, amylose, and the water-insoluble component, amylopectin, as well as to confirmation of the amylose-nature of the 'synthetic' starch obtained by Hanes in 1940 by submission of glucose-*l*-phosphate to the action of a purified phosphorylase obtained from potato tuber juice. It has led also to modern conceptions of the chemical nature of cellulose, of the pentosans, of glycogen, of mucilages and gums, of carbohydrate products of bacterial metabolism, and of the immuno-polysaccharides. The importance on the one hand of the carbohydrate sulphuric ester, heparin, the natural blood anti-coagulant stored in the liver and heart, and on the other hand of the antigenic polysaccharides derived *inter alia* from pneumococci, tubercle bacilli, and the relation of carbohydrate haptens to the polyuronides (gums), are such as to emphasise medical aspects of the need for development of knowledge of polysaccharide chemistry. The advancing front of chemical study of polysaccharides of diverse origin is now such that there has been real need for a concise readable resumé of modern developments in the field. This need is admirably met by the book under review. The structure of relevant monosaccharides is discussed briefly as an introduction to an account of modern methods of determination of polysaccharide structure. Chapters follow on starch and cellulose, glycogen, levans, galactose-, mannose-, amylase- and immuno-polysaccharides, and the polyuronides including hemicellulose, pectin, gums, mucilages and alginic acid. Part II of the book describes the derivatives of monosaccharides of importance in polysaccharide investigations, methods for estimation of carbohydrates, the utilisation of carbohydrates in animal and plant metabolism, the role of carbohydrates in immunology, and the identification of sugars and sugar acids. An appendix completes the survey of available literature to September, 1947. A comprehensive list of references at the end of each chapter permits extensive condensation of the text without in any way detracting from the clarity of the discussions. The book can be commended to all those who, unable to keep in touch with an ever expanding literature of a developing subject, yet desire, or have need, of an up-to-date knowledge of the methods of investigation, the problems arising and the results achieved in this important branch of chemistry. F. HARTLEY.

THE BASIS OF CHEMOTHERAPY by T. S. Work and E. Work. Pp. 435 + XX. Figs. 42. Oliver and Boyd, Ltd., Edinburgh, 1948, 26s.

It is refreshing to receive for review a new work founded upon an original idea. The book was designed to give young research workers a broader and sounder basis for the study of chemotherapy. It is my belief

that this aim will be achieved, but I would not call the work a text-book: rather, it is a scientific monograph. After a short historical survey, the succeeding chapters, Cell Metabolism; Essential Metabolites; Enzyme Inhibition; Drug Antagonism; Drug Resistance, lead logically to the final consideration of the relation of structure and activity. In each case the known facts are discussed in relation to their effect on the problems encountered in chemotherapy. The whole is very stimulating to thought, and, as one reads, research problems frequently suggest themselves. The authors themselves admit that quicker results in chemotherapeutic research may yet be obtained by the older empirical method than by following suggestions based upon knowledge of reactions taking place in living cells. For some time it will be more important to gain a more accurate knowledge of such reactions than to apply them to chemotherapy. It may be that the chemical reactions of living cells may never provide the basis for chemotherapeutical design because of the similarity of one cell with another. The cells of the host and those of the invading organism may be too much alike in chemical sensitivities to be differentiated in this way. However, no matter what the ultimate result may be, the worker in the field of chemotherapy cannot help but benefit by a fuller knowledge of those subjects covered by this book. There is one little grouse that I would like to make. This book mentions a large number of medicinal chemicals, and in most cases ignores the Pharmacopœial names for them. Do all biochemists speak of thiamin rather than aneurine? It would be better to include the official names, even though they be given in brackets. The book is remarkably free from errors, and is exceedingly well documented (the bibliography covers 54 pages), the references including work done in 1946. Perhaps it would be fair to point out that the group of workers mentioned on page 334 did not synthesise for the first time all the compounds treated in Table 34. The book affords the worker in closely related fields the opportunity of obtaining, in a summarised and palatable form, the results of research on the biochemistry of the living cell, and I have every pleasure in recommending it highly as accurate, authoritative and well written. W. H. LINNELL.

BOOKS RECEIVED

PRACTICAL METHODS IN BIOCHEMISTRY by F. C. Koch and M. E. Hanke. Pp. 420, Ballière, Tindall and Cox, 1948, 5th ed., 16s. 6d.

THE STUFF WE'RE MADE OF by W. O. Kermack and P. Eggleton. Pp. 350 and Index. Edward Arnold and Co., London, 1948, 2nd ed., 10s. 6d.

THE PRESENTATION OF TECHNICAL INFORMATION by Reginald O. Kapp. Pp. 140 and Index. Constable and Co., Ltd., London, 1948, 6s.

A MANUAL OF PHARMACOLOGY by T. Sollman. Pp. 1132. W. B. Saunders Company, London, 1948, 2nd ed., 57s. 6d.

AMERICAN PHARMACY, edited by R. A. Lyman. Vol. 1. Pp. 522, 1948, 2nd ed. Vol. 2. Pp. 328, 1947. J. B. Lippincott Company, Philadelphia.

PHARMACOLOGY, THERAPEUTICS AND PRESCRIPTION WRITING by W. A. Bastedo. Pp. 840, W. B. Saunders Company, London, 1947, 5th ed., 42s.

LETTERS TO THE EDITOR

An Identity Test for Pheniodol

SIR,—During recent work in our laboratories an identification test suitable for routine analysis was required for pheniodol, α-phenyl-β-(4-hydroxy-3 : 5-diiodophenyl)-propionic acid, but no assistance in this matter was obtained by a survey of the literature. Accordingly, we developed the following test which has proved satisfactory in the hands of several independent workers.

Dissolve 0·5 g. in 15 ml. of 10 per cent. sodium hydroxide solution, warming if necessary. Add 1 g. of zinc dust and boil the mixture under a reflux condenser for 20 minutes. Cool, filter, add excess of dilute hydrochloric acid and collect the α-phenyl-β-(4-hydroxyphenyl)-propionic acid. Wash with water and re-crystallise the product from alcohol-water mixture. The crystals, after drying at 100°C., melt at 180° to 181°C. The filtrate, from the acid after separation from the original reaction mixture, affords reactions characteristic of iodides.

It is felt that this test may be of interest to your readers.

Wellcome Chemical Works, Dartford. G. E. FOSTER.
October 21, 1948 W. D. WILLIAMS.

Silicotungstic Acid

SIR,—In the discussion at the British Pharmaceutical Conference on our paper entitled "The Chemical Determination of Aneurine in Tablets and Ampoule Solutions" (see *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 370, 423) a question was asked as to the composition of the silicotungstic acid used in our experiments. The composition may be of importance, as is reputed to be the case in the method of the Association of Official Agricultural Chemists for the determination of nicotine (*Methods of Analysis*, 6th Ed., p.74). We obtained the silicotungstic acid for our work from only one source and the suppliers have advised us that the composition varies slightly but approximates closely to $H_4SiW_{12}O_{40},24H_2O$. This corresponds to the formula quoted by the A.O.A.C. for the nicotine determination, $4H_2O,SiO_2 12WO_3,22H_2O$.

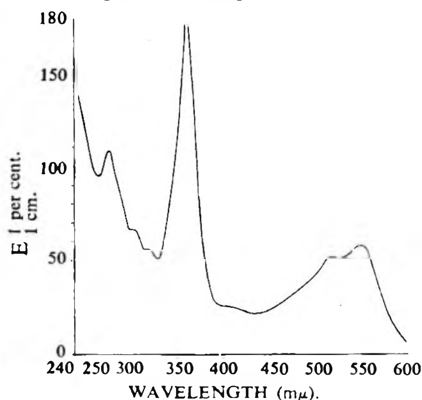
Roche Products, Ltd., Welwyn Garden City. D. C. M. ADAMSON.
November 3, 1948. F. P. HANDISYDE.

The Isolation of the Crystalline Anti-Pernicious Anæmia Factor from Liver

SIR,—Work on the anti-pernicious anæmia (A-P-A) factor present in liver. in progress in these Laboratories for some years, has led to the isolation of a red crystalline compound from anahæmin, probably identical with the vitamin B_{12} of Rickes *et al*[1] and with the crystalline A-P-A- factor of Smith and Parker[2]. The methods of purification employed by us, however, differ in certain respects from those hitherto revealed. Thus the observation that the A-P-A factor is extracted by *n*-butanol from its aqueous solutions in the presence of fairly high concentrations of ammonium sulphate[3] enabled us to effect enrichment of the fractions at various stages of the process. Chromatography was reserved only for the final purification. Columns of bentonite or aluminium silicate[3] were used, and, under carefully controlled conditions, proved eminently satisfactory by giving rise to the formation of sharply defined red bands. These, after dissection and elution, gave material which readily crystallised in small red needles from aqueous acetone.

Our crystalline product, after drying *in vacuo*, contained 4·0 per cent. of cobalt, a figure identical with that reported by Smith[4]. In aqueous solutions

it shows characteristic light absorption (see Fig.). A main band appears in the visible region of the spectrum with a maximum at 500 mμ and a "shoulder" at approximately 520 mμ, whilst two distinct maxima occur in the ultra-violet, one at 361 mμ and the other at 278 mμ, with inflections at 322 mμ and 304 mμ.



Several batches of the crystals have been hydrolysed with 20 per cent. hydrochloric acid in sealed tubes at 100°C., and the hydrolysates examined by uni-dimensional paper-strip partition chromatography, using the technique described by Consden *et al*[5]. The chromatograms obtained have consistently revealed the presence of only one substance reacting with ninhydrin. Using aqueous *iso*butyric acid as the solvent, a pronounced purple spot appears on the paper at a point approximately mid-way between the positions occupied by the α-amino-acids valine and *nor*valine. With aqueous phenol or *n*-butanol as solvents, however, the colour of the spot is greatly diminished in intensity although its position with respect to the two amino-acids remains substantially unchanged. Experiments to detect the presence of purines[6] in A-P-A hydrolysates have not, so far, been successful. Vigorous hydrolysis of the A-P-A factor with 20 per cent. hydrochloric acid under reflux for 8 hours failed to rupture the cobalt-containing complex present in the molecule. Removal of a hydrophilic fragment undoubtedly takes place as the product is readily and quantitatively extracted from the diluted hydrolysate with *n*-butanol (cf. Smith[7]). The product is an almost black, amorphous solid which is acidic in character as it is soluble in dilute alkalis and is reprecipitated unchanged on neutralisation. This "acid" is insoluble in ether, chloroform and acetone, but is rendered soluble in these solvents by the addition of a trace of hydrochloric acid. Its light absorption (measured in dioxan) shows a maximum at 557·5 mμ with a "shoulder" at 530 mμ, and is thus similar (in the visible part of the spectrum) to the absorption of the A-P-A factor itself. The retention of a cobalt co-ordination complex in the molecule after the somewhat drastic acid hydrolysis is surprising, but is paralleled by the behaviour of certain metal porphyrins. Supplies of the methyl ester of the "acid" are now being accumulated to enable us to undertake its more detailed study.

The authors thank Dr. R. E. Stuckey for the analytical and absorption data and the Directors of The British Drug Houses, Ltd., for permission to publish these results.

Research Department,
The British Drug Houses, Ltd., London, N.1.
November 24, 1948.

B. Ellis.
V. Petrow.
G. F. Snook.

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SCIENTIFIC MEETING

DRUG ACTION, IONS AND NEUTRAL MOLECULES

by ADRIEN ALBERT, D.Sc. Ph.D. B.Sc., F.R.I.C.

Summary of a paper read at the Norwood Branch of the Royal Institute of Chemistry, at the Norwood Technical Institute on December 6.

MANY drugs undergo ionisation and a number do so to different degrees at different *p*H values. The most significant changes in ionisation occur around the *p*H value at which the drug is 50 per cent. ionised, i.e., the pk_A value. Ions and molecules behave differently in their effects on drug action, especially in relation to chemical reactivity, adsorption and the penetration of membranes. An example of the first difference is shown by aniline, where the molecule is nitrated mainly in the *para* position, but the ion is nitrated in the *meta* position. Again the mono-anion of ascorbic acid is easily autoxidised, whereas the di-anion and the molecule are both quite stable. A distinction is made between general and specific adsorption. In the former, the drug is rejected by water because of a relative lack of hydrophilic groups and becomes adsorbed on any surface which offers itself. In such cases the molecule is usually more highly adsorbed than the ion, because the latter is hydrated at one end. In specific adsorption the drug, which may have many hydrophilic groups, is specifically attracted to cellular receptor-groups by reason of having a complementary structure. This often takes the form of the drug being kationic and the receptor anionic, or *vice versa.* In such a case, only the ion can be adsorbed. Generally, for any one substance, the molecule penetrates much faster than the ion. The principal barrier to the penetration of an ion is the strong attraction between its charge and the oppositely charged groups on the cell membrane. However, if ions are provided with lipophilic groups, penetration is considerably improved.

In view of these marked physico-chemical distinctions between ions and molecules, it is not surprising to find that some drugs (such as the acridine antibacterials) have ions that are many times as active as the neutral molecules. Other drugs are known (e.g. benzoic acid) where the activity is proportional to the amount of non-ionised material present. Information of this kind can be gathered from reasonably simple experiments, in which firstly a given drug is examined biologically over a range of *p*H values and then a series of related substances, differing widely in pk_A values, is examined at the physiological *p*H value (*p*H 7).

Such information is of considerable practical use. Modern knowledge of inductive constants enables the pk_A of a substance to be varied at will by the insertion of appropriate groups, so that ionisation can be either increased or repressed at a given *p*H value. In some classes of drugs, such as the sulphonamides, a more complex picture is presented, where the maximum activity is obtained when the drug is half ionised. The usual interpretation of this is that the neutral molecule is required in order to penetrate into the cell, but that the ion is regenerated within the cell (according to the law of mass action) and is, of the two, the more biologically active.

NEW REMEDIES

The asterisk (*) after the name of an article indicates that the information given is derived from the makers' publications. Further information regarding these articles may be obtained by application to the Editor.

Benecol* is an extract of mammalian intestinal mucosa, standardised on its content of erepsin, the mixture of proteolytic enzymes secreted by the intestine and responsible for the final stages of protein degradation to amino-acids. It is claimed that administration of this extract compensates for the poor intestinal functioning commonly associated with arthritis. It is administered in capsules, which pass unchanged through the stomach and disintegrate high in the small intestine, the dosage being 1 capsule 3 times daily, increased to 3 capsules 3 times daily, between meals. The treatment is continued for several months. Benecol is indicated in rheumatoid arthritis and in all types of rheumatic disease and is issued in boxes containing 100 capsules. S. L. W.

Diamidin* is a proprietary brand of 4 : 4′-diaminodiphenylsulphone disodium formaldehyde sulphoxylate, and is claimed to have given promising results in the oral treatment of leprosy. The dose is 0·33 g., increased to 1 g., daily, at least 6 months' treatment being required before clinical effectiveness can be evaluated. A combination of promin intravenously and diamidin orally has been suggested as a logical procedure. The treatment may give rise to various toxic reactions, and the blood picture must be watched carefully; it is common for patients to develop an anæmia during the initial stages of treatment. Enteric-coated tablets of diamidin, each containing 0·33 g., are supplied in bottles of 100 and 1,000. S. L. W.

Nicorbin* is a combination of aneurine hydrochloride, 1 mg., ascorbic acid, 25 mg., and nicotinic acid, 10 mg., in tablet form. 3 tablets supply the approximate daily adult requirements for these vitamins. The tablets are indicated in minor deficiency states manifested by debility and fatigue and for routine prophylaxis during pregnancy and lactation. Nicorbin is issued in bottles of 25 and 100 tablets. S. L. W.

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NEW APPARATUS

A REFLECTED LIGHT MELTING-POINT APPARATUS

MELTING-POINT determinations in organic chemistry laboratories have traditionally been made by observation of the material in a capillary tube, suspended in various liquid baths. The utilisation of the high conductivity of copper in gas-heated and later electrically-heated copper blocks, gave the advantages of determination of melting-points over wide temperature ranges, without fumes and corrosion, and of close observation of the melting material by means of a lens. Such blocks have been described by many workers[1], and when used with a thermometer calibrated in the block against A.R. chemicals such as those listed by Vogel[2], give melting-points which are reproducible and accurate. A disadvantage of blocks, however, has been that the necessary illumination of the sample has been achieved by light shining through the block and thus directly into the observer's eye. In a modification developed in the laboratories of May and Baker, Ltd., and now made available by Townson and Mercer, Ltd., two horizontal holes are drilled in the block so as to converge on the thermometer bulb. The sample is arranged immediately in front of the bulb and illuminated through one of the horizontal tubes by a shaded 6-volt bulb; the surface of the thermometer bulb aids in reflecting light through and round the sample, which is viewed by means of a lens adjusted at the other horizontal hole. This gives an extremely clear view of the behaviour of even a single crystal without eye-strain, particularly when a series of determinations is made in succession. The apparatus now commercially available as "Type 2" is compact and at the same time embodies a number of refinements in the electrical circuit designed to facilitate the determination of all melting-points. The block, adjustable for height, is mounted on a vertical rod rising from the back of a sheet metal case. The top of this case bears an ebonite panel on which is mounted a self-cleaning rotary switch, a spring-loaded push button, and a movable jack with three sockets marked H, L and M. The rotary switch selects the tappings on the transformer and thus the temperature to which the block is to be heated; the jack controls the rate of rise in temperature the sockets corresponding to high, low and medium rates, medium being regarded as normal. The spring-loaded button, when depressed, gives an approximately 50 per cent. increase in wattage input to the heating windings over the value for the maximum input from the highest tapping on the rotary switch, and thus enables quick heating of the block for readings at higher temperatures. The spring loading ensures that no damage can be done to electrical windings or thermometer, since the observer must be present while it is operated and it cannot therefore be left on by mistake. The tappings have been arranged for smooth transfer before the rate of rise on the next lowest one has become slow and the instrument has been designed to cool at such a rate that after switching off there is not an undue delay before the next determination can be made.

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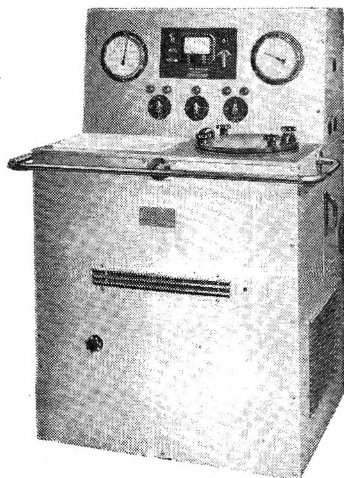

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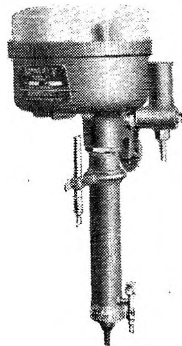

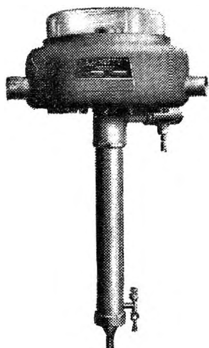

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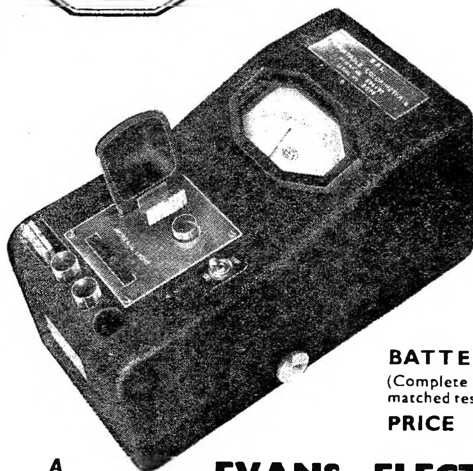

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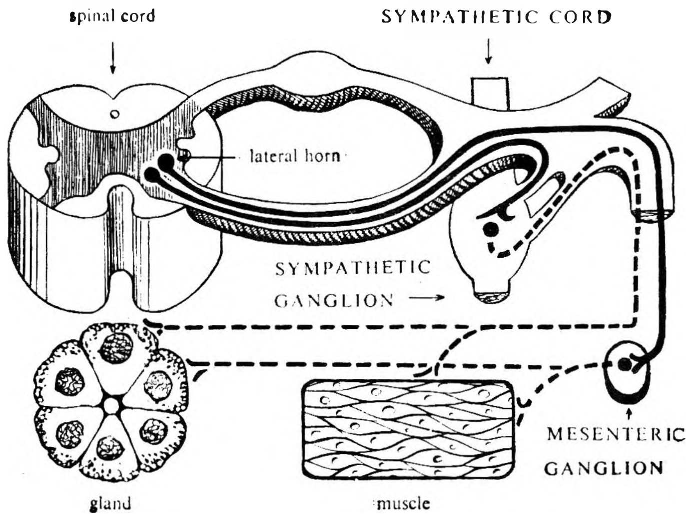

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