REVIEW ARTICLE

THE CHEMOTHERAPY OF TROPICAL DISEASES

PART II. DISEASES CAUSED BY RICKETTSIÆ, BACTERIA, SPIROCHÆTES AND WORMS

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IN Part I of this review recent developments in the chemotherapy of the tropical diseases caused by protozoa were summarised.[1] Part II deals with the more important diseases caused by rickettsiæ, bacteria and worms. The chemotherapy of virus diseases, including lymphogranuloma venereum and trachoma was reviewed recently by Findlay.[2] Gonorrhœa and syphilis, although important diseases of the tropics are outside the scope of the present review.

The most important advance in the treatment of many rickettsial and bacterial infections has been the use of antibiotics. The properties and range of activities of these drugs were summarised in this journal by Abraham.[3]

RICKETTSIAL DISEASES

Louse-borne (epidemic) and murine (endemic) typhus, scrub typhus (tsutsugamushi fever), Rocky Mountain spotted fever, fièvre bouttoneuse, rickettsialpox and Q fever are all diseases caused by rickettsiæ. These parasites are intermediate in size and organisation between the largest viruses and the smallest bacteria. In 1947, it was shown that chloramphenicol had marked activity against experimental rickettsial infections.[4] The first human cases of epidemic and endemic typhus were treated in Mexico,[5] and a team of American Army research workers headed by Smadel, took supplies of chloramphenicol to Malaya for a more extended trial against scrub typhus.[6,7] Fever is controlled very rapidly by the drug, but relapses occur if treatment is begun early in the disease, before the immunity response of the host is well-developed.[6,7,8] The action of the drug is rickettsiostatic and not rickettsiocidal; the immunity and defence mechanisms of the host finally destroy the parasite. Chloramphenicol is too expensive (and perhaps also too toxic[9]) for routine use as a prophylactic, and it is probable that the most satisfactory method of producing immunity will be by means of a living vaccine, with chloramphenicol to control the attack. Aureomycin and terramycin are effective against scrub typhus but chloramphenicol is considered to be the drug of choice.[10] Chloramphenicol is also active in Rocky Mountain spotted fever,[11,12,13] murine typhus[14] and epidemic typhus.[15,16] Aureomycin has given good results in rickettsialpox[17] and fièvre bouttoneuse,[18] and may be more effective than chloramphenicol in epidemic typhus.[15] It has given variable results in Q fever.[19,20] Terramycin is active against all rickettsial infections and has given good results in Rocky Mountain

spotted fever[21] and rickettsialpox.[22] It has high activity in experimental Q fever.[23]

Diseases Caused by Bacteria and Spirochætes

Bacillary Dysentery.

The introduction of the sulphonamides, all of which have some action against the dysentery bacilli, has greatly changed the position with regard to the treatment of this disease. Boyd[24] writes that: "The use of these drugs in bacillary dysentery is one of the major advances in tropical medicine, the advantages of which can be fully appreciated only by those who had experience of dysentery in the days before they were available."

The less readily absorbed sulphonamides such as sulphaguanidine, succinylsulphathiazole and phthalylsulphathiazole are most suitable for use in tropical climates because they have less tendency to produce renal damage when the fluid intake is low.

Plague.

A small epidemic of plague occurred in Taranto in 1945. It was satisfactorily controlled by determined public health measures for the destruction of rats and fleas.[25] In the treatment of the disease, sulphonamides have some action against the plague bacillus, but are not to be relied upon for the treatment of an established infection. They are of value for the prophylaxis of contacts; sulphadimidine and sulphadiazine are the most satisfactory derivatives.[26] The best drug used so far for the treatment of bubonic or septicæmic plague is streptomycin,[26,27,28,29,30,31,32] which rapidly aborts the attack. Streptomycin is of no value as a prophylactic because it is excreted too rapidly. Aureomycin and terramycin are effective against the plague bacillus, but all the antibiotics have the disadvantage of being too expensive for routine use on a large scale.

Cholera.

The problem of the treatment of cholera is a special one because death occurs from extreme loss of body fluids brought about by continued severe diarrhœa and vomiting. Unless the water and electrolyte balance of the patient is corrected, any chemotherapeutic treatment is worthless and may be harmful. In at least one instance, sulphonamides have actually increased the mortality in groups of patients to whom they were given, above the mortality in a control group which received no specific drugs.[33] A number of drugs are active against the cholera vibrio, but they are ineffectual unless given very early in the course of the disease, before serious fluid loss has complicated the picture. Experiments with chloramphenicol and terramycin in mice have shown that animals are protected if a small dose of drug is given within 5 hours of infection, but that enormous doses have no effect if given 7 hours after infection.[34] It has been suggested that chloramphenicol might be useful as a prophylactic in a population exposed to the risk of a cholera epidemic,[35] but this

drug is now known to be dangerous if given for prolonged periods.[9] Researches by Collier and his associates have shown a series of pteridine derivatives to be vibriostatic *in vitro*,[36] and to be effective in the early stages of infection in mice,[37] but no reports of clinical trials have yet appeared.

Enteric Fever.

Enteric fever is not confined to the tropics but is more common in warm countries in which the standards of sanitation are low and the water supplies may be suspect.

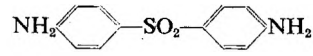
When Smadel and his team of American workers were making clinical trials with chloramphenicol against scrub typhus in Malaya, they noted that the drug also had a dramatic action upon typhoid fever.[7,38,39] At the end of the 3rd or 4th day the fever abated, and if treatment was continued for 14 days, few relapses occurred.[40] The drug has since been used in many areas with excellent results[41,42,43,44,45,46,47] and the mortality from the disease has been greatly reduced. Chloramphenicol is bacteriostatic; it does not prevent the occurrence of perforations or hæmorrhages caused by the separation of sloughs from existing lesions. With streptomycin and aureomycin available, it is now possible to treat complications of typhoid conservatively, but penicillin should not be used because it appears to have an action antagonistic to that of chloramphenicol. Polymyxin and aureomycin have no action against the typhoid bacilli.[39] Relapses are more common in people treated with chloramphenicol than in those which survive and recover from the disease without it, because it interferes in some way with the development of immunity. For the same reason, relapses are especially likely to occur in those who have been treated early in the course of the infection. Promising results have been obtained in the reduction of incidence of relapses, by intensifying the immunity response with injections of T.A.B. vaccine.[46,47] Chloramphenicol often causes vomiting; also, the release of endotoxin from dead typhoid organisms may cause an exacerbation of symptoms, with circulatory failure, early in the course of treatment. It is therefore advisable to begin with small doses of drug, and not with a large "loading dose." Cortisone reduces the effects of typhoid toxin, but increases the danger of perforation. Chloramphenicol has no permanent curative effect upon fæcal carriers of the typhoid bacillus.[39] Evidence is accumulating that in some patients, especially after prolonged administration of chloramphenicol, damage to the blood-forming tissues occurs, resulting in leucopenia or aplastic anæmia. This is likely to be a property of the nitrobenzene radical in the chloramphenicol molecule.[9]

Leprosy.

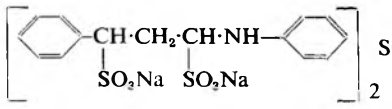
Leprosy usually takes one of two forms: the lepromatous, an active disease with nodules of granulation tissue in the skin and mucous membranes, and the neural in which the peripheral nerves are the main site of attack. Lepromatous leprosy has been treated for many years with chaulmoogra oil and its preparations. When used in large doses

for long periods the disease is controlled, but relapses are very common. Sulphapyridine was tried in leprosy; it produced an erythema nodosum (lepra) reaction, but had no beneficial action in doses which had toxic side-effects. However, the fact that a lepra reaction was produced, gave promise that among compounds related to sulphapyridine a specific for the disease might be found. The leprosy bacillus *Mycobacterium lepræ* is closely allied to *M. tuberculosis* and it is not surprising that the new drugs which have shown promise in tuberculosis have all found their way to clinical trial in leprosy.

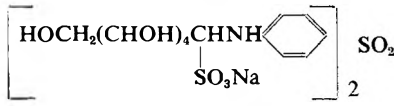
Sulphones. Dapsone (I, diaminodiphenylsulphone) was first tested against streptococcal infections in mice but was considered too toxic for use in man.[48] The less toxic derivatives solapsone (II sulphetrone, cimédone, 3668 R.P.), promin (III) and diasone (IV) followed and were shown to be effective in the treatment of lepromatous leprosy.[49,50,51,52,53,54,55,56]



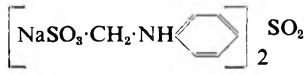
Dapsone (I)



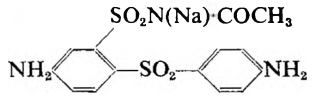
Solapsone (II)



Promin (III)



Diasone (IV)



Promacetin (V)

The substituted sulphones are expensive and large doses are required if they are given by mouth. They are broken down in the alimentary tract to the parent sulphone[57,58] and are wasteful. A return has therefore been made to dapsone itself, which, although more toxic, is cheap and is effective in small doses.

Although clinical improvement often occurs within a few weeks, the sulphones act slowly upon leprosy bacilli. First a change occurs in the morphology of the organisms, their multiplication is suppressed and the lesions regress. The patient frequently shows a lepra reaction, which is a good prognostic sign. The final disappearance of acid-fast material from the lesions may take years of continuous treatment and to ensure that cure is permanent, it is necessary to wait for a further period of 5 or more years because the recovery of surviving organisms and relapse of the disease is also a slow process. Neural leprosy and lepromatous eye lesions respond only poorly to sulphone therapy.

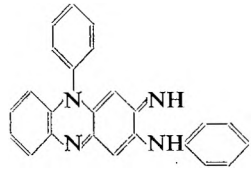
Lowe[59,60,61,62] has used dapsone by mouth in Nigeria and finds it to be cheap, effective, and free from serious toxic side-effects. Similar good results have been reported by Muir from India,[63,64,65] and by French workers.[66,67,68,69] However, the use of dapsone is not without its dangers, and the effective dose is not far removed from the toxic range. Apart from the lepra reaction, exfoliative dermatitis, anæmia, abdominal and joint pains quite frequently occur[67,70,71] and in W. Africa, mononucleosis,[71] toxic hepatitis[72,73] and psychosis have been reported. The patients are weak and depressed during the 2nd month of treatment although many become acclimatised during the 3rd to 8th months.[74] Lepra reactions may be rendered less troublesome by the use of antihistamine drugs, adrenocorticotrophic hormone or cortisone.[56,64,75] Some workers find it most convenient to give dapsone by injection in aqueous suspension,[76] or dissolved in oil, in chaulmoogra oil or its esters.[77,78] In India, where the anti-leprotic oils are much cheaper than the sulphones, advanced lepromatous cases have been given dapsone for 6 months to decrease their infectivity, and thereafter treatment continued with hydnocarpus oil.[79] Cochrane[80,81,82] finds dapsone to be suitable only if patients can be watched carefully for toxic reactions, and considers it too dangerous for mass-treatment in out-patient clinics. He agrees that it is wasteful to use substituted sulphones by mouth, but finds solapsone to be less toxic, and no more expensive than dapsone if it is given by injection. The fate of substituted sulphones given by injection is not yet clear. Solapsone partly dissociates in solution to form a mono-substituted derivative and is excreted as such, without appreciable breakdown into dapsone. It may be that monosubstitution is the normal method of detoxication of dapsone in the body, and if so it would be reasonable to give the substituted derivative by injection instead of risking the toxic effect of the parent sulphone by mouth.[82,83] French workers have reported similar properties for the monosubstituted succinyl derivative "1500 F."[84,85,86] "Sulphone cilag" (4:4′-diaminodiphenylsulphone-*N*-sulphate) and promacetin (V) are also effective; it has been shown that promacetin does not break down appreciably to dapsone in the body.[87]

Antibiotics. Although too expensive for general use, and too toxic for prolonged administration, streptomycin and dihydrostreptomycin have been found to be useful adjuncts to sulphone therapy. They are of particular value in tuberculoid leprosy, in cases with eye lesions, and in people with iodiosyncrasy to the sulphones.[56,88,89,90,91] Aureomycin is also useful for mucous membrane, skin and eye lesions[89,92]. Chloramphenicol is of little value.[93]

Thiosemicarbazone. Thiosemicarbazone is less active in leprosy than the sulphones and although clinical improvement frequently occurs, the bacteria remain and new skin lesions may develop during treatment.[94,95,96,97] It is a useful adjunct, especially in patients who are sensitive to sulphones.[91,98,99] The derivative amithiozone (4-acetylaminobenzalthiosemicarbazone) is also reported to have promising activity.[56]

Other Drugs. *p*-Aminosalicylic acid is not very active in leprosy, but is of use as an adjunct in some patients.[89] It is too early to judge the

value of isoniazid (isonicotinic acid hydrazide) but clinical improvement of lepromatous cases has been reported.[100] The phenazine dye "B283" (VI) has recently been used in Nigeria. Its action appears to be rapid and there have been no toxic side-effects, but it is too early to assess its value with certainty.



B.283 (VI)

Yaws.

This spirochætal disease usually responds rapidly to treatment with neoarsphenamine. A new arsenical preparation "STB" (4-oxy-3-acetyl-aminophenylarsenoxide), which is effective by mouth has recently been used with success,[101,102,103] but side-by-side comparisons with other methods of treatment have not been made. Antibiotics are also valuable in the treatment of yaws, particularly in cases which do not respond to the arsenicals. Procaine penicillin with aluminium stearate in oil is useful for mass treatment and is particularly effective in early cases.[104,105] Aureomycin[106,107,108,109] and chloramphenicol[110,111] are effective by mouth but not in every case. Results with terramycin have also been variable.[112,113]

The antibiotics, both locally and systemically, have given excellent results in the treatment of tropical ulcers.[114,115,116]

Relapsing Fever.

Louse-borne relapsing fever caused by *Treponema recurrentis* usually responds well to treatment with arsenicals. The tick-borne disease, caused by *Tr. duttoni*, is a much more difficult problem. Treatment with neoarsphenamine is rarely successful, unless given very early in the course of the disease.[117] Penicillin has proved disappointing in both the tick-borne and louse-borne infection.[117,118] Streptomycin has been reported effective against a strain of *Tr. duttoni* which was resistant to arsenicals and penicillin,[119] and aureomycin has also given promising results.[120,121] Tests with laboratory infections of *Tr. duttoni* in mice and *Tr. persicum* in rats have shown terramycin to have high curative activity,[122,123] and there is hope that the human infection may also be sensitive to this antibiotic.

Diseases Caused by Worms

Schistosomiasis.

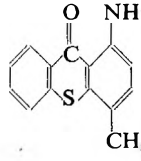
Schistosomes have a life-history similar to that of the liver fluke. The adult worms are found in the blood vessels of man, and the intermediate hosts are snails which live in irrigation canals and water-holes. *Schistosoma hæmatobium* infests mainly the veins of the bladder, and the passage of the spiny eggs through the wall causes inflammation, pain, hæmaturia and anæmia. *S. mansoni* infests mainly the large bowel, and heavy

infections also give rise to pathological changes in the liver and lungs. *S. mansoni* is common in S. America, and both species are widespread among the native populations of Africa and the Middle East; a recent estimate gave a figure of 12 million infected people in Egypt alone.[124] *S. japonicum* infests the small and large intestines and causes similar effects to those of *S. mansoni*; it is a serious public health problem in the Far East and the Pacific area.[125]

Many chemicals have been used to attack the parasite at various stages of its life-history; repellants such as dimethyl phthalate are effective for a time in preventing penetration of the skin by cercariæ, and many substances are used in campaigns against snails.

Organic Antimonials. Until recently, the only drugs of any value in the treatment of the infection in man were the organic antimonials. Tartar emetic, given by intravenous injection is a cheap but toxic remedy; work in Rhodesia has shown that it can be given intensively in short courses of large doses with surprisingly few serious accidents.[126] However, in patients with liver damage resulting from heavy *S. mansoni* infections the toxic effects have been more severe. Less toxic, but rather less efficient remedies are stibophen (fuadin) and anthiomaline which are widely used in Egypt, S. America and the Pacific Islands. Stibophen was introduced by Schmidt[127] in 1930 and the first reports of its use against *S. mansoni* and *S. hæmatobium* in Egypt were encouraging. Later, however, the proportion of failures increased,[128] and the drug has given varying results in the hands of different workers.[129,130,131,132] Against *S. japonicum* stibophen has also given variable results,[133,134,135,136] but schedules of treatment have also varied a great deal. The drug stops the production of eggs by the female worms while it is being given, but if the course is not sufficiently prolonged, the worms recover and relapses occur. Stibophen has the advantage of being less irritant than tartar emetic, and it is given intramuscularly. Anthiomaline has properties similar to those of stibophen, and has given similar results. Quinquevalent antimonials are of very little use in schistosomiasis, but the tervalent compound "triostam" corresponding to sodium stibogluconate (which is used in leishmaniasis[1]) has recently been used in Egypt,[137] Iraq[138] and Brazil. It appears to be less toxic than tartar emetic, but has not yet been used extensively enough for a true assessment of its value to be made.

Xanthone Derivatives. Kikuth and his colleagues have studied the action of a great many substances upon experimental *S. mansoni* infections in mice. They showed that members of a series of xanthone and thioxanthone derivatives were effective when given by mouth.[139,140,141] The most active compound was the thioxanthone derivative lucanthone (VII, miracil D, nilodin, R.P. 3735); the other related xanthone derivatives



Lucanthone (VII)

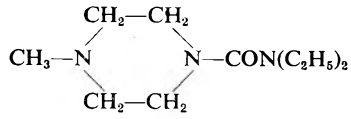
miracils A, B and C were either less potent, or too toxic at therapeutic dose levels to be of use. The series showed a high degree of specificity of action, and if the 4-methyl group of lucanthone was lacking, or was replaced by a chloro- or methoxy-group, the activity was lost. Also, unlike antimalarial drugs which have the same basic side-chain as lucanthone, any alteration in the length of the chain greatly reduced, or abolished activity.[142] Lucanthone has been found to be active against *S. hæmatobium* and *S. mansoni* in man when administered by mouth, provided that sufficiently long courses of treatment are given.[143,144,145,146,147] It has been less successful in *S. mansoni* than in *S. hæmatobium* infections, and from the small number of trials so far made, it appears to be of little value against *S. japonicum*. The drug has the disadvantage of causing gastrointestinal irritation, with nausea, vomiting and other side-effects in therapeutic doses. The side-effects are very much more troublesome among some peoples such as the Egyptians, than among others, and in susceptible individuals it is difficult to administer sufficient drug to cure the disease. A very full study of the action of different preparations of lucanthone was made by the Bilharzia Unit of the Medical Research Council and the Egyptian Ministry of Public Health between 1947 and 1950.[144] The sparingly soluble methylene-bis-hydroxynaphthoate caused less toxic reactions, but was less effective than the hydrochloride; the salicylate gave promise of being the most suitable salt. Enteric-coated tablets caused fewer side-effects, but among some peoples such as those of Rhodesia, produced fewer clinical cures than the uncoated tablets.[148,149] It is a very difficult matter to be certain that a patient has been cured of schistosomiasis, especially under conditions in which there is a constant risk of reinfection. Newsome states that: "If no serious idiosyncrasies appear and care is taken in the treatment of patients with badly damaged livers and kidneys, our impression is that a suitable miracil preparation will prove as effective but much less dangerous than tartar emetic, and more effective and less dangerous than fuadin."[144]

Filariasis.

Filariasis occurs in almost all tropical countries. The adult worms of *Wuchereria bancrofti* live in lymphatics and connective tissue; the larval forms or microfilariæ circulate in the blood. Long-standing occlusion of lymphatic drainage may result in elephantiasis, and where this is advanced, only surgical treatment is of value. *Onchocerca volvulus* lives in nodules in the skin and the microfilariæ are found in the tissue surrounding the nodules. For many years no satisfactory drugs were known for the treatment or prophylaxis of filariasis. Studies upon *Dirofilaria immitis*, a filarial worm of the dog,[150] indicated that organic antimonial compounds reduced the number of microfilariæ, but results of treatment with stibophen and other antimonials in man have been on the whole disappointing. Since 1944, *Litomosoides carinii* of the cotton rat has been used in the study of possible filaricides, and Culbertson[151,152,153] found neostibosan and neostam to be of value both in the laboratory infection and against *W. bancrofti* in man. Neostibosan

received only limited clinical trials because further extensive work with the cotton rat infection by Hewitt and his colleagues[154] led to the discovery of the action of the more active and less toxic piperazine derivatives.

Diethylcarbamazine. The most active compound of this series so far is diethylcarbamazine (VIII, hetrazan, banocide, R.P. 3799), which is now in use in many parts of the world. The drug is active by mouth



Diethylcarbamazine (VIII)

and causes rapid disappearance from the blood of the microfilariæ of most species of filarial worms. The most satisfactory salt to use is the dihydrogen citrate. Hawking, Sewell and Thurston[155] noted that in cotton rats which had received a dose of diethylcarbamazine, the microfilariæ were trapped in the liver sinusoids and attacked there by leucocytes. A similar action takes place with *W. bancrofti*[156] and *Loa loa*[157] in man. The action of the drug upon the larvæ resembles opsonisation, making them susceptible to attack by the host's defence mechanisms. In Puerto Rico,[158] Brazil,[159] Indochina,[160] E. Africa,[161,162] and the Dutch E. Indies,[163] diethylcarbamazine has proved effective in clearing the microfilariæ of *Wuchereria* from the blood; in some early cases elephantiasis has been reduced. There is still doubt as to whether the drug has a lethal effect upon adult worms,[164] but studies in Puerto Rico[165] and the Virgin Islands[166,167] showed that mass treatment of a population with diethylcarbamazine caused a drastic reduction in the percentage of mosquitoes carrying the infection and that the reduction of infection in both man and mosquito was maintained for a considerable time without further treatment. This suggests that there is a prolonged or permanent effect upon the adult worms, because otherwise they would have recovered and produced further broods of microfilariæ to infect the insect vectors.

Diethylcarbamazine sometimes produces headache, nausea and vomiting, but these side-effects are not usually serious. It also has the disadvantage (which must be shared by all drugs which kill filarial worms), that the death of large numbers of worms and the consequent release of foreign proteins may be accompanied by an allergic reaction. This is not usually severe with *W. bancrofti*, but is more troublesome with *W. malayi*[168] and with *Onchocerca.*[169,170] The reaction may be dangerous in *Onchocerca* infections, particularly in cases in which the eye is involved. The incidence of serious reactions may be lessened by administering an antihistamine drug; some workers give the antihistamine prior to the first dose of diethylcarbamazine.[171] Although microfilariæ of *Onchocerca* rapidly disappear from the tissues, diethylcarbamazine has very little action upon the adult worms of this species, which continue to live in the skin nodules.[156,172,173,174] Recent work in Guatemala[175,176] and the Gold Coast[177] has shown that suramin, or suramin together with diethylcarbamazine, are more effective than diethylcarbamazine alone in the

treatment of onchocerciasis. *Loa* infections respond well to diethylcarbamazine; both microfilariæ and adult worms are killed, and the proportion of allergic responses is high.[178,179,180,181] *Acanthocheilonema perstans* infections (which are non-pathogenic) are much more resistant and the drug has very little action upon either microfilariæ or adults.[161,182,183]

Other Drugs. A very large number of compounds has now been tested in experimental filariasis; a few have shown activity warranting clinical trial. Arsenamide (*p*-[bis-(carboxymethylmercapto) arsino] benzamide) which is active against *D. immitis* and *L. carinii*,[184] has been found of use in *W. bancrofti* infections,[185] and is considered to be worthy of further trial in onchocerciasis.[172] The organic antimonial M.Sb[1] has a prolonged prophylactic action in the cotton rat,[186] but has not yet been tried clinically. A number of styrylquinoline and cyanine dyes are effective in experimental filariasis[187,188] but so far none has had any activity in human infections.[188,189] Hawking[189] uses the example of the dye, methylene violet, to point out the desirability of making early clinical trials with any new series of chemotherapeutic substances.

Other Helminth Infestations.

A review of the chemotherapy of helminth infestations has been given by Wigand.[190] Nothing is yet known to act upon hydatids, paragonimus, trichinella, or the circulating larvæ of ascaris.

Tæniasis. Extract of male fern is the standard treatment for tape worm, but other drugs have recently been used. Mepacrine has given good results in some cases,[191,192] but the effective dose is very large and often produces vomiting. *Tænia saginata* has also been successfully treated by the introduction of an emulsion of hexylresorcinol directly into the duodenum.[193] *Diphyllobothrium latum* is sometimes resistant to treatment with male fern, and thymol has been advocated for this parasite.[194]

Ascariasis. Oil of chenopodium is still widely used for expelling round worms; it is effective, but toxic if used in large doses.[195] Diethylcarbamazine has been tried, but reports of its efficacy are conflicting.[196,197]

Enterobiasis. Threadworms are found both in tropical and temperate areas. Gentian violet and diphenan are used against them but neither is entirely satisfactory. Phenothiazine is still used in Germany,[198] although this drug is generally regarded as too toxic for use in man. Recently benzene hexachloride has been reported to give good results.[199]

Ankylostomiasis. Ankylostoma infests a very high proportion of native peoples in the tropics. Like many other helminthic diseases its control is largely a public health problem. Halogenated hydrocarbons such as carbon tetrachloride and tetrachlorethylene are the drugs most widely used against the worm, but are not without danger; it is not considered justifiable to use them for mass-treatment. There is need for an effective and non-toxic substance to eradicate these worms.

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[*Useful summaries, reviews and works of reference are marked with an asterisk.*]

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RESEARCH PAPERS

FACTORS AFFECTING THE POTENTIATION OF BARBITURATE ACTION BY TETRAETHYLTHIURAM DISULPHIDE*

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It has been demonstrated clinically[1] that ascorbic acid will alleviate the reaction induced by ethanol in a patient sensitised by prior dosage with tetraethylthiuram disulphide. It has also been shown[2] that sodium ascorbate, *in vitro*, will reverse the potent inhibitory effect of tetraethylthiuram disulphide on the dehydrogenation of acetaldehyde by liver aldehyde dehydrogenase. In the latter reaction, reduced glutathione was found to be considerably more active than sodium ascorbate.

More recently it has been observed that prior administration of tetraethylthiuram disulphide to rats or guinea-pigs[3] or to mice[4] will markedly potentiate the narcotic effect of barbiturates. It was suggested[3] that this potentiation might come about through inhibition of aldehyde dehydrogenase by both drugs since Persky *et al.* have suggested[5] that aldehyde dehydrogenase inhibition may be, at least in part, the mechanism of barbiturate action. Giarman *et al.* have postulated[4] that tetraethylthiuram disulphide may potentiate thiopental anæsthesia in mice by virtue of its ability to inhibit rat liver xanthine oxidase.[6] Giarman *et al.* also reported[4] that tetraethylthiuram disulphide showed no potentiating effect until it had been administered daily for 3 days. This is not in agreement with the work of Graham *et al.*[3] who demonstrated barbiturate potentiation in rats and guinea-pigs following a single dose of tetraethylthiuram disulphide.

It was of interest therefore to determine whether or not the administration of sodium ascorbate or of reduced glutathione would decrease the intensity of barbiturate-induced narcosis or decrease the degree of potentiation which is induced by prior administration of tetraethylthiuram disulphide. Experiments also were conducted to ascertain whether or not tetraethylthiuram disulphide potentiation of barbiturate narcosis was increased by repeated administration of the drug as compared with the effect of a single dose.

Experimental Method and Results

Female albino rats in the weight range 120 to 144 g. were assigned at random to 12 groups of 10 animals. Glycerol was given to 60 of these rats by stomach tube at the rate of 0·5 ml./100 g. of body weight: glycerol containing 80 mg. of tetraethylthiuram disulphide per ml. was similarly

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administered to the remaining 60 rats. Approximately 24 hours later, the rats were dosed intraperitoneally with freshly prepared sodium ascorbate solution at the rate of 0, 0·5, or 1·0 g./kg. of body weight, followed, 30 minutes later, by either 65 or 94 mg. of sodium cyclural per kg. The sleeping times[3] of these animals were recorded and these results, together, with the experimental design, are shown in Table I along with the analysis of variance of the findings.

TABLE I

THE EFFECT OF SODIUM ASCORBATE ON TETRAETHYLTHIURAM DISULPHIDE POTENTIATION OF BARBITURATE-INDUCED NARCOSIS IN FEMALE RATS

Sodium ascorbate mg./kg.	Sodium cyclural mg./kg.	Tetraethylthiuram disulphide	
		0 mg./kg.	400 mg./kg.
		Mean sleeping time in minutes	
0	65	49	97
500	65	50	98
1000	65	49	100
0	94	84	140
500	94	79	162
1000	94	79	153

ANALYSIS OF VARIANCE*

Source	Degree of freedom	Mean square	F
1. Tetraethylthiuram disulphide ..	1	2·4357	37
2. Sodium cyclural	1	1·1780	18
3. Sodium ascorbate	2	·0018	<1
4. Interaction 1 × 2	1	·0064	<1
5. Interaction 1 × 3	2	·0038	<1
6. Interaction 2 × 3	2	·0012	<1
7. Interaction 1 × 2 × 3	2	·0040	<1
8. Within groups	106	·0065	

* The observed sleeping times were transformed into logarithms prior to statistical analysis.

As was previously observed[3] tetraethylthiuram disulphide significantly prolonged the period of narcosis caused by sodium cyclural. However, sodium ascorbate at this level of administration failed to affect the results in any way. A similar experiment was conducted in which male rats were injected with 0, 50 or 100 mg. of reduced glutathione per kg. in place of sodium ascorbate. The weight range of these rats was 103 to 146 g. and the sodium cyclural dosages were 75 and 109·4 mg. per kg. of body weight. The results obtained in this experiment are recorded in Table II. The conclusions drawn from this and succeeding experiments were substantiated by statistical analyses of the results.

There was no significant effect of reduced glutathione in shortening sleeping time induced by sodium cyclural in the presence or absence of tetraethylthiuram disulphide. A repetition of this experiment on female rats with doses of 100 or 200 mg. of reduced glutathione likewise gave negative results. Further experiments were conducted in which reduced glutathione was injected subcutaneously 30 minutes after the tetraethylthiuram disulphide administration or 30 minutes prior to dosage with sodium cyclural, or both. Even at the higher rate of 400 mg. of reduced

TABLE II

EFFECT OF REDUCED GLUTATHIONE ON TETRAETHYLTHIURAM DISULPHIDE POTENTIATION OF BARBITURATE-INDUCED NARCOSIS IN MALE RATS

Reduced glutathione mg./kg.	Sodium cyclural mg./kg.	Tetraethylthiuram disulphide	
		0 mg./kg.	400 mg./kg.
		Mean sleeping	time in minutes
0	75	20	31
50	75	15	33
100	75	19	30
0	109·4	27	56
50	109·4	26	45
100	109·4	23	48

glutathione per kg. of body weight (divided dose) no shortening of sleeping time was observed.

To study the effect of repeated dosage with tetraethylthiuram disulphide on barbiturate-induced narcosis, 120 female rats weighing 110 to 139 g., were assigned at random to 12 groups of 10 rats. 4 groups were given 0, 0·05, 0·1 or 0·2 g. of tetraethylthiuram disulphide per kg. of body weight orally in glycerol on each of 3 successive days, 4 groups were similarly dosed on days 2 and 3, and the remaining 4 groups were dosed only on the third day. On the fourth day sodium cyclural was administered intraperitoneally to all the rats at the rate of 65 mg./kg. of body weight. Sleeping times were recorded. A similar experiment was carried out with male rats. Statistical analysis of the results of both experiments revealed no significant effect of repeated dosage with tetraethylthiuram disulphide. Giarman *et al.* used a rather large dose of tetraethylthiuram disulphide in mice (approximately 1·25 g. per kg.) in order to demonstrate the effect of multiple dosing. This level is high for rats of this colony and it was found previously[3] that little increase in potentiation occurred when single doses of tetraethylthiuram disulphide above 0·5 g. per kg. were given. However, the experiment was repeated with female rats using 0, 0·1, 0·2 and 0·4 g. of tetraethylthiuram disulphide per kg. and the sodium cyclural was increased to 80 mg. per kg. The results of this experiment, shown in Table III, were analysed statistically.

TABLE III

EFFECT OF REPEATED DOSAGE WITH TETRAETHYLTHIURAM DISULPHIDE ON THE POTENTIATION OF BARBITURATE-INDUCED NARCOSIS IN FEMALE RATS

Tetraethylthiuram disulphide mg./kg.	Number of doses of tetraethylthiuram disulphide		
	1	2	3
	Mean	sleeping time in	minutes
0	61	55	53
100	73	79	60
200	97	95	111
400	113	118	110

The experiment showed quite clearly that rats given single doses of tetraethylthiuram disulphide are just as susceptible to subsequent action of barbiturate as rats given 3 such doses on successive days.

Discussion

While sodium ascorbate will reverse the inhibitory effect of tetraethylthiuram disulphide on liver aldehyde dehydrogenase *in vitro* and will alleviate the ethanol-tetraethylthiuram disulphide reaction in man, it will not, under the conditions employed in this work, decrease the potentiating effect of tetraethylthiuram disulphide on barbiturate narcosis in rats. While ferrous iron and ascorbic acid effectively counteract the ethanol-tetraethylthiuram disulphide reaction,[7] Giarman and his co-workers[4] were unable to find any effect of this combination on the tetraethylthiuram disulphide potentiation of thiopental narcosis in mice. Similarly although reduced glutathione was found to be very potent *in vitro* in reversing the inhibitory effect of tetraethylthiuram disulphide on liver aldehyde dehydrogenase, *in vivo* reduced glutathione, as used in these experiments, had no ability to shorten the prolonged narcosis in rats given tetraethylthiuram disulphide and sodium cyclural. It would appear therefore, that the mechanism by which tetraethylthiuram disulphide causes sensitisation to ethanol is different in some respect from that by which it potentiates the effect of barbiturates. The elucidation of this difference is a matter for further study.

Contrary to the findings of Giarman *et al.* with mice, repeated dosage of rats with tetraethylthiuram disulphide failed to increase their susceptibility to barbiturate. The inference is that rats can metabolise these amounts of tetraethylthiuram disulphide daily, exhibit some regulation of absorption, or can regenerate active enzyme to the extent that there is no cumulative effect and the maximum response is elicited by a single dose.

Summary

1. Neither sodium ascorbate nor reduced glutathione will decrease the potentiating effect of tetraethylthiuram disulphide on barbiturate-induced narcosis in rats.

2. Repeated administration of tetraethylthiuram disulphide failed to make rats more sensitive to barbiturate than did a single dose.

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DIURNAL VARIATION IN THE CARDIAC ACTIVITY OF LEAVES OF *DIGITALIS PURPUREA* L.

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VARIATION in the activity of digitalis leaf was first observed by William Withering[1] who concluded that it was a seasonal effect. Subsequent observers[2,3,4,5,6,7,8,9,10] have sought to show that it may depend on such factors as season, soil, temperature, age of plant, and the like. The results of these investigations have been mainly inconclusive, but the suggestions put forward by Dafert[7] seemed of sufficient interest to merit further study, and it is a consideration of them which has led to the present work.

Dafert[7] was interested in the diurnal movement of plant reserves and had shown that they accumulate in the leaves of plants during the daytime. As he considered glycosides to be reserve substances he suggested that the maximum glycoside content would occur in digitalis leaves in the late afternoon. He concluded from some experiments that this was so. Little reliance can be placed on his results, partly because he collected his material only twice daily—at shortly before dawn and between 5 and 6 p.m.—but chiefly because of the inadequate method of biological assay which he used.

Whilst it is not generally accepted that glycosides are plant reserves, it nevertheless seemed possible that diurnal variation in glycoside content might occur in the leaf. Our experiments were designed to determine this point, for, if such a diurnal variation exists, it will clearly be necessary to take account of it in any investigation of such factors as season, soil, or climate, which might also affect the activity.

MATERIAL AND METHODS

16 seedlings were transplanted from the foot of a solitary wild parent to a cultivated plot, as uniform as possible in soil, shade, drainage, etc. Soil heavy clay, calcium deficient. The plants were arranged in a 4 × 4 square. Material was collected in July of the second year at the onset of flowering. Batches of leaf were gathered at 20.00 hours G.M.T. and thereafter at 3-hourly intervals until 20.00 hours on the following day. 9 gatherings were made, each consisting of 1 cauline and 1 radical leaf from each of 4 plants. At each gathering leaves were collected from one plant in each row and column of the square. The plants sampled varied from batch to batch, but, owing to the odd number of gatherings taken, 12 of the plants were sampled twice and 4 of them three times. Immediately after collection the leaves were set to dry in an oven at about 56° C. The drying took from 1½ to 2 hours. The batches of dried material—each weighing 2 to 3 g.—were separately reduced to No. 60 powder on the following day and stored in sealed glass ampoules.

Each batch was evaluated by one of us (J.G.D.) by the B.P., 1948

process, based on the method first described by Knaffle-Lenz,[11] in which the intravenous lethal dose for cavies is determined. The potency of each batch was estimated with reference to the Laboratory Standard Preparation.

Extracts of the batches were prepared by continuous extraction with absolute ethanol for 6 hours.[12] Excess of ethanol was then removed by distillation until 4 ml. of extract remained for each 1 g. of digitalis powder. These extracts were diluted with an equal volume of water and stored at 0° C. until required.

Burn[13] recommends that extracts be diluted before administration so as to contain 0·125 I.U./ml. Extracts of unknown potency were diluted arbitrarily and, if necessary, were adjusted after the first 2 observations to approximate to this value. The lethal dose for cavies is not directly proportional to body weight, small animals requiring a greater dose per 100 g. than large ones.[14] To obviate the difficulty arising from this, only animals weighing approximately 500 g. were used: the mean weight of all the animals was 504 g. with standard deviation ±31 g. In each observation the first 5 ml. of diluted extract was administered at the rate of 1 ml./minute and the remainder at 0·3 ml./minute. The injection rates were maintained mechanically.

Experimental Data

From the volume of diluted extract used for each cavy, the lethal dose was calculated in terms of mg. of digitalis powder per 100 g. of body weight. Lethal doses of drugs are usually distributed log.-normally,[15] hence the log.-lethal doses are given in Tables I and III.

TABLE I

Log.-lethal doses to cavies of the Laboratory Standard Preparation

	Log.-lethal doses of Laboratory Standard Preparation in mg./100 g. of body-weight			
	Extract No. 1		Extract No. 2	
Cavy No.	Aug. 19, 1949	Aug. 22, 1949	Aug. 25, 1949	Sept. 16, 1949
1	1·33846	1·44576	1·29513	1·29711
2	1·25768	1·27439	1·21985	1·40993
3	1·40654	1·33102	1·34184	1·31239
4	1·34004	1·31408	1·33364	1·34339
5	1·27692	1·31069	1·33707	1·34125
6	1·34223	1·39076	1·40976	1·36154
7			1·32939	1·30362
8			1·30188	1·36136
9			1·28285	1·30620
10			1·26787	
11			1·37015	
Log-means	1·32698	1·34445	1·31722	1·33742

Grand mean log.-dose = 1·32984

Table I gives the data from which the grand mean log.-lethal dose of the Laboratory Standard Preparation was calculated. This mean was obtained from observations made on 4 different days, using 2 independently prepared extracts. The worth of the assays of the experimental

batches is dependent on (i) the consistency of the lethal dose, (ii) the consistency of the extraction process, and (iii) the stability of the extracts when stored for a short period. The analysis of variance presented in Table II shows that there is no significant difference between the 4 mean lethal doses: thus the mean lethal dose was constant from day to day; the 2 extracts were of equal potency; they were unaffected by storage.

TABLE II

ANALYSIS OF VARIANCE OF LOG.-LETHAL DOSES FOR THE STANDARD PREPARATION

Sources of variance	Degrees of freedom	Sums of squares	Mean squares
Between days ..	3	0·00360	0·00120
Within days	28	0·07116	0·00254
Total	31	0·07476	

Table III gives the data for all the observations on the experimental batches, the mean log. dose for the Laboratory Standard Preparation being the grand mean from Table I. The potency of each batch is expressed in I.U./g. with fiducial limits for P = 0·95.

TABLE III

LOG.-LETHAL DOSES TO CAVIES OF THE BATCHES OF DIGITALIS LEAF, AND THE ESTIMATED POTENCIES OF THE BATCH

	Log.-lethal doses of digitalis leaf in mg./100 g. of body-weight									
		July 4, 1949		July 5, 1949						
Cavy No.	Laboratory standard	20·00 hr. batch	23.00 hr. batch	02.00 hr. batch	05.00 hr. batch	08.00 hr. batch	11.00 hr. batch	14.00 hr. batch	17.00 hr. batch	20.00 hr. batch
1	See Table I for individual observations	1·56633	1·55630	1·47928	1·62066	1·49831	1·32553	1·40603	1·51161	1·55437
2		1·53059	1·66171	1·49122	1·57910	1·53606	1·45438	1·38131	1·57899	1·50638
3		1·54715	1·65562	1·48657	1·62582	1·60821	1·36380	1·44138	1·57531	1·57519
4		1·€1045	1·55991	1·48898	1·54008	1·60402	1·38057	1·29292	1·58195	1·61888
5		1·€0466	1·51134	1·47026	1·59174	1·44544	1·35025	1·50555	1·58838	1·53135
6		1·€0217	1·68071	1·43917	1·53983	1·53007	1·38525	1·50624	1·56503	1·53995
7		1·57646	1·69285	1·46628	1·64885	1·60423	1·39076	1·53845	1·55763	1·50051
8		1·56217		1·41398	1·51001	1·46015	1·37291	1·54383	1·45803	1·59726
9		1·38632		1·49304	1·47783	1·55835	1·43520	1·53262	1·51228	1·58001
10				1·51627		1·55895				1·53262
11				1·51654						
12				1·62870						
13				1·52802						
14				1·44372						
Mean log. dose	1·32984	1·55403	1·61692	1·49015	1·57044	1·54038	1·38429	1·46093	1·54769	1·55365
Potency in I.U./g.	11·2	6·68	5·78	7·74	6·44	6·90	9·88	8·28	6·78	6·69
Fiducial limits in I.U./g. for P = 0·95		6·07–7·36	5·20–6·43	7·14–8·40	5·85–7·09	6·29–7·56	8·98–10·88	7·52–9·12	6·16–7·47	6·10–7·34

Results and Discussion

The results in Table III show the most active material to be that collected at 11.00 hours G.M.T., and the least active that collected at 23.00 hours G.M.T. That there is a highly significant difference between the activity of these 2 batches is evident from the graph, in which the potency in I.U./g. and the fiducial limits for each batch are shown.

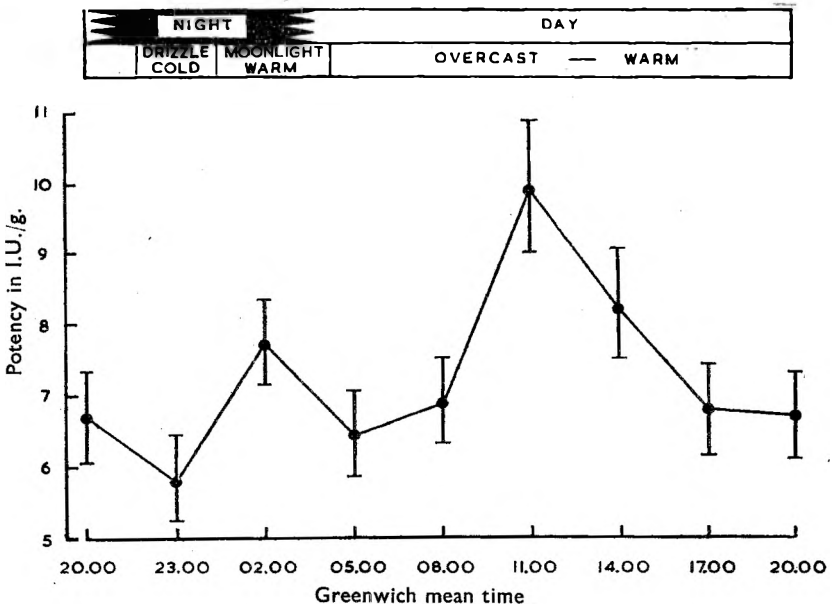

Fig. 1. Showing the estimated cardiac activities of 9 batches of digitalis leaf collected at 3-hourly intervals during 24 hours. The vertical line through each point indicates the fiducial limits (P = 0·95) of that estimate.

With the exception of the batch collected at 02.00 hours, there is a continuous increase in activity from about midnight to a maximum about noon, after which the activity steadily declines. Although there is a large variation in activity throughout the 24 hours, this follows a regular cycle, in which the first and last batches, collected at the same time of day, are equi-active. Furthermore, the Laboratory Standard Preparation, which was prepared from the remaining leaves of the plants collected at 11.00 hours on the following day, had an activity of the same order as that of the 11.00 hours experimental batch although it was somewhat more active. This greater activity may depend on the fact that the Standard material was collected on a day of continuous bright sunshine, whereas the corresponding experimental material was collected on a sunless day.

It is evident from these results that consideration of other factors which may affect the cardiac activity of digitalis must take account of the hour at which the material is collected. Even in so comprehensive an investigation as that of Watson and James[9] this factor was ignored, and it may

be that much of the variation they observed, and for which they could not account, was due to it.

The anomalous activity of the 02.00-hour material cannot be explained, but may be caused by a nocturnal alteration in metabolism, by the considerable improvement in the weather between 23.00 hours and 02.00 hours on the night in question, or by sampling variation. The last however does not seem likely, because there is no noticeable variation between batches collected from different plants at the same time of day, nor is there any evidence of irregularity in the rise and fall in activity observed in the material collected after 02.00 hours.

Summary

1. Samples of *Digitalis purpurea* leaf were collected from the same group of plants at 3-hourly intervals throughout 24 hours and dried under uniform conditions. The cardiac activity of the samples was estimated biologically.

2. The activity was found to vary significantly throughout the day, being maximal about noon and minimal about midnight.

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THE APPLICATION OF PAPER PARTITION CHROMATOGRAPHY TO THE STUDY OF THE METABOLISM OF SALICYLATE IN THE RAT

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SALICYLATES have been found to cause a reduction in the glycosuria and hyperglycæmia of the alloxan-diabetic rat.[1] Conjugation of the salicylates with glucuronic acid may have contributed to depletion of available glucose in these animals and was considered as a possible mechanism to explain the observed effects. Although glucuronic acid conjugates form a high proportion of salicylate metabolites in man[2] and the dog[3] the evidence for their occurrence in the rat is conflicting. Lutwak-Mann[4] reported that the ability of the rat's liver to form conjugated glucuronides from salicylate was negligible and could not detect such glucuronides in the urine of rats receiving salicylates. Schayer[5] studied the metabolism of C^{14} carboxyl salicylic acid in the rat by paper chromatography and in addition to identifying salicylic, salicyluric and gentisic acids obtained two unidentified substances, of which one of low R_F value could have been a glucuronide. In the present work the metabolism of salicylate in the rat has been investigated by a paper chromatographic method which gave a complete separation of the known metabolites which were quantitatively estimated. Previous observations in this species have only been qualitative in nature.

The presence of glucose in the urine of rats receiving salicylates was reported by Lutwak-Mann[4] and this observation is of great interest because of the reduction of glycosuria caused by salicylates in rats made diabetic either by partial pancreatectomy[6] or alloxan.[1] The urines of the rats which had been given salicylates were therefore examined for glucose by a paper chromatographic technique capable of detecting 1 μg. of the sugar.

METHODS

Paper chromatography of salicylate metabolites. A descending method using one-dimension strips of Whatman No. 4 filter paper and a mixture of *n*-butanol 40, glacial acetic acid 4 and water 56 (all per cent. v/v) in an atmosphere of ammonia (0·2 per cent. w/v aqueous ammonia) was used. The solvents were based on those employed by Bray, Thorpe and White[7] and Consden and Stanier.[8] Each chromatogram was run for 16 hours and the salicylate compounds visualised by means of their fluorescence in ultra-violet light[8]; a Hanovia Chromalite lamp was used as the light source. For the qualitative identification of the metabolites in the urine of rats receiving salicylate, 5 μml. of each urine specimen was chromatographed and run in conjunction with a solution containing salicylic, salicyluric and gentisic acids as marker substances. Blood

specimens were collected in oxalated tubes and the plasma separated after centrifuging. To 1 ml. of plasma was added 2 ml. of 20 per cent. aqueous trichloracetic acid and the mixture shaken and centrifuged. The supernatant liquid was neutralised to *p*H 7 with 10 per cent. sodium hydroxide solution and 50 μml. used for each run. The total salicylate in each blood sample was estimated by the method of Smith and Talbot.[9]

The ultra-violet absorption spectra of the salicylate metabolites in distilled water show maxima as follows: salicylic acid, 295 mμ; salicyluric acid, 320 mμ; and gentisic acid, 320 mμ. For the quantitative estimation of these metabolites in urine 50 μml. of urine were used in each run and the areas of paper containing the substances, as determined by their fluorescence in ultra-violet light, were cut out and placed in ground glass-stoppered test tubes together with 3 ml. of distilled water. The tubes were shaken for 15 minutes in a microid flask shaker (Griffin and Tatlock), the mixture filtered through a small sintered glass filter and the optical densities of the filtrates measured at the appropriate wavelengths in a Uvispek ultra-violet spectrophotometer. A portion of the chromatogram not containing any salicylate metabolites was treated in the same way to provide the control solution. This paper blank had a negligible optical density at 320 mμ but gave much higher values at 295 mμ which however were uniform enough to enable measurements of salicylic acid to be made. Recoveries of 95 to 100 per cent. of salicylic acid and 90 to 95 per cent. of gentisic acid were obtained by elution of chromatograms of known amounts of these substances dissolved in the urine of rats not receiving salicylates.

Paper chromatography of glucose. A descending method using one dimension strips of Whatman No. 1 filter paper and a *n*-butanol-acetic acid-water mixture[10] was employed. Each chromatogram was run for 24 hours and the spraying reagent was aniline hydrogen phthalate (0·9 ml. of redistilled aniline dissolved in 100 ml. of 0·1M phthalic acid) which gave a yellow-brown spot with glucose having an intense greenish-blue fluorescence in ultra-violet light.[11] 50 μml. of urine was used for each chromatogram.

Experimental

4 male rats of the Wistar strain, weighing approximately 300 g. and maintained on a diet of commercial rat cubes (Thompson), were placed in metabolism cages and 24-hour collections of urine made. The rats were given, by stomach tube, 117 mg. of sodium salicylate (≡100 mg. of salicylate ion) dissolved in 5 ml. of water. Successive 24-hour urine specimens were chromatographed for salicylate compounds and glucose for 2 days before and 3 days after the injections.

2 male rats of the same colony were given a solution of 117 mg. of sodium salicylate in 1 ml. of distilled water by intraperitoneal injection and killed 2 hours later by the intraperitoneal injection of 2 ml. of 10 per cent. w/v thiopentone solution. Blood was obtained by decapitation and collected in oxalated bottles.

RESULTS

Identification of salicylate metabolites in urine. The R_F values of a number of the possible metabolites of salicylate and their fluorescence in ultra-violet light are given in Table I.

TABLE I

R_F VALUES AND FLUORESCENCE IN ULTRA-VIOLET LIGHT OF SALICYLATE COMPOUNDS

Substance	R_F value (not corrected for temperature)	Fluorescence in ultra-violet light
Salicylic acid	0·76	Violet
Salicyluric acid	0·65	Blue-violet
Gentisic acid	0·57	Intense blue
Salicylamide	0·88	Blue-violet
Gentisamide	0·75	Turquoise

Good separations on the chromatogram of the first 3 substances were made from a mixture and they were easily distinguished by their fluorescence. The fluorescence of gentisic acid in ultra-violet light is especially intense and as little as 0·1 μg can be detected by this means.[8]

The pattern found in the urine of rats receiving sodium salicylate is given in Table II; an estimate of the intensity of the fluorescence is indicated.

TABLE II

SALICYLATE METABOLITES IN THE URINE OF RATS RECEIVING SODIUM SALICYLATE

Collection	Identified substances			Other fluorescent spots
	Salicylic	Salicyluric	Gentisic	
1st 24 hours	+ + +	trace	+ + +	Blue-violet fluorescent spot R_F value 0·08 observed on days 1 and 2
2nd „ „	+ +	none	+ +	
3rd „ „	none	none	none	Absent

TABLE III

URINARY EXCRETION OF SALICYLIC AND GENTISIC ACIDS AFTER THE ADMINISTRATION OF 117 MG. OF SODIUM SALICYLATE (≡100 MG. OF SALICYLATE ION) TO 4 RATS. (THE FIGURES REPRESENT THE RANGE OF VALUES FOUND)

24-hour urine collection	Salicylic acid mg./24hours (≡ per cent. of ingested dose)	Gentisic acid	
		mg./24 hr.	per cent. of ingested dose
1st	24 to 35	11·0 to 15·5	12 to 18
2nd	17 to 28	5·1 to 14·0	6 to 15·5
Combined 1st and 2nd ..	41 to 63	16 to 30	18 to 33·5

No fluorescent spot with a larger R_F value than that of salicylic acid (0·76) was observed in any chromatogram although Schayer[5] had reported an unidentified compound of high R_F value.

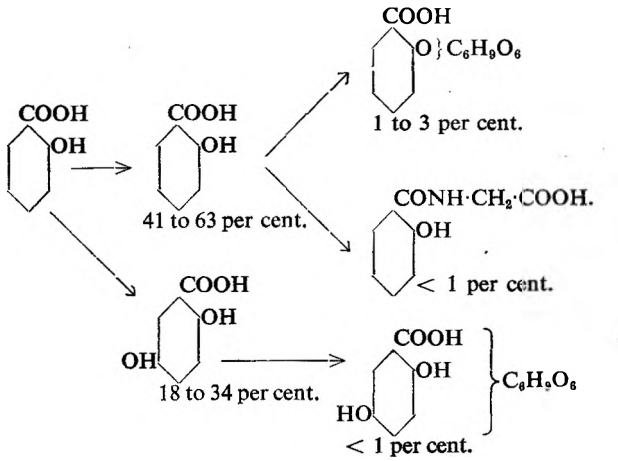
Identification of spot with R_F *value of* 0·08. 3·6 ml. of a 24-hour urine collection from a rat receiving salicylate was applied to paper, with intermediate drying, as a series of 50 μml. spots. The development of the chromatogram and the visualisation of the unknown spot were carried out as before and the areas containing the unknown substance were cut out and eluted with water in the apparatus described by Consden,

Gordon and Martin[12] the eluate being run into test tubes and the completeness of elution being checked by screening with ultra-violet light. The combined eluates (60 ml.) were concentrated by distillation under reduced pressure to a volume of 2 ml. 1 ml. of the concentrate was added to 1 ml. of N sulphuric acid and the mixture refluxed for 1 hour on a boiling water bath. The mixture was then neutralised to *p*H 7 with N sodium hydroxide and 50 μml. quantities chromatographed as before. Examination of the chromatograms under ultra-violet light showed the presence of salicylic acid and a trace of gentisic acid. The acid hydrolysate gave a strong positive naphtha-resorcinol test for glucuronic acid.[13]

The spot with an R_F value of 0·08 therefore consisted of acid labile salicyl glucuronide with a small amount of an acid labile conjugate of gentisic acid, probably a glucuronide. Two types of salicylic glucuronides occur in human urine after salicylate administration, in one of these the glucuronic acid is conjugated in an ester linkage with the carboxyl group of the salicylic acid and in the other the conjugation is an ether linkage with the hydroxyl group of the salicylic acid. The first type contains a free phenolic group and the salicylic glucuronide spots from the rats' urine were therefore sprayed with ferric chloride solution. No blue colour developed and therefore the glucuronide must have been an ether linked type.

Quantitative estimation of salicylic and gentisic acids in urine. The chromatograms showed only a trace of salicyluric acid in the first 24-hour urine collection in each rat and quantitative estimation of this substance was therefore not attempted. The figures for the excretion of salicylic and gentisic acids for the 4 rats are shown in Table III.

It is seen that the ratio of salicylic to gentisic acid is approximately 2:1. Only traces of salicyluric acid were seen in the first 24-hour collection and a rough assessment from the area and degree of fluorescence of the salicyl and gentisyl glucuronide spots was that about 1 to 3 per cent. of the ingested dose of salicylic acid was excreted on each day as these substances. The metabolites of salicylic acid occurring in the urine of the rat may be summarised as follows:



Identification of salicylate metabolites in blood. Chromatograms of the plasma filtrates showed only the presence of a salicylic acid spot, the total plasma salicylate of the rats being 69 and 62 mg./100 ml. respectively. The spot of high R_F value reported to occur in the plasma by Schayer[5] was not observed in any of the chromatograms.

Examination of the urine for glucose. The 24-hour urine collections when tested with Benedict's reagent showed only traces of reducing substances in 3 of the specimens. Examination under ultra-violet light of the chromatograms sprayed with aniline hydrogen phthalate revealed the presence of glucose. Comparison of the intensity of the fluorescence with that given by chromatograms from a series of glucose solutions of known strengths showed that less than 5 μg. was present in 0·05 ml. of the urine collections. The volumes of the 24-hour urines were between 16 and 25 ml. and this means that the rats excreted between 1·6 and 2·5 mg. of glucose per day. Lutwak-Mann[4] reported that stronger reduction occurred in urine collections made 0 to 4 hours and 4 to 7 hours than in the 7- to 24-hour collection after the administration of salicylate. 4 male rats were therefore given an intraperitoneal injection of 117 mg. of sodium salicylate in 1 ml. of water and urine collections made at similar time intervals. Testing with Benedict's and chromatographic analysis did not show any excess of glucose in the early specimens and the total 24-hour excretion was of the same order as the 24-hour specimens previously examined.

Discussion

Lutwak-Mann[14] isolated gentisic acid from the urine of rats receiving salicylate and Schayer identified salicylic, salicyluric and gentisic acids in the urine and salicylic acid in the plasma.[5] In addition the latter worker reported two unidentified compounds in the urine and one in the plasma. The results of the present work confirm the presence of salicylic, salicyluric and gentisic acids in the urine and salicylic acid in the plasma and also show that the rat excretes small amounts of an ether linked glucuronide of salicylic acid and a conjugated gentisic acid, probably a glucuronide, after the administration of salicylate. Schayer's unidentified substance of low R_F value may well be identical with this glucuronide mixture; no substances of high R_F values corresponding to his unknown substances in urine and plasma were observed. The rat is therefore similar to the dog in excreting an ether-linked glucuronide of salicylic acid in the urine and differs from man and the rabbit which excrete two types of salicyl glucuronides in one of which glucuronic acid is linked to the carboxyl group of salicylic acid and in the other it is linked to the hydroxyl group. The quantities of metabolites excreted in the urine of the rat were measured and Table IV summarises the metabolites of salicylate found in the urine of various species.

The rat has been found to excrete only 1 to 3 per cent. of the ingested dose of salicylate as glucuronide and this means that a 100 mg. dose of salicylic acid requires only a few mg. of glucose to supply the necessary glucuronic acid. The daily injection of 100 mg. of sodium salicylate to

TABLE IV

METABOLITES OF SALICYLIC ACID IN THE URINE OF VARIOUS SPECIES

Species	Metabolites (figures represent per cent. of ingested dose)						Reference
	Salicylic acid	Salicyluric acid	Gentisic acid	Salicylglucuronides		Other substances	
				Ether	Ester		
Dog	50	10	4 to 5	25	absent		Alpen, Mandel, Rodwell and Smith[3]
Rat	present	present	present			2 unidentified compounds	Schayer[6]
	41 to 63	<1	18 to 34	1 to 3	absent	Conjugated gentisic acid probably gentisyl glucuronide	Present work
Rabbit	85 Ether soluble acid fraction	5	4 to 5	5 to 14	3 to 4	(*a*) 2:3 dihydroxybenzoic acid (*b*) Conjugated gentisic acid	Bray, Ryman and Thorpe[16] Bray, Thorpe and White[7]
Man	20	55	4 to 8	25		Uraminosalicylic acid	Kapp and Coburn[8]
	10 to 85	0 to 50	1	15 to 40		No uraminosalicylic acid detected	Alpen *et al.*[3]

alloxan-diabetic rats causes a reduction of an average of 5 g. per day in the glycosuria.[1] Conjugation of the salicylate as glucuronide cannot provide an explanation of this effect.

Lutwak-Mann[4] reported that the administration of salicylates to normal rats caused an almost complete disappearance of glycogen from the liver 4 to 7 hours after the injections and this was accompanied by the excretion of glucose in the urine. The effect of salicylate on the liver glycogen content has been confirmed[1] but in the present work the normal rat when injected with salicylate only excretes traces of glucose (up to 2·5 mg.) in the urine in 24 hours.

SUMMARY

1. The metabolism of salicylic acid in the rat has been studied by a paper-partition chromatographic method.

2. After the administration of a single dose of sodium salicylate only salicylate could be detected in the plasma. The urine contained salicylic, salicyluric and gentisic acids together with an ether-linked salicylglucuronide and an acid labile conjugate of gentisic acid which was probably a glucuronide.

3. Quantitative estimation of the metabolites showed that 41 to 63 per cent. of the ingested salicylate was excreted in the free form, 18 to 34 per cent. as gentisic acid, 1 to 3 per cent. as salicyl glucuronide and traces as salicyluric acid and conjugated gentisic acid.

4. Small amounts of glucose (less than 2·5 mg. per 24-hour collection) were found in the urine of rats receiving salicylates.

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THE SEPARATION AND IDENTIFICATION OF SOME SYMPATHOMIMETIC AMINES BY PAPER PARTITION CHROMATOGRAPHY

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In pharmaceutical analysis micro methods are sometimes required for the identification of sympathomimetic amines. In a previous publication[1] one of the authors has reported the optical crystallographic properties of some commonly used salts of these amines. The observation of the optical data of pure crystalline substances is a good micro-identification method, especially when combined with micro melting point determinations.

The purpose of this paper is to report some results obtained by the authors in chromatographic experiments with some sympathomimetic amines. The technique elaborated is believed to be useful in pharmaceutical analysis, since identification of a substance by paper partition chromatography does not require its isolation in the pure crystalline state. The micro-identification of amphetamine and desoxy-ephedrine should be of particular interest, because these substances are sometimes wrongly used to produce a stimulating effect. The technique of paper partition chromatography may probably be used also in biochemical analysis for the detection of small quantities of sympathomimetic amines. Richter[2] has shown that amphetamine and ephedrine are not oxidised to any appreciable extent in the human body. The free amines could be extracted unchanged from the urine by organic solvents. The paper chromatographic separation of adrenaline and noradrenaline has been described in several publications,[3,4,5,6] and ephedrine has been included in a chromatographic study of alkaloids.[7] James[3] has also developed chromatograms of some synthetic sympathomimetic amines, which like adrenaline are catechol derivatives (corbasil, epinine). With the exception of adrenaline and ephedrine the sympathomimetic amines listed in Table I, have not as yet been subjected to a general chromatographic study, as far as the chemical literature is known to the authors.

We have developed chromatograms of 9 sympathomimetic amines with different organic solvents, water being the stationary phase. 4 of the solvent systems which have been examined in the present work, gave a satisfactory separation of the amines. The composition of these solvent systems and the observed R_F values are shown in Table I. The R_F values given in Table I are mean values from series of chromatograms where 25 μg. of the amines was employed. The temperature was maintained between 18° and 20° C. during the experiments. It is seen from Table I that the solvent systems have no resolving capacity on racemates (substances 2, 3, 4, 7) and that the separation of the two diastereoisomeric

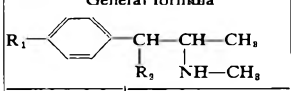
TABLE I

Substance number	Pharmacopœial or commercial name	R_F values at 19° ± 1° C. I: *n*-Butanol-water-acetic acid 40:50:10	II: *n*-Butanol-toluene-water-acetic acid 100:100:50:50	III: Ethyl acetate-water-acetic acid 30:30:10	IV: Chloroform-water-acetic acid 100:50:40
1	Adrenaline (*l*)	0·37	0·04	0·27	0·00
2	Oxedrine (*d,l*) (Sympathol)	0·53	0·11	0·37	0·01
3	Supriphen (*d, l*)	0·60	0·15	0·42	0·02
4	Isodrine (*d,l*) (Veritol)	0·69	0·25	0·51	0·14
5	Pseudoephedrine (*d*)	0·73	0·35	0·57	0·52
6	Ephedrine (*l*)	0·75	0·37	0·58	0·55
7	Amphetamine (*d,l*)	0·78	0·47	0·68	0·69
8	Desoxyephedrine (Pervitine)	0·81	0·52	0·71	0·84
9	Tuamine	0·84	0·58	0·75	0·80

substances, *l*-ephedrine and *d*-pseudoephedrine, is not sufficient to resolve a mixture of them.

With the exception of tuamine, all the sympathomimetic amines listed in Table I, have the phenylethylamine structure. On chromatograms run with the 4 different solvent systems, the R_F values of the phenylethylamine derivatives will always arrange themselves in the same order from No. 1 to No. 8. The movement of a sympathomimetic phenylethylamine derivative seems to depend upon the number of hydroxyl groups in the molecule, and also upon the character of a hydroxyl group present—if phenolic or alcoholic. The effect of the hydroxyl groups on the observed R_F values is shown in Table II. Substance 4 is supposed to move more slowly than substance 6, since the presence of a phenolic hydroxyl group will give the amine an amphoteric character, and thus probably change the partition coefficient in favour of the water phase.

TABLE II

Substance number	General formula  R_1	R_2	Relative movement at 19° ± 1° C. The R_F value of the slowest moving substance is taken as unity: Solvent system I	Solvent system II	Solvent system III	Solvent system IV
3	OH	OH	1·0	1·0	1·0	1·0
4	OH	H	1·15	1·66	1·21	7·0
6	H	OH	1·25	2·46	1·38	27·5
8	H	H	1·35	3·46	1·69	42·0

The observations shown in Table II are, of course, too limited to permit the establishment of exact rules for the relative movement of phenylethylamine derivatives on chromatograms. But it should be mentioned that James[3] has observed a considerably higher R_F value for epinine (adrenaline without the alcoholic hydroxyl group) than for adrenaline on chromatograms developed with a phenol-water system, water being the stationary phase.

For the detection of the spots we have employed 3 different spraying

reagents. (i) All the amines included in this study have sufficient strength as bases to give blue spots on spraying with bromocresol green dissolved in ethanol, when the chromatograms have been run with a solvent system containing acetic acid. (ii) By heating of the chromatograms after spraying with ninhydrin all the amines except amphetamine and tuamine may be localised as violet spots on a white background. (iii) If the chromatograms are sprayed with diazotised *p*-nitraniline and then passed through an ethanolic solution of sodium hydroxide, all the amines with phenolic character will appear as spots with characteristic colours, and, further, amphetamine and tuamine will appear as red spots.

Since absolute R_F values are not given in this paper, it will be necessary for the definite identification of one of the substances listed in Table I, to include reference substances in the chromatograms of the unknown substance. For identification purposes replicate chromatograms should be run with at least 2 of the solvent systems. Further, it will be advisable to spray replicate chromatograms with the various spraying reagents described in this paper.

Experimental

Apparatus and technique. The same technique for descending chromatograms has been used as in a previous paper,[8] where more details are given.

Adrenaline was dissolved in 5 per cent. acetic acid to obtain a 0·5 per cent. solution. The other amines were applied as free bases in a suitable solvent (ethanol or ether) on the "starting line" drawn on a sheet of Whatman filter paper No. 1, 56 × 20 cm. Quantities of 25 μg. to 50 μg. were used in a volume of 5 to 10 μl.

With the exception of *d*-pseudoephedrine (B.D.H.) and adrenaline (Danish Pharmacopœia standard), the amines were all available as salts, which were identified according to one of the Scandianavian pharmacopœias or by crystallographic methods. Desoxyephedrine (pervitin) was only available as hydrochloride in tablets and in solution for injection from Temmler Werke, Berlin. The free amines were obtained from the commercial salts by an appropriate technique (precipitation, extraction).

The chromatograms were run with the organic phase of the following solvent mixtures. The proportion of the solvent systems are given by weight.

I. *n*-Butanol (b.pt. 116° to 117° C.)-water-acetic acid (95 per cent.), 40:50:10. This solvent system has been employed in previously published work on the chromatographic behaviour of adrenaline[5,6] and of ephedrine.[7]

II. *n*-Butanol (b.pt. 116° to 117° C.)-toluene (b.pt. 109° to 110° C.) -water-acetic acid (95 per cent.), 100:100:50:50. It is shown in Table I that the addition of toluene, a liquid with relatively low polarity, to the components of solvent system I, will cause a decrease in the observed R_F values and simultaneously assure a better separation of some of the amines. If the organic phase of the mixture toluene-water-acetic acid, 100:50:40, is used as the mobile phase, the movement of the amines on the chromatograms will be negligible.

III. Ethyl acetate (Norwegian Pharmacopœia standard)-water-acetic acid (95 per cent.), 30:30:10.

IV. Chloroform-water-acetic acid (95 per cent.), 100:50:40. The R_F values given in Table I for this solvent system, were obtained by the use of freshly prepared anhydrous and ethanol-free chloroform. Chromatograms were also run with commercial chloroform (B.P.), stabilised with ethanol. The ethanol content of the chloroform will cause a decrease in the observed R_F values of about 5 to 10 per cent. but it will not affect the satisfactory separation of the amines. The mobile phases are allowed to flow down the paper to a point about 45 cm. from the "starting line." This movement will require at, 18° to 20° C., about 18 hours for the solvent system I, about 6 hours for the solvent systems II and III, and 3 to 4 hours for the solvent system IV.

Spraying reagents.

1. Bromocresol green 0·5 g. is dissolved in 100 ml. of ethanol. When the chromatograms, carefully dried in the air at ordinary temperature, are sprayed with this solution, the amines (or more precisely the amine acetates) will appear as blue spots on a greenish yellow background. If the paper is afterwards exposed to acetic acid vapours, it will turn yellow, and thus a greater contrast between spots and background may be obtained. The spots are, however, easily distinguished without this operation.

2. The dried chromatogram is sprayed with a solution of 0·20 g. of ninhydrin in 5 ml. of concentrated acetic acid and 95 ml. of *n*-butanol. The sprayed paper sheet is then heated during 5 minutes at 105° to 110° C. when strongly coloured violet spots are given by substances 2, 3, 5 and 6 and weakly coloured spots are given by substances 1, 4 and 8. Tuamine and amphetamine do not give coloured spots, even when quantities of about 100 μg. have been applied.

3. The dried chromatogram is sprayed with a solution of diazotised *p*-nitraniline. 0·25 g. of *p*-nitraniline is dissolved by gentle heating in 25 ml. of N hydrochloric acid and the solution is diluted with ethanol to 50 ml. 0·10 g. of sodium nitrite is added to each 10 ml. of this solution before spraying (cooling under running water). The sprayed chromatogram is allowed to dry in the air for 3 to 5 minutes and then it is passed through a 0·5N solution of sodium hydroxide in ethanol. Excess of the sodium hydroxide solution should be removed by means of clean filter paper. The phenolic substances appear as differently coloured spots: adrenaline, dark blue; oxedrine, dark red; supriphen, dark red; isodrine, greenish grey. Further, amphetamine and tuamine appear with a characteristic pink colour. Substances 5, 6 and 8 give no coloured spots on the yellow background.

The colour reaction between amphetamine and diazotised *p*-nitroaniline has been employed as a colorimetric determination of amphetamine which was isolated from biological materials by steam distillation.[9] Tuamine will give the same red colour with this reagent (Samdahl, private comunication).

Summary

1. The separation of 9 sympathomimetic amines by paper partition chromatography with 4 different solvent systems is described and the observed R_F values are reported. The relation between the R_F values and the constitution of phenylethylamine derivatives is briefly discussed.

2. The spraying reagents described may be used to differentiate some of the amines and thus facilitate their identification.

3. The results are considered useful for micro-identification purposes.

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THE DIRECT ESTIMATION OF THE BASE CONTENTS OF ALKALOIDAL HALIDES

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It is well known that acetic acid is a suitable solvent for acidimetric titration of bases too weak to be titrated in aqueous solution. The titrations are usually carried out with perchloric acid which is the strongest acid known in this solvent. The method generally used has recently been discussed by Ekeblad[1] who introduced some new indicators. A complete review of the literature on the method is given by Riddick.[2]

This "acetous perchloric acid method" also permits a direct titration of many organic and inorganic salts. Even salts of such strong acids as nitric and phosphoric can be titrated in this way and sulphates can be titrated to acid sulphates. Recently, work on this line has been published by Higuchi and Concha.[3]

The hydrogen halides in acetic acid, however, are too strong acids to allow a direct titration of their salts. Two different ways have been proposed to overcome this difficulty.

Higuchi and Concha[4] boiled the solutions during the titration in order to remove the undissociated hydrogen halide, a method analogous to the accurate titration of carbonates in aqueous solution. However, this method offers difficulties in practice, and many organic compounds are decomposed during the heating.

Pifer and Woolish[5] converted the halide salts into acetates by adding an excess of mercuric acetate dissolved in acetic acid. The mercuric acetate and the mercuric halides are practically undissociated in acetic acid solution and do not react with the perchloric acid. The authors titrated the halide salts in acetic acid solution but a 0·1N solution of perchloric acid in dioxan was used for the titration. The end-points were usually indicated potentiometrically. In some cases visual end-point determinations with crystal violet were possible.

This method *inter alia* permits a rapid estimation of the base content in samples of alkaloidal halides without previous separation of the base. As the procedure described by Pifer and Wollish requires rather large quantities of the solvents and as the solution of perchloric acid in dioxan has to be standardised daily, a modified method has been worked out in this laboratory which permits the titration to be made with a solution of perchloric acid in acetic acid. With this method most alkaloidal halides can be titrated visually with the indicator Blue BZL previously proposed.[1]

Titrations with 0·1N Perchloric Acid

Reagents used.

Anhydrous acetic acid. Acetic acid of pharmacopœia quality and containing less than 0·15 per cent. of water when determined by the Karl

Fischer method. The water in the commercial acetic acid is readily removed by the freezing-out method.

0·1*N acetous perchloric acid.* 14·3 g. of 70 per cent. aqueous perchloric acid is diluted with 200 ml. of anhydrous acetic acid. 31 g. of acetic anhydride is added and the solution diluted with anhydrous acetic acid to 1 l. After 1 day the solution should contain less than 0·05 per cent. of water, determined by the Karl Fischer method. If not, more acetic anhydride must be added. 1 g. of water is equivalent to 5·7 g. of acetic anhydride. If the solution contains no titratable amount of water, 0·02 per cent. of water must be added and a new water determination is made after 1 day. Even a slight excess of acetic anhydride can interfere with the titration of primary and secondary amines.

Blue BZL solution. Dissolve 0·5 g. of blue BZL (a dye from Ciba, Basle, catalogue number CIBA 22062 S), in 100 ml. of anhydrous acetic acid.

Crystal violet solution. Dissolve 0·5 g. of crystal violet, hexamethyl-*p*-rosaniline chloride, in 100 ml. of anhydrous acetic acid.

Acetous mercuric acetate solution. 3 per cent. w/v solution of reagent grade mercuric acetate in anhydrous acetic acid. The solution can easily be prepared without heating. The solution should comply with the following test:—to 10 ml. of acetous mercuric acetate solution add 2 drops of Blue BZL solution; not more than 0·02 ml. of 0·1N acetous perchloric acid should be needed to change the colour from blue to violet or red.

The acetous mercuric acetate solution is approximately 0·2N and can be used as solvent for the salts being titrated. Usually the salts are dissolved in such proportion that the solution is 0·1N with respect to the salt. About 50 per cent. of the mercuric acetate is thus converted into the mercuric halide.

Procedure.

Standardisation of the acetous perchloric acid. The perchloric acid is standardised against potassium acid phthalate. 0·4000 g. of potassium acid phthalate is dissolved by heating in 20 ml. of anhydrous acetic acid. When acetic acid solutions are heated silica gel tubes must always be used to prevent adsorption of moisture from the air. After cooling, 1 drop of crystal violet solution is added and the solution is titrated with the perchloric acid. The first colour change from violet to blue is the end-point. The equivalent weight of potassium acid phthalate is 204·2.

Titration of halide salts. The accuracy of the method is demonstrated in Table I by two titrations on pure sodium chloride (the argentimetric standard substance of Ph. Suec. Ed. XI). The sodium chloride was dissolved by boiling in 20 ml. or 10 ml. of the acetous mercuric acetate solution. After cooling to room temperature and adding 2 drops of the Blue BZL solution, the solutions were titrated with the acetous perchloric acid.

In Table II are listed results from titrations of some alkaloidal salts. The results are expressed in percentage of anhydrous salt found. In those cases

TABLE I

TITRATION OF SODIUM CHLORIDE

Amount weighed mg.	0·0989N perchloric acid ml.	Amount recovered mg.
101·6	17·59	101·7
57·2	9·91	57·3

where the samples contained water it was determined by the Karl Fischer method. Amounts corresponding to approximately 10 ml. of 0·1N acetous perchloric acid have been used. The salts were first dissolved or suspended in 2 to 5 ml. of anhydrous acetic acid. When 10 ml. of the acetous mercuric acetate solution was added most of the samples insoluble in acetic acid dissolved rapidly. In a few cases gentle heating was required to obtain complete solution. 2 drops of the Blue BZL solution were used. Generally, this indicator gives a distinct colour change from blue to red at the end-point. In some cases, however, especially when precipitation occurs, the colour change may not be sharp. In these cases the titrations have been carried out to an intermediate reddish violet colour. Even in these cases (e.g., morphine hydrochloride) the accuracy of the results is sufficient for practical purposes.

TABLE II

Sample	Amount g.	Perchloric acid ml.	Perchloric acid normality	Anhydrous salt per cent.		Water (Karl Fischer) per cent.	Anhydrous salt + water per cent.
Æthylmorphine hydrochloride.	0·4660	12·18	0·0989	90·45	90·5	9·7	100·2
	0·4606	12·05	0·0989	90·53			
"	0·3826	9·50	0·1041	90·44	90·7	9·4	100·1
	0·3997	9·97	0·1041	90·86			
Atropine methobromide.	0·3814	9·50	0·1042	99·74	99·8	—	99·8
	0·4193	10·45	0·1042	99·80			
Carbacholum.	0·2344	12·28	0·1041	99·61	99·7	—	99·7
	0·2159	11·34	0·1041	99·87			
Cocaine hydrochloride.	0·3460	9·66	0·1041	98·76	98·7	0	98·7
	0·3987	11·12	0·1041	98·66			
"	0·3530	9·89	0·1041	99·10	99·1	0	99·1
	0·3469	9·71	0·1041	99·01			
Ephedrine hydrochloride.	0·1785	8·46	0·1041	99·53	99·7	—	99·7
	0·2006	9·54	0·1041	99·87			
"	0·2146	10·19	0·1041	99·70	99·7	—	99·7
	0·1830	9·69	0·1041	99·71			
Hyoscine hydrobromide.	0·3513	7·66	0·1041	87·2		11·7	98·9
Morphine hydrochloride.	0·4106	10·50	0·1042	85·75	85·9	14·2	100·1
	0·4177	10·72	0·1042	86·06			
Narcotine hydrochloride.	0·4497	9·66	0·0989	95·58	95·7	3·9	99·6
	0·4605	9·91	0·0989	95·75			
Oxicon hydrochloride (Eukodal).	0·3961	9·78	0·0988	85·84	85·7	14·6	100·3
	0·3595	8·84	0·0988	85·49			
"	0·3686	8·64	0·1041	85·85	86·1	13·8	99·9
	0·3503	8·25	0·1041	86·25			
Papaverine hydrochloride.	0·4467	11·35	0·1041	99·41	99·6	—	99·6
	0·3574	9·11	0·1041	99·73			
"	0·4061	10·38	0·1041	100·00	100·1	—	100·1
	0·3725	9·53	0·1041	100·10			
Pilocarpine hydrochloride.	0·2709	10·59	0·1040	99·49	99·6	—	99·6
	0·2537	9·93	0·1040	99·61			
"	0·2830	11·06	0·1040	99·46	99·7	—	99·7
	0·2547	10·00	0·1040	99·92			
Pyridoxine hydrochloride.	0·2090	9·76	0·1041	99·95	100·2	—	100·2
	0·2028	9·51	0·1041	100·37			

Titrations with 0·01N Perchloric Acid

Procedures for titration of amines in acetic acid with 0·01N perchloric acid have been described by Keen and Fritz.[6] These authors use methyl violet for visual titrations, titrating to a clear blue colour. When titrating halide salts on this scale, the mercuric acetate solution may have a blank value of importance. As the colour change intervals of acid-base indicators in acetic acid are greatly affected by changes in the ionic strength, the correction obtained is not reliable even if a blank is run on the reagents. In the procedure described below, this difficulty is overcome by a previous "neutralisation" of the mercuric acetate solution at approximately the same ionic strength as in the titration.

Reagents used.

0·01N acetous perchloric acid. 1·43 g. of 70 per cent. aqueous perchloric acid is diluted with anhydrous acetic acid to 1 l. The water content is adjusted to less than 0·05 per cent. in the same manner as described above for the 0·1N acetous perchloric acid.

Crystal violet solution. A 0·05 per cent. solution in anhydrous acetic acid.

Mercuric acetate—R. To 100 ml. of the acetous mercuric acetate solution described above are added 10 ml. of a 0·1N triethylamine solution in anhydrous acetic acid* and 5 ml. of the crystal violet solution. 0·1N acetous perchloric acid is added till the first change to green occurs in the intermediate blue colour.

Procedure.

Standardisation of the acetous perchloric acid. The 0·01N acetous perchloric acid is standardised against potassium acid phthalate (Table III). The weighed quantity of potassium acid phthalate is dissolved by heating in 2 ml. of anhydrous acetic acid. After cooling, 1 drop of the crystal violet solution is added, and the solution is titrated with 0·01N perchloric acid to a clear blue colour.

TABLE III

Standardisation of the 0·01N acetous perchloric acid against potassium acid phthalate

Amount weighed mg.	Perchloric acid used ml.	Normality found
35·17	16·58	0·010388
40·54	19·09	0·010400
	Normality: 0·01039	

The standardisation was carried out at 21° C. As the coefficient of cubic expansion of acetic acid is about 0·001 per ° C. and the following titrations were carried out at 20° C., the normality was corrected to 0·01040.

Titration of halide salts. Aureomycin hydrochloride, diphenhydramine hydrochloride, terramycin hydrochloride, and tubocurarine chloride were

* This solution is used in this laboratory as the standard base solution for back titrations in the acetous perchloric acid method.

selected for testing the method on this scale. The following procedure was used. An amount corresponding to not more than 10 ml. of the 0·01N perchloric acid was weighed and suspended in 1 ml. of anhydrous acetic acid. 1 ml. of mercuric acetate R was added. The solution obtained was titrated to the first change to green in the intermediate blue colour. In the titration of terramycin hydrochloride it was necessary, due to the yellow colour of the compound, to add 2 drops of the crystal violet solution and titrate till no red tint could be seen in the green colour. Aureomycin hydrochloride failed to dissolve on the addition of mercuric acetate, and reproducible results were not obtained with this substance.

TABLE IV

DIPHENHYDRAMINE HYDROCHLORIDE

Equivalent weight: 291·8

Amount weighed mg.	0·01040N perchloric acid ml.	Found per cent.
29·78	9·78	99·7
32·49	10·70	99·9

TABLE V

TERRAMYCIN HYDROCHLORIDE, CRYSTALLINE

Molecular weight[7]: 499 (± 5).

The sample contained 3·4 per cent. of water (Karl Fischer method)

Amount weighed mg.	0·01040N perchloric acid ml.	Equivalent weight found (calculated on anhydrous basis)
39·79	7·32	504·9
36·81	6·71	509·5

TABLE VI

TUBOCURARINE CHLORIDE

Equivalent weight of anhydrous salt: 347·8.

The sample contained 6·0 per cent. of water (Karl Fischer method).

Amount weighed mg.	0·01040N perchloric acid ml.	Anhydrous salt found per cent.
31·49	8·25	94·8
29·28	7·59	93·8
	Mean value 94·3	

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THE PURITY OF VITAMIN B_{12}

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It has been variously reported that hydroxycobalamin, vitamin B_{12b}[1,2] nitrito-cobalamin, vitamin B_{12c}[3] and the thiocyanate analogue[4] all possess hæmatological activity and are all equally effective with vitamin B_{12} in causing remission of the symptoms of Addisonian pernicious anæmia.

It is perhaps unfortunate, therefore, that only cyanocobalamin thus far has received official recognition, for this tends to imply that the therapeutic activity of the substance is in some way associated with the cyanide grouping in the molecule. Apart from this anomaly, if cyanocobalamin only is considered suitable for therapeutic use, then the method of estimation of purity should be specific, within small experimental error, for this particular form of the vitamin. The present method, recommended by the U.S.P. XIV, consists essentially in determining, spectrophotometrically, the extinction coefficient $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 361 mμ at which wavelength cyanocobalamin possesses its maximum peak. Unfortunately for this recommended procedure, the absorption curve for hydroxycobalamin is similar to that for cyanocobalamin and the maximum absorption peak for this analogue is quite close (351 mμ) to that for the cyano-compound. It follows that quite considerable amounts of the hydroxy compound can be present in commercial samples of vitamin B_{12} without substantially lowering the purity as spectrophotometrically determined, and this has, in fact, been demonstrated in these laboratories. As regards the source of vitamin B_{12b}, the present writer has shown by partition chromatography that this is present in natural fermentation liquors, e.g., of *Streptomyces griseus* to the extent of *ca.* 40 per cent. of the total vitamin B_{12}. Furthermore, hydrolysis of cyanocobalamin to the hydroxy compound can occur at any stage of subsequent processing, especially in the presence of light and heat even if, at one stage, all B_{12}-like substances have been converted to the cyano-type of vitamin. As normally prepared, vitamin B_{12} is crystallised from aqueous organic solvent mixtures, usually water and acetone, but even with repeated crystallisation it is frequently difficult to bring the purity to a high level. This is understandable in view of the non-specific nature of such solvents. Recrystallisation from aqueous solution, however, readily frees cyanocobalamin from the hydroxy analogue and from other water-soluble amorphous impurities and provides the simplest means of preparing the pure compound.

Should confirmation be forthcoming of the reported finding that a pseudo-vitamin B_{12}[5] possessing all the characteristics of cyanocobalamin including microbiological but *not* clinical activity has been produced by fermentation, an even more cogent reason would exist for not placing

absolute reliance on this sole criterion of purity. In the case of pseudo-vitamin B_{12} the only reported difference between it and the authentic vitamin is a difference in partition between organic and aqueous phases —a property which likewise differentiates vitamins B_{12} and B_{12b}. It would appear necessary, then, to apply a secondary test, viz., distribution of the vitamin between water and an organic solvent in order to avoid contamination. A simple and reproducible technique is outlined below for determining the partition coefficient of commercial vitamin B_{12} preparations.

Experimental

Preparation of purified vitamin B_{12}. Approximately 2 g. of vitamin B_{12} crystals prepared by deep fermentation of cultures of *Streptomyces griseus* and of *ca.* 90 per cent. purity was recrystallised from water and, after standing at 5° C. overnight, collected on a Buchner funnel and washed with ice-cold water. It was then sucked dry in air and finally dried *in vacuo* over phosphorus pentoxide. When dry to constant weight a sample was set aside for distribution experiments and also for purity and moisture determinations. The purity of this material was estimated at 97 per cent. on the basis of the spectrophotometric absorption at 361 mμ. The remainder was again subjected to an aqueous recrystallisation procedure and analysis of this twice recrystallised material showed a purity of 99 per cent., i.e., theoretical within the experimental error of the method. Both sets of crystals were then subjected to partition experiments as described below.

Distribution experiments were carried out using specially purified solvents. The method consisted in preparing an aqueous solution of the vitamin of appropriate concentration. In the case of benzyl alcohol, using the samples of recrystallised cyanocobalamin, a concentration of 125 μg./ml. was found to be suitable. Of this solution, 50 ml. was shaken vigorously for 2 minutes with an equal volume of solvent in a 250-ml. separating funnel and the mixture was allowed to stand and separate for 5 minutes. The whole process, shaking and standing, was repeated twice in order to ensure equilibration of the phases, the latter being separated finally by centrifugation. The separated phases were next assayed spectrophotometrically at 361 mμ. against the appropriate blank phases, e.g., water-saturated benzyl alcohol and benzyl alcohol-saturated water. Similar experiments were carried out with hydroxycobalamin but in this case the solutions of purified vitamin were assayed at 351 mμ. The distribution coefficient for vitamin B_{12} between butanol and water was also determined; it was not possible to determine, with accuracy, Cs/Cw for hydroxycobalamin owing to its extremely low value.

Discussion

The results in Table I show that there was little difference in partition coefficient between the two aqueous recrystallised specimens of vitamin B_{12}. The value for this coefficient between benzyl alcohol and water was, for the twice recrystallised cyanocobalamin, 0·78. This value is in good agreement with that (0·79) reported by Anslow *et al.*[6] for the

distribution of vitamin B_{12} between benzyl alcohol and water but does not agree too closely with that (0·84) of Buhs *et al.*[7] In the case of hydroxycobalamin, there is wide discrepancy between the partition coefficient as determined by the latter authors and the value reported in the present paper; this latest value, however, is in good agreement with the qualitative assessment of partition reported by Anslow *et al.*[6]

TABLE I

SOME PARTITION COEFFICIENTS OF CYANO- AND HYDROXYCOBALAMIN

Author	Solvent	Temperature °C.	*p*H	Cs/Cw
Cyanocobalamin (Vitamin B_{12b})—				
Anslow *et al.*[6]	Benzyl alcohol	?	4·0	0·79
Buhs *et al.*[7]	,, ,,	?	?	0·84
Present study	,, ,,	21	6·0	0·76*
	,,	,,	,,	0·78†
	Butanol	,,	,,	0·045
Hydroxycobalamin (Vitamin B_{12d})—				
Anslow *et al.*[6]	Benzyl alcohol	?	4·0	almost wholly in aqueous phase
Buhs *et al.*[7]	,, ,,	?	?	0·13
Present study	,, ,,	22	6·2	0·055

* Corrected mean of 8 determinations on once recrystallised cyanocobalamin.
† Corrected mean of 6 determinations on twice recrystallised cyanocobalamin.

It may seem somewhat academic to draw attention to the possible existence of hydroxycobalamin in pharmaceutical preparations of the vitamin if this "impurity" is equally effective clinically and equally non-toxic. On the other hand, there would appear to be little point in stressing purity determinations at 361 mμ. if substantial amounts of a material possessing a maximum absorption peak at 351 mμ. are thereby allowed to be present. It may, therefore, be considered worthwhile permitting several official forms of cobalamin and laying down purity tests for each form. If, notwithstanding, cyanocobalamin is to become the official form of vitamin B_{12} in the British Pharmacopœia[8] it should be produced by a final aqueous recrystallisation in the absence of organic solvents and the partition coefficient between, say, benzyl alcohol and water should be within prescribed limits. There would seem to be, however, little justification for singling out any one analogue to be the subject of the official monograph. Finally, it is at least arguable that hydroxycobalamin, vitamin B_{12b}, is the truly active natural vitamin.

SUMMARY

1. The anomalous position of the various therapeutically active cobalamins is discussed in relation to their possible inclusion in the British Pharmacopœia. Attention is drawn to the possible presence of other B_{12}-like bodies in commercial samples of cobalamin conforming to the U.S.P. purity standards.

2. The desirability of a final aqueous recrystallisation, or at least of distribution measurements, is stressed.

3. A simple, but specific and reproducible, method for the determination of the partition coefficient of cobalamin between solvent/water systems is described.

4. Values are reported for the distribution of this vitamin between benzyl alcohol/water and butanol/water systems.

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THE DETERMINATION OF LOCAL ANÆSTHETICS BY EXCHANGE OF IONS

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By methods based on the use of ion exchange resins a number of groups of substances of importance in pharmacy have been determined, i.e. alkaloids[1,2,3] sulphonamides,[4,5] official acids and their salts, etc.[6,7,8] It seemed possible that local anæsthetics might be determined in a similar way both as pure substances, and in the form of galenicals.

From the chemical point of view substances with local anæsthetic effect are derivatives of *p*-aminobenzoic acid or substituted amides, or substituted urethanes. Benzocaine and butesine are esters which are insoluble in water, the others are organic bases and are used in the form of salts which are soluble in water. They may be determined (1) gravimetrically,[9,10] (2) by titrating the base, precipitated by alkali and isolated by extraction or distillation, with an acid,[11,12] or (3) bromimetrically.[13,14,15,16,17,18,19] Procaine or procaine-penicillin may also be determined by volumetric methods[20,21,22]: Colorimetric or photometric determinations,[23,24,25,26,27,28,29,30,31] and biological methods have also been worked out.[32,33]

We have separated the bases of the commonly used local anæsthetics by chromatography on a column of the anion exchange resin Amberlite IRA-400. In this process the acid component was separated and remained in the column, and the basic component set free was determined volumetrically. The principal advantage of this procedure is that the laborious separation of the base by organic solvents is avoided.

Experimental

The assembly of the apparatus for chromatography, the preparation of the column, solutions used and experimental technique are the same as described by Jindra and Pohorsky.[2,3] The addition of a stopcock to the outlet of the tube to regulate the speed of the flow, is an advantage.

Method. The samples were dried to constant weight in a desiccator over concentrated sulphuric acid and 20 to 50 mg., weighed with an accuracy of $\pm 0{\cdot}01$ mg., was dissolved in 5 ml. of distilled water in a 35-ml. beaker and 15 ml. of ethanol added. Before use the regenerated and rinsed chromatographic column was washed with 5 ml. of hot ethanol (95 per cent.). When the ethanol had drained away, the solution of the substance under examination was passed through the ion-exchange column (8 to 10 g. of Amberlite TRA-400), the speed of flow being regulated by the stopcock to 60 drops per minute. The column was then washed with 30 ml. of hot ethanol (96 per cent.). The beaker containing the collected eluate was used for the titration with 0·1N hydrochloric acid, methyl red being used as indicator, or the titration being made

potentiometrically. The column was washed with a few ml. of distilled water and regenerated in the usual way. The results of our experiments are shown in Table I.

TABLE I

	Theoretical content of base, per cent.	Base equivalent to 1 ml. of 0·1N acid, mg.	Weight taken, mg.	0·1N (f = 0·9664) acid used, ml.	Content of base, per cent.	Deviation, per cent.
2-Diethylaminoethyl *p*-aminobenzoate hydrochloride (Procaine)	86·56	23·62	45·61	1·73	86·51	− 0·06
			35·07	1·33	86·49	− 0·10
			44·62	1·70	86·88	+ 0·38
			37·39	1·42	86·62	+ 0·06
			37·71	1·43	86·49	− 0·08
3-Diethylamino-2: 2-dimethylpropyl-*p*-aminobenzoate hydrochloride (Larocaine)	88·42	27·84	45·82	1·53	89·24	+ 0·92
			33·43	1·10	88·52	+ 0·11
			21·92	0·72	88·37	− 0·17
			36·71	1·21	88·67	+ 0·29
			40·94	1·34	88·06	− 0·41
3-Dimethylamino-1: 2-dimethylpropyl *p*-aminobenzoate hydrochloride (Tutocaine)	87·28	25·03	45·30	1·63	87·05	− 0·27
			38·42	1·38	86·90	− 0·44
			28·92	1·04	87·00	− 0·33
			35·31	1·27	87·01	− 0·31
			37·34	1·35	87·47	+ 0·21
2-Butoxy-4-β-diethyl-aminoethylcarbamyl-quinoline hydrochloride (Nupercaine)	90·38	34·24	39·71	1·08	90·01	− 0·41
			34·76	0·95	90·44	+ 0·07
			35·10	0·95	89·57	− 0·89
			41·60	1·12	89·10	− 1·41
			33·60	0·91	89·63	− 0·83
1-Dimethylamino-2-methyl-2-butanol benzoate hydrochloride (Amylocaine)	86·58	23·53	54·12	2·06	86·51	− 0·08
			47·84	1·82	86·45	− 0·14
			37·45	1·42	86·18	− 0·47
			28·30	1·08	86·73	+ 0·17
			36·28	1·37	86·03	− 0·64
2-Dimethylaminoethyl-*p*-*n*-butylaminobenzoate hydrochloride (Amethocaine)	87·84	26·33	42·43	1·46	87·57	− 0·30
			35·05	1·21	87·86	+ 0·02
			40·07	1·37	87·01	− 0·94
			30·56	1·05	87·45	− 0·45
			33·98	1·18	88·38	− 0·61
p-Dialloxyethenyl-diphenylamidine hydrochloride (Diocaine)	89·85	32·30	18·72	0·54	90·05	+ 0·43
			30·50	0·87	89·05	− 0·90
			49·98	1·42	88·70	− 1·28
			35·93	1·03	89·48	− 0·39
			28·16	0·81	89·80	− 0·07

Local anæsthetics in galenicals may also be determined by this method. The presence of an electrolyte is a disturbing factor as the water-ethanolic solution is more basic when the anion has been removed. As an example we describe the determination of procaine in an ointment containing 2 per cent. of procaine hydrochloride in soft paraffin.

Procedure: 2 to 3 g. of the ointment is vigorously mixed in a 50-ml. beaker with 5 ml. of boiling distilled water and 5 ml. of ethanol (96 per cent.) added. When the ointment base has settled the ethanolic solution is poured off into another beaker. The ointment base is then washed 5 times with a mixture of 5 ml. of hot water and 5 ml. of ethanol. The mixed solution and washings are then passed through the ion exchange column of Amberlite IRA-400, at 50 to 60 drops per minute. The beaker and the column are then washed quickly with 60 ml. of warm ethanol (96 per cent.). The eluate is diluted with water, 10 to 15 drops of methyl red are added and the eluate is titrated from a microburette with 0·1N

hydrochloric acid to a distinct red colour. 1 ml. of 0·1N hydrochloric acid is equivalent to 0·027264 g. of procaine hydrochloride.

Results: 2·00 g. of ointment (40 mg. of procaine hydrochloride, required 1·44, 1·45 ml. of 0·1N acid (f = 0·9664) = 38·15, 38·41 mg. of procaine hydrochloride.

Summary

1. Salts of local anæsthetics can be determined by separation of the base by means of the anion-exchange resin, Amberlite IRA-400, and titration with acid.

2. This method can be used for galenicals if no electrolyte is present.

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A NOTE ON THE ASSAY OF THE METHYL AND PROPYL ESTERS OF *p*-HYDROXYBENZOIC ACID

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According to the Danish Pharmacopœia 1948, methyl *p*-hydroxybenzoate is assayed by treatment with aqueous sodium hydroxide solution on a water bath followed by bromination in acid solution, the excess of bromine being estimated with sodium thiosulphate after the addition of potassium iodide. As six atoms of bromine are absorbed by one molecule of the ester the methanol is presumably oxidised to formaldehyde.

$$HO{\cdot}C_6H_4{\cdot}COOH + CH_3OH + 3Br_2 = HO{\cdot}C_6H_2Br_2{\cdot}COOH + CH_2O + 4HBr$$

It seemed reasonable to suppose that time could be saved by brominating the ester directly without previous hydrolysis when the reaction would be expressed by the following equation:—

$$HO{\cdot}C_6H_4COOCH_3 + 2Br_2 = HO{\cdot}C_6H_2Br_2COOCH_3 + 2HBr$$

Good results were obtained when the ester was dissolved in sodium hydroxide solution provided that the alkaline solution was brominated with 0·1 N bromine and acidified with hydrochloric acid immediately after the preparation of the solution. Bromination was not complete under 40 minutes. If the alkaline solution of the ester was not brominated and acidified immediately high results were obtained due to slow hydrolysis of the ester by hydroxyl ions and oxidation of the methanol produced thereby (see Table I).

TABLE I

Estimation of methyl *p*-hydroxybenzoate without previous hydrolysis

Age of solution	Time of bromination minutes	per cent.
Fresh	20	99·2, 99·1
"	30	98·8, 99·1
"	40	99·5
"	50	99·4
4 hours	40	101·2
8 hours	40	110·2
48 hours	40	137·4

The method of the Danish Pharmacopœia gave slightly higher figures for the same sample; it was found that the instruction to brominate in the dark was unnecessary as the same figures were obtained when the bottles were left in the light during bromination (see Table II).

A sample of propyl *p*-hydroxybenzoate when assayed without previous hydrolysis gave high figures (>103 per cent.), but two recrystallisations (one from aqueous ethanol and one from benzene) yielded a product assaying at 100·6 per cent. It seemed probable that the high figure

TABLE II

ESTIMATION OF METHYL *p*-HYDROXYBENZOATE AFTER HYDROLYSIS (DANISH PHARMACOPŒIA)

Conditions of bromination	per cent.
In the dark	99·7, 99·7
In the light	99·7, 99·8

TABLE III

ESTIMATION OF PROPYL *p*-HYDROXYBENZOATE

Sample	Time of bromination minutes	Results	
		Method of Danish Pharmacopœia per cent.	Method not involving hydrolysis per cent.
Old sample	20	99·0	102·9
	30	99·2	103·3
	40	99·3	103·2
	50	99·0	103·3
Sample recrystallised twice ..	20		100·6
	30		100·6
	40		100·6
	50		100·7
Sample recrystallised after washing with sodium bicarbonate solution	10	99·2	100·2
	20	99·4	100·2
	30	99·5	100·2
	70	99·6	
	120		100·2

resulted from the presence of free *p*-hydroxybenzoic acid in the sample, this theory being reinforced by the fact that a sample washed with 5 per cent. solution of sodium bicarbonate and then recrystallised from benzene assayed at 100·2 per cent. The last named sample, when assayed by the method of the Danish Pharmacopœia for methyl *p*-hydroxybenzoate gave low figures, even when the bromination was allowed to proceed for over an hour, presumably because the propanol resulting from the hydrolysis of the ester is less readily oxidised, than is the methanol in the case of methyl *p*-hydroxybenzoate.

Finally, the impure sample of propyl *p*-hydroxybenzoate which by the simpler process (i.e., estimation without previous hydrolysis) had assayed at over 103 per cent. when assayed by the method of the Danish Pharmacopœia for methyl *p*-hydroxybenzoate assayed at 99·0 to 99·3 per cent., which suggests that the presence of free acid is more easily detected by the simpler process than by the process involving saponification. Simple calculation supports this suggestion for it can easily be shown that a sample of propyl *p*-hydroxybenzoate containing exactly 10 per cent. of free *p*-hydroxybenzoic acid as impurity would assay at 103 per cent. by the direct bromination process but at 98·7 per cent. by the Danish Pharmacopœia process if it can be assumed that both processes yield a correct picture of the facts. The theoretically calculated figures for the methyl ester containing 10 per cent. of free acid would be 101·03 per cent. by the direct bromination process but 97·32 per cent. by the

Danish Pharmacopœia method, hence for the methyl ester the presence of free acid is more readily detected by the latter process.

All the above figures are calculated on the assumption that although a poor sample of ester might easily contain appreciable quantities of free *p*-hydroxybenzoic acid the equivalent amount of methanol or propanol produced simultaneously would have volatilised. As free *p*-hydroxybenzoic acid is a very likely impurity in any of its esters and as neither assay process readily reveals the presence of small amounts of free acid in either the methyl or the propyl ester, it seems highly desirable that the B.P.C. monograph should contain a separate test for limit of free acid.

Bromination without previous saponification is complete in 20 minutes with propyl *p*-hydroxybenzoate, whereas in the case of the methyl ester 40 minutes is required.

Proposed Method

About 1 g. of ester, accurately weighed, was dissolved in a mixture of 20 per cent. w/v solution of sodium hydroxide (2 ml.) and water (20 ml.) without the aid of heat and the volume made up to 100 ml with water. 20 ml. of this solution was treated immediately in a stoppered bottle with 0·1 N bromine (50·0 ml.) and hydrochloric acid (6 ml.) with shaking. The bottle was stoppered and allowed to stand, with frequent shaking for 40 minutes (methyl ester) or 20 minutes (propyl ester). 10 per cent. w/v solution of potassium iodide (10 ml.) was then added and the liberated iodine titrated with 0·1 N sodium thiosulphate. Each ml. of 0·1 N bromine is equivalent to 0·003802 g. of methyl *p*-hydroxybenzoate, or 0·004505 g. of propyl *p*-hydroxybenzoate.

Summary

1. It is proposed to modify the assay process for methyl *p*-hydroxybenzoate as given in the Danish Pharmacopœia by omitting the preliminary saponification process.

2. Propyl *p*-hydroxybenzoate can be determined in the same manner and gives results more in accordance with the facts than does the bromination of the products of hydrolysis, the presence of free *p*-hydroxybenzoic acid being indicated by a figure greater than 100 per cent.

3. Neither process is sufficiently sensitive to limit small quantities of free acid and a separate limit test for this very likely impurity is desirable.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***Delphinium Barbeyi* H, Alkaloids of.** W. B. Cook and O. A. Beath. (*J. Amer. chem. Soc.*, 1952, **74**, 1411.) This species of larkspur was found to contain a mixture of crystalline alkaloids (approximately 0·15 per cent. of the plant on a dry weight basis) plus smaller amounts of amorphous bases. By means of fractional crystallisation and chromatography, two crystalline alkaloids, lycoctonine and anthranoyllycoctonine comprising approximately 62 per cent. and 38 per cent. respectively of the crystalline bases were obtained. New empirical formulæ ($C_{24}H_{41}O_7N$, H_2O for lycoctonine and $C_{31}H_{46}O_8N_2$, $\frac{1}{2}H_2O$ for anthranoyllycoctonine) were assigned on the basis of elementary analysis of the bases and their salts and peripheral group studies. 9 derivatives of each of these alkaloids were prepared and their physical constants determined. The X-ray diffraction patterns and ultra-violet absorption spectra of lycoctonine, anthranoyllycoctonine and ajacine were determined. A. H. B.

ANALYTICAL

Acetic Acid, Titration of Bases in. P. Ekeblad. (*Svensk. farm. Tidskr.*, 1952, **57**, 201.) Approximately 0·1M solutions of bases in acetic acid containing not more than 0·15 per cent. of water are titrated with 0·1N perchloric acid in acetic acid. The perchloric acid solution is made anhydrous by the addition of acetic anhydride, but should contain a small amount of water, not more than 0·05 per cent., when tested by the Karl Fischer method, as this ensures absence of acetic anhydride. The solution is standardised against potassium acid phthalate. A suitable reagent for back titration is 0·1N triethylamine in acetic acid, standardised against the perchloric acid solution. Titration curves for sodium acetate, potassium acetate, nikethamide, phenazone, sulphanilamide and caffeine, determined with a glass electrode, are given. Blue BZL is a suitable indicator for nikethamide and stronger bases, while for phenazone and *iso*-propylphenazone, neutral red or Nile blue sulphate may be used. A mixture of Nile blue sulphate, 2 parts and Blue BZL, 1 part is recommended for sulphanilamide titrations. A table of colour changes of the indicators is given, but the colour change intervals in acetic acid are greatly affected by changes in the ionic strength of the solution. Results of sodium nitrite and perchloric acid titrations of sulphanilamide are compared. G. B.

***p*-Aminosalicylic Acid, *m*-Aminophenol Content of.** W. Seaman, J. T. Woods, W. B. Prescott and W. H. McComas, Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 207.) A colorimetric method has been reported for determining *m*-aminophenol in *p*-aminosalicylic acid and its sodium salt; the method involves the diazotisation of the sample with a subsequent hydrolysis of the diazo-compound to 2:4-dihydroxybenzoic acid, which couples with the unhydrolysed diazotised *m*-aminophenol to form a colour. The intensity of the colour was measured spectrophotometrically at 440 mμ and could be used to calculate the *m*-aminophenol content of the sample from its light absorption. Details of the method are given, together with conditions which gave satisfactory results at concentrations of *m*-aminophenol in *p*-aminosalicylic acid of

less than 1 per cent. The *m*-aminophenol content of 30 samples of medicinal grade *p*-aminosalicylic acid from various commercial sources were found by the proposed method to range from 0·01 per cent. to 0·20 per cent.; 16 samples of medicinal grade sodium *p*-aminosalicylate contained up to 0·11 per cent. of *m*-aminophenol. R. E. S.

Barbiturates and Sulpha Drugs Determination of, in Non-aqueous Solution. V. Vespe and J. S Fritz. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 197.) Barbiturates and sulpha drugs in various pharmaceutical preparations were determined by acidimetric titration in non-aqueous solution. The sample was dissolved in dimethylformamide and titrated with 0·1N sodium methoxide, using thymol blue as indicator; the end-point was marked by a sudden change from yellow to blue. Twelve barbiturates and a number of pharmaceutical preparations of phenobarbitone were assayed satisfactorily by this procedure; powdered tablet samples could be dissolved in dimethylformamide and titrated directly. Tablets containing sulpha drugs can be titrated directly in dimethylformamide solution with sodium methoxide- Most common tablet excipients did not interfere. R. E. S.

Benzoic Acid in Foods, Detection of. E. Rathenasinkam. (*Analyst*, 1952, **77**, 101.) A modification of Mohler's test is given. The ammonium salt of the isolated benzoic acid is first prepared by a modification of Leather's procedure (*Analyst*, 1931, **56**, 299), an ethereal solution being exposed to an atmosphere of ammonia. About 1 mg. of the acid or the sodium or ammonium salt is then nitrated by heating with 150 mg. of potassium nitrate and 15 drops of sulphuric acid, sp.gr. 1·84, in a bath of boiling water for 20 minutes; after dilution to about 30 ml. and extraction with 30 ml. of ether, the ether layer is washed with two 10-ml. quantities of water and the ether removed by evaporation. The residue is dissolved in 2 ml. of a mixture of 2 volumes of acetone and 1 volume of absolute ethanol, and 1 to 2 drops of a 10 per cent. aqueous solution of sodium hydroxide are added; after gentle mixing a purple colour develops, changing slowly to violet. *p*-Hydroxybenzoic acid gives no colour in the test; *p*-chlorobenzoic acid, a reddish colour developing slowly; salicylic acid, no colour; cinnamic acid, a dirty violet changing to brown; phenylacetic acid, a green; saccharin, no colour. To detect cinnamic acid in the presence of benzoic acid 0·5 ml. of nitric acid sp.gr. 1·42 is used for the nitration; after heating for 20 minutes, the contents of the test tube are evaporated to dryness, the residue dissolved in 2 ml. of a mixture of acetone and ethanol, and 1 or 2 drops of sodium hydroxide solution are added. Under these conditions cinnamic acid gives a positive reaction (dirty violet colour changing to brown), no colour being obtained with benzoic acid. R. E. S.

Chlorinated *o*-Cresols, Determination of, with Gibbs's Reagent. K. Gardner. (*Analyst*, 1952, **77**, 160.) A method is described for the determination of chlorinated *o*-cresols after reaction with Gibbs's reagent(2 : 6-dibromoquinone-4-chloroimide). 10 ml. of buffer solution (containing boric acid, potassium chloride and sodium hydroxide) and 4 ml. of Gibbs's reagent were added to a solution containing the appropriate cresol in 200 ml. of water, the solution was mixed (*p*H 9·40) and allowed to stand in the dark for 16 hours, the colour, after filtration with 50 ml. of *n*-butanol and filtration into a 1-cm. or 4-cm. glass cell, being measured photometrically. Experiments were made with equimolar solutions (25×10^{-8} to 400×100^{-8}M) of phenol, *o*-cresol, 4-chloro-*o*-cresol, 4 : 6-dichloro-*o*-cresol and 6-chloro-*o*-cresol; it was found that

6-chloro-*o*-cresol and *o*-cresol had a greater speed of reaction than the other chlorinated cresols, which reacted at speeds greater than that of phenol itself. Calibration graphs obtained with Ilford No. 607 and No. 608 filters and a tungsten filament lamp were linear over the range 0 to 300 × 10^{-8}M, with the exception of the graph for phenol. *o*-Chloro-substitution could be detected in *o*-cresols, if phenol itself were absent, by taking two readings with different filters, since the halogen in the *ortho*-position displaced the absorption band towards the red end of the spectrum. R. E. S.

Cortisone and Related Ketol Steroids, Colorimetric Determination of. W. J. Mader and R. R. Buck. (*Anal. Chem.*, 1952, **24**, 666.) The determination depends on the fact that alcoholic solutions of steroids which contain the primary α-ketol group reduce tetrazolium salts in the presence of tetramethyl ammonium hydroxide, forming coloured solutions. 2:3:5-Triphenyl tetrazolium chloride and 3:3-dianisole-bis-4:4′-(3:5-diphenyl) tetrazolium chloride produce colours which obey the Lambert-Beer Law over a suitable concentration range; the molecular absorption of the diformazan from cortisone acetate and dianisole bisdiphenyl-tetrazolium chloride is twice that of the formazan of cortisone acetate and 2:3:5-triphenyltetrazolium chloride. Of the 17 steroids and related compounds studied, 5 contained the α-ketol group and developed colour with both dianisole bisdiphenyltetrazolium chloride and 2:3:5-triphenyltetrazolium chloride; no colour was produced with Δ^5-3-hydroxypregnene-20-one, Δ^4pregnene-17:20:21-triol-3-one, Δ^4-androstene-3:11:17-trione, Δ^4-androstene-3:17-dione, œstradiol, œstrone, progesterone, methyltestosterone, testosterone propionate, ethinyl testosterone, cholic acid, and desoxycholic acid. In the assay of an ointment containing 25 mg./g. of cortisone the standard deviation for 8 assays was 0·3 mg. per g. with an average of 25·3 mg. per g.; the standard deviation for cortisone acetate tablets was 0·3 mg. for 10 determinations with an average of 27·5 mg. per tablet. R. E. S.

Water Content of Medicinal Chemicals and Drugs, Karl Fischer Method for. E. Brochmann-Hanssen and P. Pong. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 177.) The Karl Fischer reagent was used to determine the water content of various medicinal chemicals, powdered extracts, and ointments. A modified reagent containing ethylene glycol monomethylether was found to be more stable than one made with methanol. Details are given for procedures using a visual end-point and also for a direct electrometric titration with a "dead-stop" end-point. The direct method was not satisfactory for powdered extracts because they were not completely soluble, and the water was extracted very slowly; good results were, however, obtained by back titration. Results are quoted for the moisture content of numerous medicinal chemicals, of powdered extracts and ointments. The results obtained by the Karl Fischer method in general agreed very closely with those obtained by the official drying methods. In a few cases oven drying gave low values; titration of the oven-dried compounds accounted for this difference. R. E. S.

Metaldehyde, Identification by Kofler's Micro Method. K. Teuchner. (*Acta pharmacol. Toxicol.*, 1952, **8**, 79.) The substance under investigation is sublimed at a temperature of 130° to 140° C. on to a cover-glass which is then pressed on to a slide carrying a few particles of β-naphthol, or pyrogallol. The slide is placed on the heating apparatus of the microscope and when the eutectic point is reached, small drops of liquid are observed where the two substances meet. The temperatures at which this occurs are 128° C. for mixtures of

metaldehyde and pyrogallol and 115° C. for mixtures of metaldehyde and β-naphthol. If phenacetin is substituted for β-naphthol or pyrogallol, the m.pt. of the phenacetin is not lowered. G. R. K.

Nitrogen, Determination of. P. McCutchan and W. F. Roth (*Anal. Chem.*, 1952, **24**, 369.) A simple modification of the Kjeldahl procedure using thiosalicylic acid as the reducing agent permits the rapid determination of nitrogen in compounds such as nitrobenzene and nitromethane. It appears to be suitable for the determination of nitrogen in nitro-type compounds and in all basic and neutral forms of nitrogen compounds found in petroleum or shale oil fractions. Approximately 1 g. of thiosalicylic acid is placed in a Kjeldahl flask, 20 ml. of concentrated sulphuric acid is added followed by the requisite amount of sample which is washed down the neck of the flask with an additional 20-ml. quantity of sulphuric acid. The mixture is heated until boiling and spattering occurs (274° to 288° C.); it is then cooled to room temperature, 20 g. of potassium sulphate and 1·3 g. of clean metallic mercury are added and the determination is completed as in the regular Kjeldahl procedure with a digestion temperature of approximately 365° C. Satisfactory results were obtained in comparison with those obtained by the regular Kjeldahl and the A.O.A.C. methods. R. E. S.

Phenylmercuric Nitrate, Polarographic Characteristics of. W. L, Wuggatzer and J M. Cross. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 80.) Solutions of phenylmercuric nitrate were mixed with buffer solution and examined polarographically after the addition of gelatin to suppress maxima, and removal of oxygen by passing nitrogen through the solution. Phenylmercuric nitrate was reduced in two steps between 0·0 and − 1·53 volts measured against a saturated calomel electrode at 24° C. In acid solutions the first wave started at zero applied potential, but above *p*H 6·7, both waves were measured and found to be of equal height. Half-wave potentials of both waves became more positive with increase in hydrogen ion concentration, and both were affected by the presence of potassium nitrate or potassium chloride as supporting electrolyte. In experiments at *p*H 9·2, the diffusion current was proportional to the concentration of phenylmercuric nitrate, and quantitative measurements were made. G. B.

Proflavine Hemisulphate, Colorimetric Estimation of. W. H. C. Shaw and G. Wilkinson. (*Analyst*, 1952, **72**, 127.) A method is described for the colorimetric estimation of proflavine hemisulphate by conversion to the quinone-imine form of 2-aminoacridyl diazonium chloride. A solution of proflavine was treated with a limited excess of nitrous acid, under controlled conditions of *p*H and temperature, and the excess of nitrous acid subsequently removed by sulphamic acid; coupling in acid solution with *N*-(1-naphthyl)-ethylenediamine dihydrochloride then gave a stable purple colour. Details are given of the effect of *p*H, temperature, of light and of concentration on the quinone-imine formation. The intensity of the colour produced by proflavine hemisulphate is greater than the original quinone-imine colour, thus increasing sensitivity. The method gave a rectilinear relation of optical densities and concentrations for quantities of proflavine hemisulphate up to 300 μg. and could be applied without modification to euflavine and acriflavine in similar amounts. The procedure was inapplicable to 5-aminoacridine but could be applied directly to the B.P.C. 1949 preparations, eye-drops, pessary, solution and solution

tablets. Difficulties were sometimes experienced in complete recovery of proflavine in the presence of fatty matter, and extraction with an immiscible solvent was sometimes necessary; ethylene dichloride was preferable to other organic solvents, losses of proflavine being reduced to a minimum by maintaining the aqueous phase at an acidity of about 0·1 N with hydrochloric acid. Results of recovery experiments are given on proflavine pessaries and on proflavine cream; the mean error (approximately − 3 per cent.) was small and the method is regarded as sufficiently accurate for routine estimations. R. E. S.

Salts, Titration of, in Non-aqueous Solvents. J. S. Fritz. (*Anal. Chem.*, 1952, **24**, 306.) The determination of certain salts by titration as acids in non-aqueous solvents is outlined. The sample to be titrated is weighed accurately, neutralised solvent is added and the solution is titrated with 0·1N sodium methoxide to the clear blue colour of thymol blue; carbon dioxide must be excluded during the titration. The sodium methoxide is standardised against benzoic acid and the solution must be re-checked frequently. Mineral acid salts of ammonia and aliphatic amines, as well as aromatic amines, give sharp end-points; guanidine hydrochloride behaved as an acid, but the end point was poor; trimethylphenylammonium iodide was however not acidic to azo violet. Aqueous solutions could be titrated if diluted 15 times with ethylenediamine provided precipitation did not occur. Ethylenediamine and dimethylformamide were the most satisfactory solvents; in the latter solvent aromatic and higher aliphatic amine salts of organic and inorganic monobasic acids were soluble; many ammonium and lower aliphatic amine salts of monobasic acids are also soluble. Ammonium nitrate, ammonium bromide, and ammonium thiocyanate were soluble, but ammonium chloride, ammonium acetate and butylamine hydrochloride were insoluble. Salts of polybasic acids were generally insoluble. Thymol blue was found to be the most satisfactory indicator. The method as described was accurate to within ± 0·3 per cent., although by using larger samples and a more careful analytical technique, a better precision and accuracy could be obtained. R. E. S.

"Sulpha" Drugs and Sulphonamides, Titration of in Non-aqueous Solvents. J. S. Fritz and R. T. Keen. (*Anal. Chem.*, 1952, **24**, 308.) The SO_2NH-group found in "sulpha" drugs and other sulphonamides of primary amines is feebly acidic and sulpha drugs dissolved in basic organic solvents show acidic properties sufficient to permit direct titration with a strong base. The method is applicable to the determination of sulphaphthalidine, sulphasuxidine, and other sulphonamides which cannot be assayed by the diazo method. In the process given a sample of suitable size is dissolved in dimethylformamide or butylamine; a solution of thymol blue in methanol is added as indicator, the beaker is covered with cardboard provided with a small hole for the burette tip, and the titration with magnetic stirring is carried out to the first appearance of a clear blue colour. The solvents employed must be neutralised with sodium methoxide shortly before use. The sodium methoxide is standardised against benzoic acid using dimethylformamide as solvent and thymol blue as indicator; the titration of benzoic acid in butylamine gave erratic results due to gel formation. Results are given for the titration of numerous sulphonamides of primary amines; by observing the sharpness of the end-points in the two solvents an indication of the basic strength of the parent amine can be obtained. R.E.S.

Testosterone and Derivatives, Determination of. E. Diding. (*Svensk farm, Tidskr.*, 1952, **56**, 3.) Testosterone, methyltestosterone and testosterone propionate may be determined photometrically by dissolving about 0·5 mg. of the material in 2·0 ml of ethanol (95 per cent.) and adding 10 ml. of a 0·25 per cent. solution of 2:4-dinitro-phenylhydrazine in 2N of hydrochloric acid. After standing for 6 hours, the precipitate is collected in a sintered filter, washed with hydrochloric acid, then with water, and dried. The residue is then dissolved in chloroform to 100 ml., and the extinction is determined at 390 mμ. For oily solutions of testerone propionate, a quantity of the solution, containing about 3 mg., is shaken with 15 ml. of heptane and 10 ml. of ethanol (90 per cent.). The ethanolic layer is shaken with two further quantities, each of 15 ml., of *n* heptane, and a further 3 quantities, each of 10 ml., of ethanol are used to follow up the extraction. The last two quantities of heptane are washed with another 10 ml. of ethanol, and the final quantity again with a further 10 ml. The mixed ethanolic solutions are made up to volume and an aliquot is evaporated to dryness before continuing the determination as described above. G. M.

Tetracaine (Amethocaine) and Phenylephrine Hydrochloride, Spectrophotometric Determination of. R. I. Ellin and A. A. Kondritzer. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 71.) Amethocaine solutions may be assayed by determining the optical density at 310 mμ. Phenylephrine has a negligible absorption at this wave-length. The solution in 0·1N hydrochloric acid is extracted twice with wet benzene to remove any of the decomposition product, *p*-butylaminobenzoic acid, which would interfere in the assay. The aqueous solution is neutralised with 0·01N sodium hydroxide, the optical density determined at 310 mμ and the result calculated from the absorption data for amethocaine hydrochloride solutions. The following method, based on the formation of a red colour by diazotisation is recommended for phenylephrine. Dilute a sample containing 0·5 mg. to 10 ml., and filter if necessary. To 1 ml. add 3 ml. of a 15 per cent. solution of mercuric sulphate in 5N sulphuric acid, heat on a boiling water bath for 10 minutes, cool, add 3 ml. of 0·1N sodium nitrite solution dilute to 10 ml., determine the optical density at 495 mμ, and calculate the result by reference to a calibration graph. G. B.

Tropa Alkaloids, Assay of. A. Berggron and M. Nordberg (*Farm. Revy*, 1952, **51**, 177.) The method given is based on the hydrolysis of the alkaloids with alkali and subsequent acidification, the tropic acid formed being measured from its ultra-violet spectrum. It is assumed that any substance present other than tropic acid will have linear absorption; determination of the absorption at three wavelengths will then give data from which the tropic acid content can be calculated. The alkaloid is dissolved in sodium hydroxide and the solution is heated on a water bath for 15 minutes. After acidifying with hydrochloric acid and making up to the required volume with water the extinction is measured at 254·0, 257·5 and 261·5 mμ. The percentage of alkaloid is then calculated from the equation $X = \frac{(7{\cdot}5\,E_2 - 4\,E_3 - 3{\cdot}5\,E_1)\ \text{equiv. w.}}{427{\cdot}1}$ mg alkaloid/ml. where X is the concentration, E_1, E_2 and E_3 are the measured extinctions at 261·5, 257·5 and 254·0 mμ respectively. Results are given in which the proposed method is compared with classical methods; agreement is satisfactory. R. E. S.

Water, Hardness of, by the Versenate Method. J. E. Houlihan. (*Analyst*, 1952, 77, 158.) The method introduced by Schwarzenbach and his co-workers

and modified by Betz and Noll (*J. Amer. Wat. Wks. Ass.*, 1950, **42**, 49), in which the calcium and magnesium ions are titrated directly with a solution of an ethylenediamine tetra-acetate salt to produce un-ionised complexes in the presence of Eriochrome black T, was used for the determination of total hardness over a range of widely differing samples; tables of results are given. The Schwarzenbach method gave results a little higher than the mixed-alkali method although considering the very different techniques employed and the large number of steps required for the second method the figures were in very good agreement; the solution strengths used were such that 1 drop of titrating solution for the Schwarzenbach method on a 25 ml. sample represented 2 p.p.m. of $CaCO_3$, whereas 1 drop for the mixed alkali method on a 200 ml. sample represented 5 p.p.m. of $CaCO_3$. R. E. S.

GUMS AND RESINS

Myrrh, Some Observations on the Constitution of. L. Hough, J. K. N. Jones and W. H. Wadman. (*J. chem. Soc.*, 1952, 796.) Extraction of myrrh with 90 per cent. aqueous ethanol removed most of the resin and left a crude polysaccharide (*ca* 40 per cent. yield), which was further purified by precipitation with acidified ethanol. This product had an equivalent weight of 547, contained 6·1 per cent. of methoxyl, gave positive tests for amino-acids, and from the nitrogen content was estimated to contain approximately 18 per cent. of protein. After its hydrolysis with acid, at least 15 amino-acids were detected on the paper chromatogram. By means of precipitation with copper sulphate, oxidation studies, and the application of ion-exchange resins and paper chromatography, it was shown that the crude acidic polysaccharide isolated from myrrh contained approximately 64 per cent. of carbohydrate containing the following monosaccharides in approximately the proportions indicated: D-galactose (4 parts), L-arabinose (1 part), and 4-methyl D-glucuronic acid (3 parts). A. H. B.

ORGANIC CHEMISTRY

Acetone, Dryness and Density of. P. Thirion and E. C. Craven. (*J. app. Chem.*, 1952, **2**, 210.) A review is given of the various figures in the literature for the density and water content of acetone samples. It is confirmed that, in a relative way, density is a good guide to water content but there remains some doubt concerning the density of dry acetone; a figure ($d_4^{20°\,C.}$, 0·7899) has been found, 0·0005 lower than was previously accepted, but still 0·0005 higher than those given recently by American workers. For the determination of water the acetyl chloride-pyridine method was used and this is recommended as a primary standard for this analysis; the Karl Fischer method was not regarded as trustworthy. It is suggested that the lower values reported by American workers may be due to the presence of *iso*propyl ether from the *iso*propanol source; a satisfactory cloud point method for the determination of *iso*propyl ether in acetone is given, using the cloud temperature of acetone (10 ml.) mixed with 25 ml. of aqueous sodium chloride solution (250 g./l.).
R. E. S.

4-Amino-6-hydroxy-*iso*phthalic acid. A. B. H. Funcke, C. van der Stelt, A. M. Simonis and W. T. Nauta. (*Pharm. Weekbl.*, 1952, **87**, 65.) In the manufacture of *p*-aminosalicylic acid by the carboxylation of *m*-aminophenol, a by-product is 4-amino-6-hydroxy-*iso*phthalic acid, which may be separated from the *p*-aminosalicylic acid by its relative insolubility in dilute nitric acid. This new acid is a stronger acid and weaker base than *p*-aminosalicylic acid. On

heating it decomposes at about 200 to 237° C. The absorption spectrum shows maxima at 238, 260 (inflexion point), 282·5 and 317 mμ. Since this acid may be present in commercial *p*-aminosalicylic acid, its pharmacological properties are of importance. The toxicity appears to be similar to that of *p*-aminosalicylic acid, and it has a small, but appreciable, tuberculostatic action. G. M.

Chloramphenicol, Analogues of. J. N. Ashley and M. Davies. (*J. chem. Soc.*, 1952, 63.) Simple analogues of chloramphenicol, in which the phenyl group was replaced by an alkyl group or by a reduced ring were prepared by standard methods. The products, namely 2-dichloroacetamido–propane-1:3-diol, -2-hydroxymethylbutanol, -2-phenylpropane-1:3-diol, -2-*p*-nitrophenylpropane-1:3-diol, -4-methylpentane-1:3-diol, -3-*cyclo*hexylpropane-1:3-diol, and -3-*cyclo*hexylpropan-1-ol had no significant bactericidal or virucidal activity.

A. H. B.

Hecogenin, Source of. P. C. Spensley. (*Chem. Ind.*, 1952, 426.) An improved method is given for the isolation of hecogenin from the sisal plant. The juice of the leaves is acidified to give a 1·5N solution which is heated to near the boiling point for 3 to 4 hours; activated charcoal is then incorporated and after an hour or more is collected and washed with water. The charcoal is dried and Soxhlet-extracted with ether or carbon tetrachloride, crude sapogenin being obtained by evaporation of the solvent followed by purification; normal specimens of this sapogenin fraction can be made to yield at least 60 per cent. of hecogenin and details of methods by which the pure steroid may be obtained are being published. Sisal juice ferments rapidly when freshly collected and gradually deposits a thick greenish-yellow sediment leaving a pale yellow, almost clear, layer at the top; examination of the upper and lower layers shows that the sapogenin is concentrated in the lower layer and is present only in small amounts in the upper layer. Allowing this fermentation and discarding the upper layer affords an improved process for the extraction of the sapogenin; as a further improvement the sediment can be collected from the fermentated juice by centrifugation. By this process a greenish yellow sludge is obtained representing about 3 to 10 per cent. of the bulk of the juice from which it originated. If this sludge is dried, a brittle solid, representing about 0·5 to 2 per cent. of the original juice is obtained which yields 4 to 12 per cent. of crude sapogenin. On a large scale the dried centrifuged sludge could be produced at the sisal estates.

R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Vitamin A, Synthesis from *cyclo*Hexanone. J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker. (*J. chem. Soc.*, 1952, 1094.) 8:9-Dehydrovitamin A was prepared by two routes from 2-ethynyl-1:3:3-trimethyl*cyclo*hex-1-ene, obtained by methylation of *cyclo*hexanone and then treatment with sodium acetylide in liquid ammonia and dehydration of the resultant acetylenic alcohol. Vitamin A was obtained from 1-ethynyl-2:2:6-trimethyl*cyclo*hexanol by condensation with 6-methylocta-3:5:7-trien-2-one to give a C_{20} glycol, rearrangement, selective semi-reduction of the triple bond, and then dehydration. An alternative route *via* a C_{18} acetylenic glycol obtained by the condensation of 2:2:6-trimethyl-*cyclo*hexanone with a C_9 acetylenic alcohol, or by the condensation of 1-ethynyl-2:2:6-trimethyl*cyclo*hexanol with crotonylideneacetone, followed by rearrangement, is also described. A. H. B.

BIOCHEMICAL ANALYSIS

Aliphatic Amines, Paper Chromatography of. R. Schwyzer. (*Acta chem. Scand.*, 1952, **6**, 219.) A paper chromatographic method has been developed for the detection, separation, and identification of as little as 1 μg. of aliphatic amines in biological materials. The ascending chromatogram method was used, placing strips or cylinders of paper in small receptacles and running the chromatogram for 1 hour with Munktell paper (solvent ascends 13 to 14 cm.) or 3 hours with Whatman paper, thus minimising the losses of amines due to volatilisation; *n*-butanol saturated with 25 per cent. acetic acid was used as solvent and the amine spots were made visible by spraying with a suitable indicator solution (bromophenol blue). Blue spots appeared immediately on a yellow background, varying in the intensity of colour according to the quantity of amine present; basic amino-acids gave similar spots. Results are given and R_F values are quoted for methylamine, dimethylamine, trimethylamine, ethylamine, *n*-propylamine, *iso*-propylamine, benzylamine, phenylethylamine, cadaverine and piperidine as hydrochlorides, and arginine as free base. A combination of the method with microdiffusion techniques offers a possibility of separating amine mixtures and preparing derivatives of the components.

R. E. S.

Dinitro-*ortho*-cresol in Blood, Estimation of. D. G. Harvey. (*Lancet*, 1952, **262**, 796). Routine blood estimation is the method of choice for assessing the risk of poisoning in persons using this substance. A method is described for collecting 0·1 to 0·2 g. of blood for analysis. With simple and readily available equipment 5 to 20 μg./g. of blood can be estimated with reasonable accuracy by a modification of Parker's method (*Analyst*, 1949, **74**, 646). The estimation in urine is not a reliable guide to the blood level. S. L. W.

Lactic Acid in Urine, Microdetermination of. M. U. Tsao, M. L. Baumann and S. Wark. (*Anal. Chem.*, 1952, **24**, 722.) The method described is based on the oxidation of the lactic acid to acetaldehyde followed by the estimation of the aldehyde produced from the colour reaction with *p*-hydroxydiphenyl on sulphuric acid. An apparatus is described in detail which achieves in one step the oxidation of lactic acid with ceric sulphate and the rapid transfer of the acetaldehyde thus obtained into sulphuric acid; the colour is developed in the same tube. The effects of variations in ceric sulphate concentration, in the water content of the sulphuric acid and in the diffusion time were studied. A standard deviation of 5·5 per cent. for a single determination was found in a series of recovery experiments. A study of interfering substances showed that carbohydrates and the products of glycolysis caused the most serious interference; methods for the removal of the interfering substances are discussed.

R. E. S.

Phenols and Surface-active Agents, Fungicidal and Fungistatic Evaluation of. G. C. Walker, C. L. Porter and H. G. De Kay. (*J. Amer. pharm. Ass., Sci. Ed.* 1952, **41**, 77.) Fungistatic tests were made on 0·1 per cent. solutions of a variety of derivatives of phenol, in ethanol (95 per cent.), using the agar cup-plate method with *Trichophyton mentagrophytes* as test organism. Halogen substitution in phenol and its derivatives increased the fungistatic power, chlorine being more effective than bromine or iodine. Increased activity was also obtained by substitution of a benzyl, *cyclo*hexyl or phenyl group, but the introduction of carboxyl, methyl or hydroxyl groups had little effect. Of 20 surface-active agents tested by the same method, laurylpyridinium chloride was

the most effective fungistatic. Using *Trichophyton mentagrophytes* in a mycelial disk technique, some of the substituted phenol derivatives were fungicidal in 1 minute at concentrations of 1·0 and 0·5 per cent. Only 2:4:5-trichlorophenol and 2:3:4:6-tetrachlorophenol were effective at 0·3 per cent. Of the surface-active agents, only laurylpyridinium chloride was fungicidal in a concentration of 1 per cent. G. B.

Procaine Benzyl Penicillin, Assay of. K. R. Gottlieb. *Dansk Tidsskr. Farm.*, 1952, **26**, 1.) About 0·1 g. of the compound, dissolved in 15 ml. of ethanol, is chromatographed on 10 g. of alumina, using in all 25 ml. of ethanol for the elution. After changing the receiver, the column is then eluted with a mixture of 45 ml. of O.1N sodium acetate solution and 5 ml. of 0·1N acetic acid. This latter solution is made up to 100 ml., and the benzylpenicillin in it is determined spectrophotometrically by measuring $E_{2570} - (1/3\ E_{2800} + 1/30\ E_{3200})$. The ethanol eluate is diluted with an equal volume of boiled water and titrated with 0·1N hydrochloric acid using bromophenol blue as indicator. A similar blank test is done by eluting another alumina column with 40 ml. of ethanol, which is used for the blank titration, followed by an elution with the buffer solution which is then used for a blank in determining the extinction. The procaine content is calculated from the titration, 1 ml. of 0·01N hydrochloric acid being equivalent to 0·02363 g. of procaine base. The method is applicable only to pure procaine benzylpenicillin. G. M.

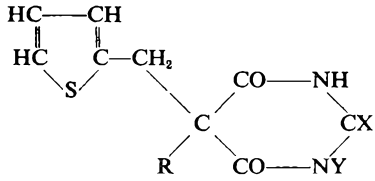
Tyrothricin, Assay of. A. K. Miller, C. Matt and J. L. Ciminera. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 23.) The tube dilution method of the U.S.P. in which the biological activity of tyrothricin against a hæmolytic streptococcus is compared with that of a standard preparation may be modified to use a young seed culture which, together with the standard dilutions of tyrothricin in propylene glycol-ethanol mixture, may be prepared in bulk and can be stored for up to 2 weeks. An 18-hour broth culture of the test organism is prepared and 0·1 ml. is used to inoculate 5 ml. of medium in a colorimeter tube. The turbidity is determined at intervals during incubation of the tube at 37° C., until the growth curve indicates that the culture is definitely in the logarithmic phase of growth. 4 ml. of this culture is mixed with 100 ml. of medium at 37° C. and growth is checked during incubation by observing a 5-ml. sample in a colorimeter tube. At the beginning of the logarithmic phase incubation is stopped and the inoculum is stored at 5° C. For the test, 80 ml. of medium, 20 ml. of bovine serum albumen and 2 ml. of inoculum are mixed at refrigerator temperature and 5-ml. quantities are placed in tubes, to which tyrothricin solutions are added in amounts equivalent to 2·6, 4, 6 and 9 mμg. of standard tyrothricin and about 5 mμg. of the tyrothricin under test. The tubes are incubated at 37° C. until growth is sufficient, as determined by turbidity measurement in check tubes. Generally 5 to 5½ hours' incubation is required and the determination can be completed during a day's work. The standard curve is prepared from which the potency of the sample is read. The method of calculation of 95 per cent. confidence limits is given. G. B.

CHEMOTHERAPY

2-Alkoxy Analogues of Procaine and Amethocaine, Local Anæsthetic, Toxic and Irritant Effects of. F. P. Luduena and J. O. Hoppe. (*J. Pharmacol.*, 1952, **104**, 40.) Local anæsthetic activity was assessed by sciatic nerve block in guinea-pigs (duration of motor paralysis) and by the rabbit cornea method.

Intradermal irritation was determined by a modification of the trypan blue test in the clipped abdominal skin of the rabbit, and acute toxicity was measured in mice, by intravenous injection. Results are tabulated for a number of derivatives of procaine (β-diethylaminoethyl 4-aminobenzoate) amethocaine (β-dimethylaminoethyl 4-butylaminobenzoate) and closely related compounds. Local anæsthetic activity was greatest in the piperidylpropyl esters, less for the corresponding diethylaminoethyl and diethylaminopropyl series and least in the dimethylaminoethyl compounds, and the local anæsthetic activity and toxicity increased with the length of the 2-alkoxy group. These compounds had little irritant effect on the tissues, but 2-propoxy and 2-butoxy substitution of piperidylpropyl 4-butylaminobenzoate greatly increased the local irritant effect. Some of the compounds, for example, diethylaminoethyl 2-propoxy- and 2-butoxy-4-aminobenzoate had greater anæsthetic action and were less irritating than amethocaine. G. B.

Barbituric Acid Derivatives as Narcotics and Anæsthetics. H. Morren, G. Dony and S. Levis. (*J. Pharm., Belg.*, 1952, **7**, 65.) A number of 2-thienyl-barbiturates of the general formula given below, were examined.



(X = O or S, Y = H or CH_3 and R = various alkyl or alkylheterocyclic residues, *cyclo*hexyl or *cyclo*hexenyl). The compounds were prepared by (*a*) reaction of sodium diethylmalonate with an organic halide, treatment with 2-thienyl chloride and sodium, and ring closure with urea, thiourea or *N*-methyl thiourea, (*b*) as for (*a*) but interchanging the first two steps or (*c*) preparing 5-(2-thienyl) barbituric acid and allowing the sodium derivative to react with the organic halide. For the derivative R = *cyclo*hexenyl, *cyclo*hexanone was used instead of an organic halide. When tested in mice by intravenous injection, observing the effective dose, time of induction and duration of sleep and nervous symptoms, the following 3 compounds appeared most suitable as anæsthetics:— (1) R = 1-methylbutyl, X = O, Y = H, (2) R = *n*-hexyl, X = O, Y = H and (3) R = 1-methylheptyl, X = O, Y = H. These compounds were tested as anæsthetics in rabbits and dogs, the effect on arterial pressure and respiration being observed in the latter animal. Compound (2) caused some agitation in all three species, but the other two compounds are to be tested clinically. G. B.

Barbituric Acid Derivatives, Synthesis and Pharmacological Properties of. M. Ruhnek and F. Sandberg. (*Svensk farm. Tidskr.*, 1952, **56**, 70, 95, 120.) The following new amphoteric barbituric acid derivatives were synthesised:— (1) 1-(β-diethylaminoethyl)-5:5-diallyl-, (2) 1-(β-dibenzylaminoethyl)-5:5-diallyl-, (3) 1-(*N*-ethyl-*N*-α-naphthylmethylaminoethyl)-5:5-diallyl-, (4) 1-(β-diethylaminoethyl)-5-*cyclo*hexenyl-5-ethyl-, (5) 1-(β-diethylaminoethyl)-5-allyl-5-phenyl-barbituric acid. Basic derivatives prepared were (6) 1-methyl-3-(β-diethylaminoethyl)-5:5-diallyl-, and (7) 1-benzyl-3-(β-diethylaminoethyl)-5:5-diallyl-barbituric acid. An account of the method of preparation, the physical constants, and the potentiometric titration curves is given, and the detailed examination of the pharmacological action of these compounds is

recorded. None of the compounds had any hypnotic or adrenolytic action. All compounds produced a fall in the blood pressure which was unaffected by atropine, antergan or vagotomy. Compounds (1), (4) and (5) differed from the other derivatives in that they produced an autonomic ganglion block and a parasympathetic neuro-effector block. The basic barbituric acid (7) exhibited a pronounced analgesic and antipyretic action, in addition to a sedative effect. In toxic doses it produced bronchoconstriction, presumably by direct action on the muscles. A. H. B.

PHARMACY

DISPENSING

Chloramphenicol, Stability of Aqueous Solutions of. C. Trolle-Lassen. (*Arch. Pharm. Chem.*, 1951, **58**, 780.) The method used for the assay of chloramphenicol was a modification of that used in the United States Pharmacopœia. Buffered solutions (0·1 per cent.) were prepared and heated for 15 minutes at 100° C., 60 minutes at 100° C. or 20 minutes at 120° C. respectively. Stability was satisfactory at a *p*H range between 2 and 7. Much decomposition occurs outside this range, although the absorption spectrum shows little change. In basic solutions decomposition is accompanied by a drop in *p*H value, owing to hydrolysis and formation of dichloracetic acid. G. M.

NOTES AND FORMULÆ

Mercumatilin Sodium (Cumertilin Sodium). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1952, **148**, 1124.) Mercumatilin sodium is a mixture in equimolecular proportions of theophylline and sodium mercumallylate (sodium 8 - (2′ - methoxy - 3′ - hydroxymercuripropyl)coumarin - 3 - carboxylate, $C_{14}H_{13}HgNaO_6$,$C_7H_8O_2N_4$,H_2O. It is not isolated from solution, which may contain a slight excess of theophylline. The mercumallylic acid used in the preparation of mercumatilin sodium responds to the following tests and standards. It is a white, odourless, bitter powder, m.pt. about 190° C. with decomposition, soluble in sodium hydroxide test solution (1 in 4·2), slightly soluble in water, ethanol, chloroform and acetic acid, and almost insoluble in ether. When refluxed with aqueous formic acid for 15 minutes, and filtered, the filtrate yields crystals of cumallylic acid (8-allylcoumarincarboxylic acid), which after washing with water and drying at 105° C. for 2 hours melt at 147° C. to 150° C. A solution in 0·1N sodium hydroxide yields no immediate precipitate or colour with sodium sulphide (absence of mercuric ions). When extracted with acetone for 4 hours, it yields not more than 2·5 per cent. of acetone–soluble extractive, dried at 105° C. for 2 hours. When dried at 105° C. for four hours, it loses not more than 5·0 per cent. of its weight; it yields not more than 0·05 per cent. of sulphated ash. It contains 39·8 to 43·1 per cent. of Hg, equivalent to 95·0 to 103·0 per cent. of mercumallylic acid, when assayed by the thiocyanate method. The solution of mercumatilin sodium must comply with an additional identity test for theophylline and with the test for absence of mercuric ions. It contains 94·0 to 106·0 per cent. of the labelled amount of mercumatilin sodium, determined by the assay for mercury described above, and 94·0 to 106·0 per cent. of the labelled amount of theophylline. Mercumatilin sodium is used as a diuretic. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

p-Acetamidobenzaldehyde Thiosemicarbazone in the Treatment of Leprosy. J. Lowe. (*Lancet*, 1952, 262, 436.) This drug has been administered daily in tablets of 50 mg. to 71 patients over periods varying from 5 to 17 months. Three types of cases were under treatment: a group of lepromatous type previously untreated, a group of tuberculoid type also previously untreated, and a group of lepromatous type previously treated with sulphones but with resulting complications. Apart from 1 case of acute agranulocytosis no serious toxic effects were observed. The drug was well tolerated and complications few and usually not severe. Clinical and bacteriological response was satisfactory. Compared with sulphones, allergy is rare, but apart from this the drug has no obvious advantage over sulphone treatment in non-lepromatous cases. In more severe lepromatous cases the complications are fewer and less severe. However, administration is more difficult, and the drug more expensive. It is suggested that if agranulocytosis is rare the drug will be a valuable alternative to the sulphone treatment. J. R. F.

Analgesic Drugs, Assay of, on Man. C. A. Keele. (*Analyst*, 1952, **77**, 111.) A review is made of the techniques available for the quantitative assessment of the value of drugs given to patients suffering pain. The effects of dithienyl analgesics on ischæmic muscle pain have been studied in two ways: (*a*) by observing the effects on maintained ischæmic pain, the drug being injected intravenously, and (*b*) by observing the effects on the number of muscular contractions required to produce ischæmic muscle pain, the drug being injected intramuscularly. The first method yielded useful results and the estimate of the analgesic potency of the dithienyl compounds places them somewhat between morphine and amidone, on the one hand, and pethidine on the other; subsequent work on patients confirmed this estimate of the relative potency of new compounds. The second method, using muscular contractions producing ischæmic muscle pain, was less reliable and an analysis of the results suggested that the figures gave more information about the subject than about the analgesic potency of the drugs used. Results are given of the frequency of side effects observed, including drowsiness, euphoria, dizziness, nausea and vomiting. Work on normal persons and on selected patients showed that the dithienylbutylamines had an analgesic action, usually associated with a definite hypnotic effect. Such properties would be most useful in treating post-operative pain or in helping to procure sleep in patients kept awake by pain during the night; an investigation on patients is being carried out. R. E. S.

Analgesic Potencies, Estimation of. A. Tye and B. V. Christensen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 75.) Analgesic potencies of a number of drugs were estimated in rats. The criterion of analgesia was the failure of the rat to move its tail when a radiation stimulus was applied from a modified Hardy-Wolff-Goodell pain threshold apparatus. Satisfactory estimates of the strengths of morphine sulphate solutions were obtained by a comparison of the AD50 for the solutions with that of pure morphine sulphate. There was no statistical difference in the results for AD50 whether or not litter mates were used in the test. Pentobarbitone and acetylsalicylic acid showed no activity when tested by this method, and pentobarbitone did not potentiate the action of codeine. Diethylaminoethanol, a hydrolytic fragment to which the analgesic effect of intravenous procaine has been attributed, produced analgesia only when administered in toxic doses. G. B.

Aureomycin, Intrathecal Administration in Meningitis. K. M. S. Ainley-Walker and F. D. Bosanquet. (*Lancet*, 1952, **262**, 433.) Systemic administration to 14 out of 15 patients did not produce therapeutic levels of the antibiotic in the cerebrospinal fluid. A fall in blood pressure in hypertensive patients and a fall of cerebrospinal fluid pressure in both normal and hypertensive patients were observed. An intrathecal preparation was used in an attempt to achieve sufficient concentration. This was administered to 3 patients with advanced penicillin- and streptomycin-resistant types of meningitis. Therapeutic levels were produced without untoward reactions and an improvement in their condition was seen although recovery was not achieved. Intraventricular or cisternal injection is considered essential to obtain a satisfactory concentration. The solution used was prepared by dissolving 100 mg. of aureomycin hydrochloride in 10 ml. of sterile water giving a *p*H of 2·6. This, when frozen solid, keeps for a minimum of 6 weeks. Immediately before use, 1 ml. is added to 9 ml. of a buffer consisting of 9 ml. of 2 per cent. glycine in water and 0·175 ml. of 0·2N sodium hydroxide solution, giving a final *p*H of 7·2 to 7·4. The solution contains 1 mg. of aureomycin per ml. and is active for 1 hour. A slight precipitation may occur. The buffer may be made in bulk and distributed in 9 ml. quantities into vaccine bottles and autoclaved. The contents will keep for at least 6 weeks. J. R. F.

Barbiturate Poisoning, Conservative Treatment of. S. Locket and J. Angus. (*Lancet*, 1952, **262**, 580.) On admission the patient receives gastric lavage with 2 pints of normal saline or tap water. This is followed by penicillin, 500,000 units 6-hourly by intramuscular injection as a prophylactic against broncho-pneumonia, until the patient has been conscious for 48 hours. Oxygen is given at the rate of at least 10 l./minute until respiration is normal, cyanosis has permanently disappeared, and consciousness is fully restored. In the event of coma lasting more than 24 hours, fluid loss is made up by giving intravenous 5 per cent. glucose saline, 1 l. in 24 hours. Catheterisation of the bladder is necessary in unconscious and some stuporose patients. Prolonged laryngeal intubation, which may occasionally produce fatal laryngeal œdema, is unjustified. The use of sympathomimetics to raise blood pressure may produce renal vasoconstriction and interfere with clearance of barbiturates with a prolonged action. In a series of 64 consecutive cases of barbiturate poisoning given this conservative treatment there were only 2 deaths. No analeptics were used and drainage by lumbar puncture was never attempted. No chest-thumping is practised, but during the entire period of coma great care is taken to ensure an adequate airway by careful attention to the tongue and mouth. S. L. W.

Cardiac Glycosides, Absorption Rate of Orally Administered. W. F. White and O. Gisvold. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 42.) Doses of digitoxin, digoxin, acetyldigoxin and lanatoside C were administered orally to unanæsthetised cats and the time of death was observed so that the survival time could be used as a measure of the absorption rate for the drug. Using solid forms (tablets of digitoxin, digoxin and lanatoside C, and capsules of acetyldigoxin) digitoxin and acetyldigoxin were absorbed fairly rapidly, but digoxin and lanatoside C were absorbed much more slowly. When the drugs were administered as solutions in aqueous ethanol, in aqueous solution with Tween 80 or in a solution of low ethanol content with Tween 80, all the glycosides were more rapidly absorbed than from the solid. Digoxin administered in this way was comparable with digitoxin and acetyldigoxin. Thus if absorption in cats and human beings is comparable the maintenance dose of 0·5 mg. of

digoxin in tablets might be reduced to one-fifth by administration of the substance in capsules containing a Tween 80 solution. Lanatoside C was slowly and incompletely absorbed even from solutions. It is suggested that acetyldigoxin in tablets should be tried clinically. G. B.

Chloramphenicol, Local Use in Wound Infections. M. H. Flint, H. Gillies and D. A. C. Reid. (*Lancet*, 1952, **1**, 541.) 30 cases of wound infection (infected burns, gravitational ulcers, pedicle flaps, grafts and abscess cavities) were treated with chloramphenicol applied as a 5 per cent. dilution in lactose or a 5 per cent. solution in propylene glycol. The majority of infections were due to penicillin-resistant organisms (*Staphylococcus aureus*, *Pseudomonas pyocyanea*, *Proteus vulgaris* and coliform bacilli) and had not responded to penicillin, aureomycin or chloramphenicol administered systemically. Bacterial clearance was obtained in an average of 4 or 5 days and improved wound healing occurred during treatment. None of the organisms showed any tendency to become more resistant to chloramphenicol. The propylene glycol solution, irrigated into the cavities or applied on wicks or gauze pads appeared to be preferable to the powder. G. B.

Citrinin, Therapeutic Tests on. L. Leusch. (*J. Pharm., Belg.*, 1952, **7**, 77.) Ointments containing 0·5 per cent. of citrinin, an antibiotic obtained from *Penicillinum citrinum* Thom. were very effective in cases of streptococcal and staphylococcal skin infections, whereas ointments prepared with sodium citrinate were ineffective. In the treatment of tropical ulcers the initial effect of the ointment was good, but after the first week, progress was slow. Treatment of the tropical ulcers with gauze dressings impregnated with a 0·5 per cent. suspension of citrinin in water, followed at a later stage by the application of a powder containing 0·5 per cent. of citrinin in boric acid, cleared the infection and the curative action continued without delay until healing was complete. 25 cases of ulcers infected with fusiform bacteria and borrelia were successfully treated by this method, the average time of hospitalisation in this series, which included cases complicated by œdema etc., being 18 days. Simple cases were cured in 12 to 15 days. G. B.

Daraprim, a New Antimalarial; Trials in Human Volunteers. L. G. Goodwin. (*Brit. med. J.*, 1952, **1**, 732.) In a group of 13 volunteers, daraprim (2:4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine) in doses of 50 mg. twice weekly for 3 months produced no significant effects on the blood counts, sedimentation rate or urine. Slight gastro-intestinal upset was observed in 2 persons. In one individual, daily doses of 5 mg. administered continuously for one year produced no toxic symptoms and no abnormalities in the blood and bone-marrow or urine. This dose was an effective suppressant of *P. falciparum* malaria infections. G. B.

Daraprim in Treatment of Malaria. I. A. McGregor and D. A. Smith. (*Brit. med. J.*, 1952, **1**, 730.) 25 children and 4 adults infected with *P. falciparum* and 3 children with *P. malariæ* were treated with daraprim (2:4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine), a tasteless substance administered in a single oral dose of 0·25 to 0·5 mg./kg. in the form of a suspension or a syrup. The blood was freed from asexual parasites within 96 hours for *P. malariæ* infection and in 72 hours in all but 2 cases of *P. falciparum* infection. The drug was equally effective in patients without or with a considerable immunity. No skin rashes, toxic renal damage or depression of hæmopoiesis were observed, but

vomiting occurred in 2 children. The drug appears to act mainly during schizogony at the stage of plasmodial development when the chromatin is in active division, but, after a single dose, the blood concentration remains high enough to be lethal to all asexual forms even if some do not reach the vulnerable phase for 72 hours after the drug has been administered. G. B.

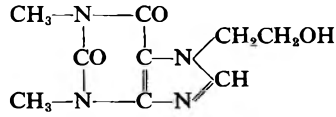
Dinitro-*ortho*-cresol Poisoning, Prevention of. P. L. Bidstrup, J. A. L. Bonnell and D. G. Harvey. (*Lancet*, 1952, **262**, 794.) The estimation of blood levels in workmen subjected to the risk of absorbing dangerous amounts of this substance would prove a valuable additional measure in the prevention of acute poisoning. This should be done at least at weekly intervals and the man should not return to work with this substance until the results are known. If the level in blood taken 8 hours after the last exposure is 20 μg./g. or above, the workman should be removed from further contact with the substance for at least 6 weeks. At this level the only symptom is an exaggerated feeling of well-being, but at levels of 40 μg./g. or more symptoms of headache, lassitude and general malaise occur. Where the blood level is 10 to 20 μg./g. strict supervision is necessary to ensure that all the recommendations for safe handling are observed, and if it has risen further after 2 days the man should be removed from contact with the compound. With levels below 10 μg./g. no extra precautions are necessary. The main route of absorption is by inhalation, and respirators should be worn, especially by spray operators and others concerned in the spraying of cereal crops. Determination of dinitro-*o*-cresol in the urine is unlikely to prove a reliable test of absorption when the blood level is about 20 μg./g. S. L. W.

Fungistatic Powers of Phenindamine and Asterol Dihydrochloride. E. E. Seale. (*Canad. med. Ass. J.*, 1951, **65**, 582.) *In vitro* studies on fungistatic properties are reported on the antihistamic drug phenindamine (thephorin) and a new compound, asterol dihydrochloride, 2-dimethylamino-6-(β-diethylamino-ethoxy-benzothiazole). Three methods were used in the investigation, namely, the mycophil broth sensitivity test, the agar plate technique to establish the critical fungistatic dilution, and the penicillin cylinder assay method. The results obtained suggest that both substances possess *in vitro* fungistatic properties for pathogenic fungi. Using the mycophil broth sensitivity test, asterol dihydrochloride in a concentration of 0·3215 mg./ml. inhibited the growth of the dermatophytes, the effective inhibitory concentration of phenindamine varying with the species. Both compounds showed fungistatic properties with the penicillin cylinder assay technique using *Tricophyton mentagrophytes*. With the agar plate method resistance to asterol dihydrochloride developed in cultures of *Microsporon audouini* isolated from ringworm of the scalp, when treatment with the compound had been continued for 6 to 7 weeks. S. L. W.

Hydrocortisone (Free Alcohol), Hydrocortisone Acetate and Cortisone (Free Alcohol) Antirheumatic Effects of, Compared with Cortisone Acetate. E. W. Boland. (*Brit. med. J.*, 1952, **1**, 559.) The compounds were administered orally to patients with rheumatoid arthritis and results assessed by observing the response to large suppressive doses, and by comparing equivalent maintenance doses. Cortisone (free alcohol) and cortisone acetate were equally effective. Hydrocortisone (free alcohol), i.e., 17-hydroxycorticosterone, in initial suppressive doses acted more rapidly and lowered the erythrocyte sedimentation rate more quickly. By comparison of maintenance doses, this

compound was 50 per cent. more active than cortisone. The smaller maintenance doses of hydrocortisone (free alcohol) induced fewer side effects than the cortisone treatment. Hydrocortisone acetate was much less active, possibly because its lower solubility may lessen alimentary absorption. G. B.

Hydroxyethyltheophylline, Clinical Experience with. O. W. Lürmann and W. Böres. (*Dtsch. med. Wschr.*, 1952, **77**, 15.) As an alternative to the present method of increasing the solubility of theophylline by the addition of amines, soluble derivatives of theophylline have been tried. One such product is 7-(β-hydroxyethyl)theophylline



Clinical experience with this compound, in a large number of cases, has shown that its action is somewhat less than that of the other preparations, probably owing to the absence of the additional effect of the amine on the heart and circulation. It is, however, more readily tolerated. G. M.

Insulins, Comparison of Duration of Action of Different Forms of. F. Gerritzen. (*Brit. med. J.*, 1952, **1**, 249.) The duration of action of 10 different products was investigated on young healthy student volunteers. They were administered, in equal doses of 20 units, to groups varying in numbers from 4 to 12, with a control group of 6 who did not receive insulin. In the control group no significant deviation was found between the average blood sugar values at different times over 24 hours. The other groups responded as follows:—Regular insulin; blood sugar reaches its lowest point after 1 hour and returns to its starting-point in 8 hours. Protamine zinc insulin; duration of action 18 hours, lowest point reached in 5 to 8 hours. Di-insulin; duration of action 16 hours, greatest decrease in blood sugar during first 3 hours. Iso-insulin; duration of action 16 hours, rapid decrease in the first hour. Globin insulin; action identical with that of di-insulin. N.P.H. 50 (two samples); duration of action 11 hours, lowest point after 3 (or 4) hours. Insulin mixtures (proprietary); duration of action would appear to depend on the regular insulin content only so far as the decrease during the first 3 hours is greatest with the mixture containing most regular insulin (a 3:1 mixture) and that thereafter the blood sugar takes longer to come back to the starting-point than with 2:1 and 1:1 mixtures. S. L. W.

Kemadrin in Postencephalitic Parkinsonism. E. Montuschi, J. Phillips. F. Prescott and A. F. Green. (*Lancet*, 1952, **262**, 583.) Kemadrin (1-*cyclo*hexyl-1-phenyl-3-pyrrolidino-propan-1-ol) is a spasmolytic chemically related to artane. A trial of the drug in 50 cases of postencephalitic parkinsonism showed that it is of considerable value in relieving some of the symptoms of the disease, particularly the rigidity, depressed emotional tone, and lack of expression. It was administered for periods varying from 3 to 11 months in doses of 5 to 20 mg. 3 times daily, the average dose being 15 mg. 3 times daily. Side-effects (giddiness) occurred in only 3 of the patients, and was relieved by reduction of the dose; one patient complained of visual disturbance. In no case did toxic effects necessitate withdrawal of the drug. There would appear to be no significant difference in the therapeutic actions of kemadrin and artane, and it was found possible to change patients from one drug to the other without

causing any apparent change in their clinical condition. No contra-indications to the use of the drug were found; in particular, hypertension is not a contra-indication. As kemadrin has no greater mydriatic effect than artane it is likely that, with due care, it can be used in patients with glaucoma. S. L. W.

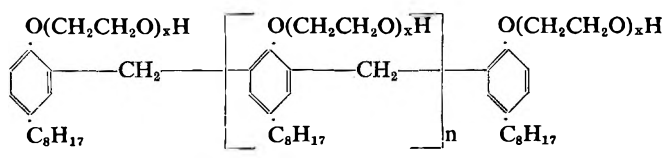
Methonium Compounds: Oral Use in the Treatment of Hypertension. A. J. M. Campbell, J. G. Graham and R. D. H. Maxwell. (*Brit. med. J.*, 1952, **1**, 251.) The authors have used methonium compounds for almost 2 years in the treatment of hypertension, and the treatment of an unselected group of 35 patients with severe symptoms, whose ages ranged from 29 to 67 years, is described. Tablets of comparable methonium content were used, that is, a 250 mg. tablet of hexamethonium bromide, which is equivalent to a 350 mg. tablet of the bitartrate and to 125 mg. of M. and B. 1863 bromide (a homologue of hexamethonium). Oral dosage began with a single tablet, followed by a progressive increase in frequency and quantity to a maximum of 12 to 16 tablets a day. At no time was more than 4 g. of hexamethonium bromide given daily. In 23 of the 35 patients there was a good symptomatic improvement with regression of the signs. Hexamethonium also produced a symptomatic improvement in 5 patients with severe chronic nephritis and 2 with malignant hypertension. S. L. W.

1-Methyl-2-mercaptoimidazole, Treatment of Thyrotoxicosis with. R. L. Kendrick, K. Balls and E. Rose. (*Arch. intern. Med.*, 1952, **89**, 368.) A mixed group of 17 patients was treated with 1-methyl-2-mercaptoimidazole with the object of inducing remission of thyrotoxicosis without operation, and a group of 15 women was treated pre-operatively. A daily dose of 30 mg. for adults and 15 mg. for children was given initially and reduced as thyrotoxicosis subsided. Responses were good in 26 cases, fair in 5 and poor in 1. In 15 patients on prolonged therapy with complete remission, the minimum time required to induce complete remission of symptoms was 4, the maximum 12 and the average 7 weeks. The only untoward effects were sensitivity reactions (vesicular skin eruptions, urticaria and pruritus) in 3 patients. G. B.

Morphine, Liberation from Codeine in the Rat. T. K. Alder and F. H. Shaw. (*J. Pharmacol.*, 1952, **104**, 1.) Rat-liver slices were incubated at 38° C. in Krebs–Ringer bicarbonate solution containing 1 to 2 mg. of codeine, nitrogen or oxygen, with 5 per cent. of carbon dioxide, being passed in and the preparations agitated. The solution after precipitation of protein with trichloracetic acid and extraction with chloroform under acid and alkaline conditions to remove codeine, etc., was saturated with sodium bicarbonate and the phenolic metabolite extracted with ethanol–chloroform mixture. The product was identified as morphine by spectrophotometer and by the X-ray diffraction pattern and ultra-violet spectrum of the derivative formed with 2:4-dinitrochlorobenzene. Codeine in the metabolites was estimated by a modification of a method depending on the opacity produced after adding silicotungstic acid. Under ærobic conditions about 40 per cent. of the codeine disappeared and the morphine formed was equivalent to less than half this quantity. Under anærobic conditions about 6 per cent. only of the codeine was destroyed and no morphine could be detected. The morphine was shown to undergo conjugation which could be inhibited by M/50 sodium fluoride or M/1500 monoiodoacetic acid, but the latter compound interfered with the metabolism of the codeine. G. B.

Mysoline in the Treatment of Epilepsy. R. Handley and A. S. R. Stewart. (*Lancet*, 1952, **262**, 742.) Mysoline, an anti-convulsant drug closely related to phenobarbitone, is 5-phenyl-5-ethyl-hexahydropyrimidine-4: 6-dione. It is a white, crystalline substance, practically tasteless, chemically stable, and sparingly soluble in water. It has an extremely low toxicity, both acute and chronic, in all species of laboratory animals tested, and has no hypnotic action in doses many times that required to protect animals against electrically-induced convulsions. Of 40 patients suffering from major epilepsy, 32 (80 per cent.) were improved and 12 (30 per cent.) were completely freed from all types of attacks, under mysoline therapy. The optimum daily dosage rarely exceeds 1·6 g., and this dosage produces only very mild and transient side-effects and does not make the patients sleepy. Hypertrophy of the gums and abnormal blood changes did not occur in this series. The change from old to new treatment was uneventful; the previous drug should be withdrawn gradually over 2 weeks. 0·25 g. of mysoline twice daily should be added for 3 days and then increased by 0·25 g. every 3 days to a daily total of 1 g., with further increases to a daily total of 1·6 g. if necessary. Withdrawal of previous treatment should begin on the 4th day and be complete by the end of the second week. S. L. W.

Surface-active Agents, Anti-tuberculous, Depression of Tuberculin Sensitivity in Guinea-pigs by. P. D'Arcy Hart, D. A. Long and R. J. W. Rees. (*Brit. med. J.*, 1952, **1**, 680.) Surface-active polyoxyethylene ethers of low toxicity were prepared by polymerisation of ethylene oxide with condensation products of formaldehyde with *p-tert.*-octylphenol. They had the general formula given below.



The following were chemotherapeutically active in the tuberculous mouse:—(1) a mixture in which $n = 0$ upwards, $x = 20$ (average value); (2) a similar mixture with a smaller proportion of lower members of the series and (3) as for (1), but $x = 10$ (average value). An isolated $n = 1$ compound and a mixture with $x = 60$ (average value) were inactive. Single subcutaneous or intravenous injections of the chemotherapeutically active compounds, but not the inactive ones, decreased the sensitivity of BCG-sensitised guinea-pigs to tuberculin, in a degree comparable to that for cortisone. The desensitising action, unlike that of cortisone, was independent of dietary factors and thyroid activity. It is suggested that (1) for these surface active compounds the mechanism of chemotherapeutic action and depression of sensitivity are interlinked, although there is little evidence that the antituberculous effect is due to desensitisation and (2) for cortisone the mechanism of desensitisation is different, being related to diet etc., and the cortisone-induced mesenchymal depression equals or outweighs the benefits of desensitisation, since cortisone has no anti-tuberculous action. G. B.

Terramycin in Infections in Infants and Children. B. Wolman and A. Holzel. (*Brit. med. J.*, 1952, **1**, 419.) Cases of pneumonia responded rapidly, fever disappearing in 24 to 72 hours. Of 35 patients, only 3 showed no response

to the drug. Good results were also obtained in upper respiratory tract infections and in tonsilitis. Treatment with terramycin was effective in pyuria where sulphonamides, streptomycin and chloramphenicol had given only temporary improvement. In newborn infants with purulent conjunctivitis, terramycin was useful when the infecting organism had become resistant to penicillin or streptomycin. No toxic reactions were observed. The drug was conveniently administered to children in a dose of 50 mg./kg., as a palatable elixir, and a solution with sodium chloride and sodium borate in distilled water was used for ophthalmic cases. G. B.

Triethylene Melamine in Neoplastic Diseases. J. C. Wright, A. Prigot, L. T. Wright and I. Arons. (*Arch. Intern. Med.*, 1952, **89**, 387.) 42 patients with neoplastic diseases were treated with triethylene melamine, administered orally in an average dose of 5 mg. daily for 3 days. When an initial course produced no toxic effects and no therapeutic effect after 15 days, the treatment was repeated. Pyridoxine (50 mg.) was administered orally at the same time as the drug to reduce the incidence of nausea and vomiting. Citrovorum factor was used in 2 cases to correct leucopenia after the treatment. Improvement occurred in 18 patients and was marked in lymphosarcoma, Hodgkin's disease, chronic myelogenous leukæmia and chronic lymphatic leukæmia, and moderate in fibrosarcoma, reticulum-cell sarcoma and mycosis fungoides. No improvement occurred in cases of carcinoma. When triethylene melamine showed marked inhibition of a patient's tumour in tissue culture, the drug was also active *in vivo*. G. B.

Vitamin B_{12}, Side-effects of a Preparation of. P. D. Bedford. (*Brit. med. J.*, 1952, **1**, 690.) The following preparations were tested for skin reaction following intradermal injection and for side-effects following deep intramuscular injection:—(1) a commercial solution of vitamin B_{12} factors obtained by extraction from *Streptomyces griseus* fermentation liquors, (2) a preparation of vitamin B_{12} (cyanocobalamin) derived from liver and (3) a solution of vitamin B_{12c} obtained from streptomyces cultures but highly purified by crystallisation. Of 100 patients, 14 had a positive skin reaction to (1), 2 to (3) and none to (2), 6 showed side effects to (1) and none showed side effects to (2) and (3). Both cutaneous reactions and side-effects to intramuscular injection were twice as common in patients who had previously been treated with antibiotics. Attention is drawn to the fact that as non-specific hypersensitivity to fungi or mould products may cause a significant proportion of unusual responses to antibiotic therapy, the use of impure vitamin B_{12} preparations derived from mould cultures is liable to induce idiosyncrasy to antibiotics. G. B.

BACTERIOLOGY AND CLINICAL TESTS

Alkaloids, Toxicity to Bacteria. C. C. Johnson, G. Johnson and C. F. Poe. (*Acta pharmacol. Toxicol.*, 1952, **8**, 71.) Various species of *Escherichia* and *Aerobacter* were incubated at 37° C. for 72 hours in media containing various concentrations of berberine, sanguinarine, and physostigmine, and the volume of gas produced after 24, 48 and 72 hours was compared with that produced in controls. Berberine and sanguinarine were more toxic for *Escherichia* than for *Aerobacter*, although the difference was not sufficient to allow separation of the genera by growth in media containing the alkaloids. In media containing 1 in 900 of berberine, no gas was formed during 48 hours by cultures of *Escherichia*; cultures of *Aerobacter* required a concentration of 1 in 400 to produce

[*continued on page* 672

LETTERS TO THE EDITOR

The Actions of Drugs on the Isolated Trachea

SIR.—The isolated tracheal preparation has been much used recently.[1] Instead of simply tying the rings together, we have opened each ring and recorded only the movement of the circular muscle, thereby increasing the magnification at least three times. In the table are shown the minimal concentrations of histamine and acetylcholine necessary to produce constriction of various mammalian preparations. It will be noted that tracheæ of the dog are extremely sensitive to acetylcholine; those of the cat, rabbit and rat are insensitive to histamine. In these latter preparations, histamine reduces the acetylcholine response and that due to potassium chloride; a trachea contracted by acetylcholine, however, is partly relaxed by histamine whereas one contracted by potassium chloride is unaffected. It is suggested that permeability of the cell is affected.

SENSITIVITY OF TRACHEAL PREPARATIONS

Constrictor	Guinea-pig	Human	Dog	Cat	Rabbit	Rat
Acetylcholine ..	10^{-7}	10^{-5}	10^{-9}	10^{-8}	10^{-6}	10^{-6}
Histamine ..	10^{-7}	10^{-5}	10^{-6}	—	—	—

In all the species studied, calcium chloride potentiates the acetylcholine response but blocks the potassium stimulation. Magnesium chloride is much more effective in blocking the potassium stimulation than it is in reducing the acetylcholine response.

If only one tracheal ring is used, rhythmical activity may sometimes be seen. We have confirmed that this type of activity is present in the dog,[2] especially if small doses of histamine, acetylcholine or potassium chloride are introduced into the bath. It is present also in the rabbit and cat when the muscle is affected by small doses of acetylcholine or potassium chloride.

This smooth muscle preparation may be of value in assessing the activity of parasympathetic blocking agents, detecting the presence of minute quantities of acetylcholine in biological fluids, and studying the penetration of drugs through cell membranes.

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July 11, 1952.

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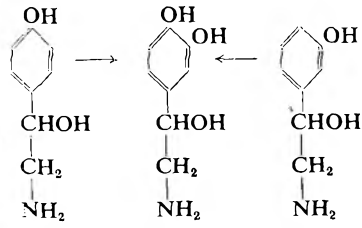
The Formation of Noradrenaline Using Ultra-violet Irradiation

SIR.—Extracts of posterior salivary glands of *Octopus vulgaris* contain a material named "octopamine" which has been identified as *p*-hydroxyphenylethanolamine (norsynephrine).[1] When this substance is irradiated with ultraviolet radiation in the presence of air, "hydroxyoctopamine" (i.e., noradrenaline) is formed.[1]



p-Norsynephrine Noradrenaline *m*-Norsynephrine

We have now shown that both the *p*- and the *m*-norsynephrine can form noradrenaline under such conditions. The irradiation was carried out using a Hanovia Fluorescence Lamp, Model 11, and the best yields were obtained after two hours irradiation at *p*H 5 with a 1 in 10,000 solution of the amine. Proof that noradrenaline was in fact the material formed was obtained by running concurrently chromatograms of the irradiated solutions and of noradrenaline. Further chromatograms allowed of elution of the corresponding areas and biological examination of the eluates on the blood pressure of a spinal cat and the isolated rabbit intestine.[2]

In the biosynthesis of noradrenaline, therefore, *p*- or *m*-norsynephrine may be formed at an intermediate stage, and it may be possible to identify one or other of these substances in extracts of mammalian suprarenal glands. Their formation from tyrosine or tyramine is conceivable, a step not requiring the production of dihydroxyphenylalanine or hydroxytyramine. Further work on this approach to the synthesis of adrenaline is in progress.

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July 11, 1952.

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ABSTRACTS (*Continued from page* 670)

inactivation. Of the three alkaloids, sanguinarine was the most toxic to the bacteria studied, and physostigmine the least. Berberine at a dilution of 1 in 10,000 showed some stimulation of growth. In media containing berberine and sanguinarine, gas was produced in a number of experiments after an inactive period of as much as 10 days, confirming evidence of bacteriostasis. Experiments designed to test the germicidal activity of the alkaloids in relation to that of phenol showed that media saturated with salts of the alkaloids did not inhibit the growth of *E. typhi* and *Staph. aureus* after 15 minutes exposure, and that *Staph. aureus* was not inhibited by the following concentrations until after the times stated: berberine hydrochloride (saturated), 43 minutes; physostigmine hydrochloride (1 in 11), 10 minutes; and sanguinarine sulphate (1 in 40), 24 minutes. G. R. K.

H. B. MACKIE

Chairman, 1952