REVIEW ARTICLE

THE CHEMOTHERAPY OF TROPICAL DISEASES

PART I. PROTOZOAL INFECTIONS

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THE chemotherapy of tropical diseases has seen great advances during the last ten years. The need to maintain armies in the tropics during the war, and increasing interest in Colonial development, has stimulated the search for new drugs. The latest edition of Findlay's *Recent Advances in Chemotherapy*[1] has had to be accommodated in four volumes, the first two of which deal almost exclusively with tropical diseases.

The assessment of the value of new drugs in the tropics is not a simple matter. The course of a disease in a native population is influenced by social and hygienic conditions, the state of nutrition, the presence of other infections in the same host and many other factors. Improvement of hygiene and the destruction of intermediate hosts of disease often make it difficult to estimate the value of chemotherapy given concurrently. After treatment, exposure to reinfection is often the rule; it is also difficult to follow treated patients for any length of time. The practice of using experimentally infected human volunteers, as in the study of malaria, is yielding very valuable results and reliable comparisons of the activities of drugs.

MALARIA

Fairley and his team of workers at Cairns, N. Australia, made important tests with antimalarial drugs upon experimentally infected normal men.[2] These tests paved the way for further controlled comparisons of drugs, especially in the U.S.A., and for many field trials. They also gave further evidence for the existence of tissue stages in the development of plasmodia infecting man. Shortt and his colleagues recently demonstrated[3] that the earliest stages of development of *Plasmodium vivax* and *P. falciparum* occur in the cells of the liver. These stages are very important from the point of view of chemotherapy.[4] All of the drugs used against malaria cause the death of trophozoites in the red blood cells; only a few have activity against primary or secondary tissue forms, or against the gametocytes which infect mosquitoes. The present views upon the value of drugs in the treatment of malaria are summarised in a number of reports.[5,6,7]

Quinine. Quinine is still used in the treatment of acute malaria because it acts so rapidly. It acts mainly upon trophozoites and has no appreciable effect upon *P. falciparum* gametocytes or upon tissue parasites. Quinine has the disadvantage of precipitating attacks of blackwater fever in some circumstances.

Mepacrine. Mepacrine has been widely used as a suppressant, and also for the treatment of acute malaria. It acts only upon trophozoites.[8]

It rarely causes toxic side-effects, although these have been well documented.[9]

Proguanil. The research upon the antimalarial activity of pyrimidine compounds which led to the discovery of proguanil is well known.[10] Since the war, the suppressant and curative properties of this drug have been tested in many parts of the world. It has a wide margin of safety, it cures most strains of *falciparum* malaria and suppresses all other species. It has some inhibitory effect upon the primary tissue forms of *P. vivax*,[11,12] but is not a certain cure, and relapses frequently occur when the medication stops.[13] Its action is slower than that of quinine, mepacrine and the new 4-aminoquinoline derivatives, and it is therefore not a good drug for the initial treatment of acute malaria.[14,15,16,17,18] Strains of parasite vary greatly in their sensitivity to proguanil, and unduly resistant strains have been encountered in West Africa,[17,19,20] Eritrea,[21] the Philippines,[22] and elsewhere. The sensitivity of the parasite to the drug may also be decreased by the presence of secondary infections such as infestation by worms.[23] By giving increasing sub-curative doses of proguanil it is easy to produce strains that are very resistant to the action of the drug. This has been shown with *P. gallinaceum*[24,25] in chicks, *P. relictum*[26] in pigeons, *P. cynomolgi*[27,28] in monkeys and *P. vivax*[29,30] and *P. falciparum*[31] in man. The resistance survives repeated and rapid passage,[32] and mosquito transmission.[33,34,35] Prolonged exposure of the exoerythrocytic forms of *P. gallinaceum*[36] or *P. vivax*[37] to proguanil (by giving full suppressive doses) has failed to produce detectable drug-resistance in the strain when it relapsed after the end of treatment; the resistance is therefore only produced in the trophozoites. Recent work with *P. cynomolgi*[38] suggests that the exoerythrocytic forms of this species may also become resistant. There is some evidence that human malaria in some parts of the world may be increasing slightly in resistance to proguanil.[39,40,41,42] In Malaya it has been recommended that other drugs should be used alternately with proguanil as suppressants, to minimise the risk of making drug-fast strains.[43] Gametocytes are not morphologically affected by proguanil in the mammalian host, but lose their power to develop in the mosquito.[44]

4-*Aminoquinolines.* A number of years ago German workers prepared a series of quinoline derivatives which included sontochin (I, nivaquine C, M, or R) and resochin (II, chloroquine, aralen, nivaquine B).[45] This series was re-examined in the U.S.A. during the war, and careful comparisons of activity and toxic side-effects were made. A new synthesis of chloroquine was devised[46] and large amounts of the drug are now manufactured. Sontochin and chloroquine are about equal in activity to mepacrine, and act more rapidly in acute malaria.[47,48,49,50] They have fewer toxic side-effects than mepacrine, and do not stain the skin. Chloroquine is usually effective against strains resistant to proguanil; it may be given by intramuscular[51] or intravenous[52] injection if the patient is too ill to swallow the dose. It is a good suppressant,[53,54,55] and in some areas is superior to proguanil.[55,56] The action of sontochin and chloroquine is upon the trophozoites, and there is no effect on

exoerythrocytic parasites or gametocytes. Camoquin (III), another member of the series, has similar properties to chloroquine but acts slightly less rapidly.[57] It has given good results as a suppressant and for treatment of acute malaria in Egypt,[58] South America,[59,60,61] the Philippines[62] and India.[63]

8-*Aminoquinolines.* This series of drugs has the property (which is not shared by any other known group of antimalarials) of killing the tissue stages of *P. vivax*. Pamaquin has long been known to do this, and when used with quinine or another schizonticide, effectively puts an end to relapses of *vivax* malaria in about 90 per cent. of cases.[64,65] Pamaquin has toxic effects and must be used with care. Pentaquine (IV) has properties similar to pamaquin and has been used with quinine or chloroquine for the cure of *vivax* malaria.[66,67,68,69] Isopentaquine (V) has activity equal to pamaquin but is less toxic.[70] The primary amine, primaquine (VI) is less toxic still, and is probably the best of the series of 8-aminoquinolines so far discovered.[70]

Other Drugs. A great survey of drugs was made in the United States during the war,[71] and many groups of compounds were shown to have antimalarial activity. Sulphonamides act upon young trophozoites in the blood cells; sulphapyrazine has been shown to be a useful suppressant.[72] Metachloridine [*N*′-(5-chloro-2-pyrimidyl) metanilamide] has an effect upon the sporozoites and early tissue forms of *P. gallinaceum*[73] but has proved very disappointing in human malaria. A new derivative of proguanil has been prepared which has higher activity upon exoerythrocytic parasites in experimental animals.[74] Proguanil itself is metabolised in the body to produce a highly active dihydro-triazine derivative; recent work has shown that the related compound, 2:4-diamino-1-(3:4-dichloro-phenyl)-1:6-dihydro-6:6-dimethyl-1:3:5-triazine is about 100 times as active as proguanil against *P. gallinaceum*.[75] One of a new series of 2:4-diamino-pyrimidines has shown activity 50 to 200 times as great as that of proguanil[76] in laboratory infections; preliminary field trials in West Africa show that it also has very great activity against the schizonts of *P. falciparum*.[77]

There are still many problems to be attacked in the chemotherapy of malaria. There is as yet no prophylactic which affords complete protection against *P. vivax*, and no satisfactory drug is known which will protect by killing sporozoites as they are injected by the mosquito. There is also the great unsolved problem of the best way of treating native populations in hyperendemic areas, where the aim must be to assist the children to reach a stage of premunition or tolerance to the infection, without rendering the strains of parasite resistant to antimalarial drugs. Attempts have been made to do this in Java,[78] Indochina,[79] and elsewhere, but there is much truth in the closing remarks of a review of hyperendemic malaria by Bagster Wilson, Garnham and Swellengrebel[80]: "The optimum dosage of antimalarial drugs, and the choice of drug in the treatment of infants in order so to modify their attacks that mortality is eliminated and morbidity reduced to a minimum (without extermination of parasites) is in urgent need of study; for at present there is wide diversity of practice in this respect."

Trypanosomiasis

African Trypanosomiasis

The problem of the treatment of trypanosomiasis with drugs is threefold: (*a*) treatment of early cases, (*b*) treatment of advanced cases, and (*c*) prophylaxis. A number of drugs are effective in early infections but there are very few which penetrate into the central nervous system to kill the trypanosomes in advanced cases. Prophylaxis is now being tried in many areas with apparent success, but it is as yet too soon to know to what extent this will lead to cryptic infection. A useful general review of trypanocides was given recently by Walls.[81]

Suramin. Suramin has been in use for many years and is effective in the early stages of trypanosomiasis. It is especially valuable in *Trypanosoma rhodesiense* infections. It has no action in advanced sleeping sickness but it is used to supplement treatment with tryparsamide (see below). Suramin combines with plasma proteins and remains in the circulating blood for long periods; a dose given as a prophylactic is effective for 3 months. Suramin also has an inhibitory effect on enzymes; its properties have been studied by Wormall and his collaborators in a systematic attempt to discover the mode of action of the drug.[82,83]

Aromatic Diamidines.

Pentamidine. This drug has a powerful effect in early cases of *T. gambiense* sleeping sickness.[84,85,86,87,88,89] In *T. rhodesiense* infections it is said to be less effective than suramin,[90] but there are very few reports upon the use of pentamidine in this form of the disease. Against "intermediate" cases of *T. gambiense* sleeping sickness, pentamidine has given good results in some areas,[91,92] but it is not to be relied upon when an increased cell-count and protein content of the cerebrospinal fluid indicates that the central nervous system has become involved.[93,94] There is always a danger in such cases that a cryptic infection will continue and produce nervous lesions without trypanosomes appearing in the blood or lymphatics. Pentamidine is often effective in relapses after arsenical treatment,[95] but in advanced cases it is of no value alone, and must be given together with tryparsamide.[87,88,92] Pentamidine has some effect upon early cases when given by mouth, but produces diarrhœa and vomiting in some patients; the parenteral route is preferable. Pentamidine is now used widely as a prophylactic in African populations exposed to the risk of trypanosomiasis. Although in laboratory animals the protective effect of an injection lasts only for a month, it appears that in man it will protect for 4 to 12 months.[96,97,98,99,100,101] However, it is very important that a full survey of the population is made before the first prophylactic injection, because there is a likelihood that some people will be already harbouring the disease. These must be treated at once with a full course of drugs, or the infection may become cryptic and the opportunity for curing the patients easily will have been missed. Pentamidine is given by intramuscular or intravenous injection, either as the isethionate or the methanesulphonate (Lomidine). It has an irritant

effect when given intramuscularly but this is to be preferred to the alarming symptoms which occasionally follow intravenous injection and which are caused by the fall in blood pressure which the drug produces. It is dangerous to give the drug intrathecally.[86] It has recently been shown that many of the immediate pharmacological effects of pentamidine are greatly reduced when it is given together with suramin. The therapeutic action of the two trypanocides in combination is not likely to be affected, because they kill trypanosomes over much longer periods and at much lower concentrations in the blood than are required for the immediate effect upon smooth muscle and neuromuscular transmission.[102]

Propamidine. Propamidine is more toxic than pentamidine and may cause abortion when given to pregnant women.[103,104] Prophylaxis with propamidine has been reported to produce a high incidence of cryptic infections,[105] and the drug is now rarely used in trypanosomiasis.

Arsenicals.

Tryparsamide. Tryparsamide, which is reduced in the body to the active trivalent form, has the power of crossing the "blood-brain barrier" and killing trypanosomes in nervous tissue. It is still the mainstay of treatment for advanced cases, and is usually given together with pentamidine or suramin.[87,88,92,106] Unfortunately there is an increasing number of strains of trypanosomes found to be resistant to tryparsamide, especially in the Belgian Congo.

Melarsen, Melarsen Oxide, Mel. B. Organic arsenical compounds containing the melamine nucleus were introduced by Friedheim.[107,108] Melarsen (VII) is very active in early and intermediate cases[96] but has given variable results in more advanced cases.[109,110] Doses large enough to be useful sometimes produce serious toxic side-effects.[101,110] Melarsen oxide (VIII) is also of value in early cases,[111] and against tryparsamide-resistant strains,[101] but there is diversity of opinion as to its effectiveness in the later stages. Melarsen and melarsen oxide have been tried orally with good results in some cases, but they are more certainly effective when injected.[110] Mel. B (IX) is a compound of melarsen oxide with dimercaprol (B.A.L.); it has given good results in advanced cases and in infections which are refractory to all other forms of treatment.[92,112,113,114] Mel. B is usually effective against tryparsamide-resistant strains,[115,116] but will itself produce drug-resistance if the dose given is not large enough. Inadequate treatment with melarsen or melarsen oxide also renders trypanosomes resistant to Mel. B, and such strains are resistant to tryparsamide.[116] The disadvantage of Mel. B is that it sometimes produces serious toxic side-effects and in some trials, a high proportion of fatalities has occurred.[92,114,117] Attempts to control the toxic effects have been made using novocaine or *p*-aminobenzoic acid in animals, and by supplementary injections of dimercaprol itself in man, but there is no doubt that the drug should be given under close supervision in hospital and that it is unsuitable in its present form for general use in field dispensaries.[92,115] Some of the toxic effects and variations of therapeutic action of this group of drugs may be caused by difficulties in the

manufacture of batches of uniform quality. It is important that every batch should be controlled by biological tests.[118] It is too early to judge the future of the compounds, but Mel. B. is likely to be the most useful member of the series.[115,118] A melaminyl derivative of antimony "M Sb." (X) has shown promise in laboratory animals as a prophylactic[119] but there have been as yet only limited clinical trials.[113,115]

Butarsen (XI). This was introduced by Eagle[120] as a result of extensive experiments in the laboratory. The results of clinical trials have been disappointing because although it is active in the early stages of *T. gambiense* infections butarsen has no effect when the central nervous system is involved.[88,109,121]

South American Trypanosomiasis

Infection with *T. cruzi* is responsible for deaths in children and chronic heart disease in adults. It is found in South American populations living under poor conditions where the bug which is the vector of the disease flourishes. No drug is known which is really effective against the chronic stage of the disease. "Bayer 7602 (Ac)" (XII)[122] has proved to be of value in the acute stage in children,[123] but the drug is very irritant on injection and it has no effect on trypanosomes in the heart muscle. Butarsen has some effect, in the early stages of the disease only. In laboratory infections of *T. cruzi* in mice, activity has been shown with pentaquine,[124] and with some phenanthridine derivatives.[125,126] Only one of these compounds, 3-amino-9-*p*-carbethoxyaminophenyl-10-methylphenanthridinium sulphate, has so far been tried clinically.[127]

Trypanosomiasis in Cattle

The outstanding problem in cattle trypanosomiasis is the control of infections of *T. congolense* and *T. vivax*. These parasites are practically unaffected by all the trypanocides used in human sleeping sickness, and until the introduction of phenanthridinium compounds, the only drugs of any value were tartar emetic, stibophen and surfen C. The antimonials were of low activity and surfen C had undesirable toxic side-effects. The two phenanthridine derivatives phenidium, "897" (XIII) and dimidium, "1553" (XIV) were first used in the field about 10 years ago; dimidium was found to be the more effective drug.[128,129] The trypanocidal effect of dimidium has been proved in many areas, but the drug has the disadvantage of being irritant at the site of injection. Also, in some areas therapeutic doses cause considerable losses due to a toxic action, probably upon the liver, which may be accompanied by symptoms resembling photosensitisation of the skin. Phenanthridine compounds have no real prophylactic value. Further compounds of the series have recently been tried on a limited scale in the field.[118] The introduction of antrycide (XV)[130] gave reason to hope that the scourge would at last be effectively controlled, and that a mixture of antrycide methylsulphate and chloride given at 6-monthly intervals might be useful as a prophylactic. Antrycide has given good results as a curative drug,[130,131] but it is not without toxic side-effects in ill-nourished animals, and the effect

of the prophylactic mixture lasts for less than 90 days in areas where the tsetse fly density is high.[118] Also, resistant strains of trypanosomes are making their appearance and there is evidence that these are also resistant to dimidium.[132] Antrycide probably acts as a growth inhibitor of trypanosomes, and produces changes in the nucleoproteins of the cytoplasm.[133] It inhibits different enzyme systems from those affected by suramin. Trypanocidal cinnoline derivatives have recently been prepared,[118,134] but have not yet been tried in the field. There is still much to be done in the prophylaxis and treatment of bovine trypanosomiasis.

Leishmaniasis

Visceral Leishmaniasis (*Kala azar*)

Quinquevalent organic antimonials are still the most commonly used drugs for the treatment of kala azar. Aromatic diamidines are also effective, and of especial value in infections resistant to antimony. These two groups of compounds serve for the cure of almost all cases of kala azar.[135] In spite of much research upon laboratory infections no other series of drugs has yet been found to have any action in visceral leishmaniasis.

Antimony Compounds.

Derivatives of Stibanilic Acid. Probably the most widely used stibanilic acid derivative is urea stibamine, which is manufactured and used in India in large quantities. The drug is a mixture of compounds of phenylstibonic acid, and batches must be controlled for toxicity by biological tests. It is usually given by intravenous injection twice weekly; it has recently been given intensively in an intravenous drip. Other well-established phenylstibonic acid derivatives are neostibosan and stibamine glucoside. A new member of the series introduced recently is pentastib, the *p*-aminophenylstibonate of *N*-methylglucamine. This has had only limited trials.[136]

Quinquevalent "Emetic Type" Compounds. In stibanilic acid, the antimony atom is joined directly to a carbon atom of the phenyl ring. In tartar emetic the antimony atom is joined to carbon through oxygen. Antimony pentachloride reacts with a number of organic compounds rich in hydroxyl groups, such as gluconic acid, to form quinquevalent "emetic type" compounds of high solubility in water, and low toxicity. Such compounds are excreted rapidly, and are active in leishmaniasis. The first of these compounds to be used in kala azar was solustibosan, which had high activity in experimental leishmaniasis,[137] in spite of the fact that its action upon trypanosomes was very slight. Sodium stibogluconate replaced solustibosan during the war, and is now used extensively under various trade names. It is effective in all forms of kala azar,[138,139,140] even the Sudanese variety which is notoriously difficult to cure with antimony.[141] Schmidt[142] has taken pains to point out that sodium stibogluconate B.P.C. is "an uncertain approximation to solustibosan." It nevertheless gives good results. Sodium stibogluconate

rarely produces serious toxic side-effects, although rigors have been observed in one series of cases. An oily suspension of solustibosan has been used in Spain.[143,144] A similar "emetic type" compound is glucantime, the antimoniate of *N*-methyl glucamine. This has been used mainly in Algeria,[145,146,147] but has also been tried in Italy[148] and India.[149] Glucantime is superior to the corresponding stibanilic acid derivative, pentastib. It is very rapidly excreted in the urine.[145,150] Enormous doses of antimony have been given as glucantime with safety, but occasional toxic effects have been recorded. Tartar emetic itself was recently tried in large doses in an attempt to give a short, intensive, curative course for kala azar.[151] Apart from its cheapness, tartar emetic has little to recommend it now that safer remedies are available.

Aromatic Diamidines.

The diamidine first used in kala azar was stilbamidine, but although it was effective, especially in antimony-resistant cases, it was found that exposure to light rendered solutions of stilbamidine very toxic.[152,153] The toxic substance was shown to be the dimer, 1:2:3:4-tetraphenyl*cyclo*-butane.[154,155] Even freshly prepared solutions of stilbamidine have a toxic action on nervous tissue,[156] and the clinical use of the drug sometimes produces neuropathy, especially of the trigeminal nerve, which appears during the year following treatment with the drug and seems to be permanent.[157,158,159] For this reason, stilbamidine is now very rarely used. Pentamidine is used instead[135,160,131] and has approximately the same activity. It does not produce nerve lesions and is more stable. Phenamidine has also been found effective in kala azar.[162]

Another important development is the treatment of cancrum oris, a fatal complication of kala azar, with penicillin.[163,164] This controls secondary bacterial infection, and greatly improves the prognosis.

Mucocutaneous Leishmaniasis (Espundia)

South American Leishmaniasis is more resistant than kala azar to chemotherapy. Antimonials have been tried, sometimes with success, but relapses frequently occur.[165,166] A similar condition found in the Sudan usually responds to treatment with antimonials or pentamidine. In South America, a large variety of drugs has been tried, including mepacrine, sodium formaldehyde sulphoxylate and arsenicals. Penicillin has no effect in this condition.

Dermal Leishmaniasis (Oriental Sore)

Leishmaniasis of the skin has been treated with X-rays, local injections of berberine, or by intravenous antimonials. More recent treatments include the local injection of mepacrine or of solustibosan. None is entirely satisfactory.

AMŒBIASIS

Papers and review articles upon methods of treatment for amœbiasis frequently appear in the press.[167,168] This is evidence that although there are many remedies for the disease, none is entirely satisfactory. A fair

statement of the present position is given in the review by Anderson and Hansen.[167] There are three main types of amœbic infection:—

(1) The symptomless carrier state in which the host passes cysts in the fæces.
(2) Amœbic dysentery in which the amœbæ form extensive ulcers in the bowel wall.
(3) Spread of the infection from the intestine to the liver, producing amœbic hepatitis or abscess.

A number of drugs are effective against amœbic dysentery, and when supplemented with antibiotics to control secondary bacterial infections, the clinical response to treatment is nearly always good. However, relapses are common, and the host often passes cysts for many years and is a potential danger to the community. Such chronic infections are difficult to eradicate.

Emetine. This alkaloid is still the most potent remedy known against *Entamœba histolytica.* It is toxic to the host in therapeutic doses and produces unpleasant side-effects upon the gastrointestinal tract and the heart. When given by injection emetine is effective in acute amœbic infections and in amœbic hepatitis, but rarely produces permanent cures. Treatment must be supplemented with doses of emetine bismuth iodide, or emetine in enteric coated capsules[169] by mouth. A large number of schemes have recently been devised in which emetine, halogenated hydroxyquinolines, arsenicals, sulphonamides and antibiotics are all given in a "blunderbuss" treatment.[170,171]

Conessine. Kurchi (*Holarrhena antidysenterica*) and its constituent alkaloid conesine have been favourite remedies in India for many years. Recently French workers have used the alkaloids extracted from *H. africana*[172] and *H. floribunda.*[173] There are a number of enthusiastic reports upon the efficacy of conessine,[174,175,176] but in most of these trials the patients were not examined for long enough to ensure that the infection had been eradicated. A number of workers have observed toxic effects[176,177,178] and some have used phenergan to counteract the sleeplessness caused by conessine.[176] It appears that conessine will be of value when given together with emetine[178] or other amœbicides, and also in patients who are unable to tolerate emetine. The hydrobromide is considered to be the most suitable salt.[176]

Halogenated Hydroxyquinolines. Vioform, chiniofon and diodoquin are widely used in amœbic infections. Diodoquin (XVI) is of low toxicity and is probably the best drug at present available for the treatment of chronic infections.[179,180] It is often given together with sulphonamides and with other amœbicides. Studies upon the blood iodine levels produced by doses of these iodoquinolines showed that all were absorbed to some extent,[181,182,183] and that diodoquin gave the highest blood level.[182] It is likely that the main action of the compounds is upon the amœbæ and the bacterial flora in the lumen of the bowel.

Arsenicals. Carbarsone and acetarsone are well-tried remedies and are usually given together with other amœbicidal drugs. A bismuth

derivative of *p*-N-glycolylarsanilic acid (XVII) was introduced under the name of "Wia" in 1943,[184] and has recently been re-examined as "Milibis" or "Win 1011."[185,186,187] It is claimed to have good effect in chronic cyst-passers but is of little value in acute amœbiasis. Other new arsenical preparations are the thioarsenites "CC914" (XVIII) and "CC1037" (XIX) introduced by Anderson,[188,189] and the sulphonamido derivative (XX) tested by Schneider and Montezin.[190]

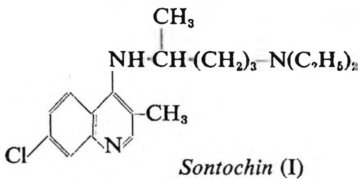
Antibiotics. The invasive power of *E. histolytica* is probably related to the presence of certain bacteria in the gut. Amœbic ulcers become secondarily infected with bacteria and the local tissue reaction may prevent access of amœbicides in adequate concentration to kill the amœbæ. The introduction of penicillin and sulphonamides to supplement treatment with emetine and other amœbicides[191] was a great step forward in the control of acute infections. Most of the available antibiotics have now been tried. Chloramphenicol is of little value,[192] but streptomycin and bacitracin[192,193] are useful. Aureomycin is the most promising antibiotic tried so far, because not only does it affect the bacterial flora of the gut, but it probably has a direct amœbicidal action of its own.[194] Treatment with aureomycin rapidly alleviates acute dysentery, but relapses frequently occur.[195,196,197,198] It is best to use aureomycin together with other amœbicides. Terramycin has also been tried,[199,200,201] but it does not appear to be more active than aureomycin.

Chloroquine. Chloroquine, like emetine, is selectively concentrated in liver tissue. It has proved to be very useful in the treatment of amœbic hepatitis and liver abscess,[202,203,204,205] although it is of little value in intestinal amœbiasis. Chloroquine is much less toxic than emetine, and has given good results in cases in which treatment with emetine and other remedies had failed. Sontochin is also active in hepatic amœbiasis.[206]

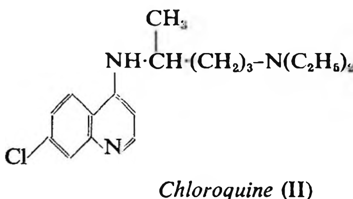
In spite of a great deal of work upon *E. histolytica* in experimental animals and in culture, a safe and powerful remedy for chronic amœbic infections is still lacking.

Antimalarials

New 4-aminoquinoline derivatives

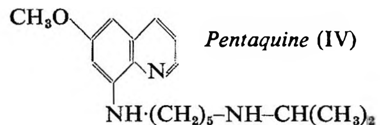


Sontochin (I)

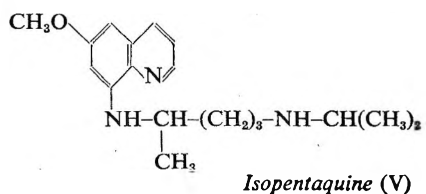


Chloroquine (II)

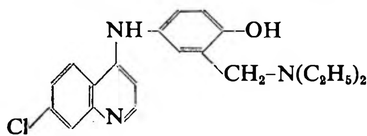
New 8-aminoquinoline derivatives



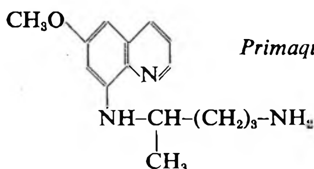
Pentaquine (IV)



Isopentaquine (V)

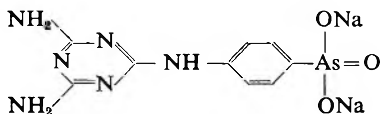


Camoquin (III)

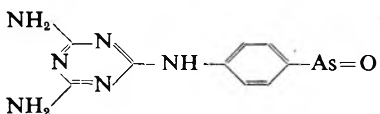


Primaquine (VI)

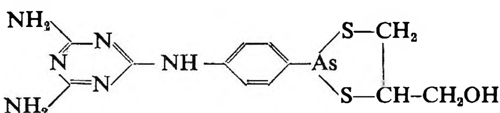
Trypanocides



Melarsen (VII)



Melarsen oxide (VIII)



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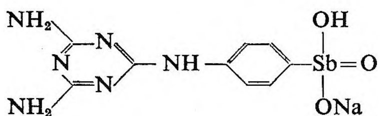
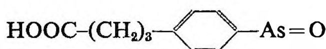


MSb. (X)



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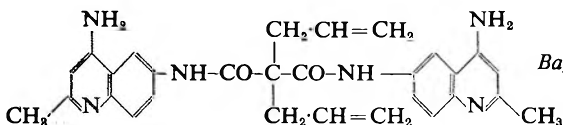
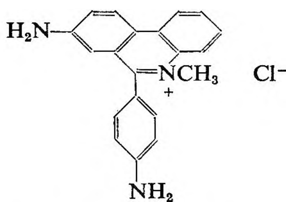


Bayer 7602 (*Ac*) (XII)



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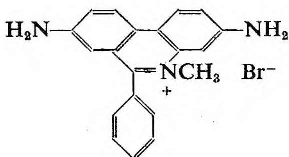

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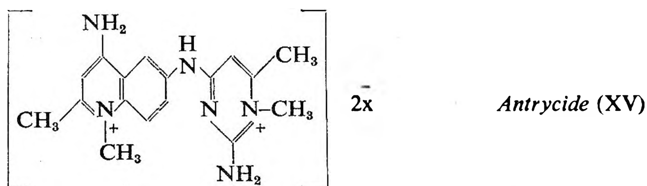
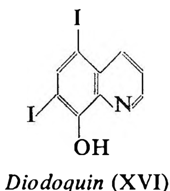
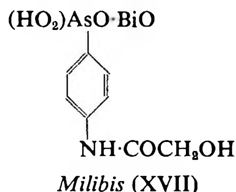


Antrycide (XV)

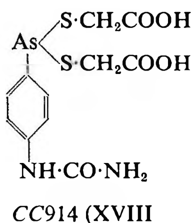
Amœbicides



Diodoquin (XVI)



Milibis (XVII)



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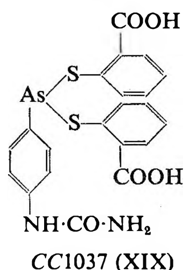

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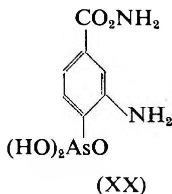

(XX)

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RESEARCH PAPERS

THE DISTRIBUTION OF ERROR IN MOUSE INSULIN ASSAYS

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To specify the accuracy of a bio-assay technique some knowledge of the nature of the distribution of errors between tests of the same type is required, since it is the uniformity of variance from test to test as much as the mean variance which will determine the usefulness of the method. To investigate the distribution of errors in an assay involving a probit response, the mouse insulin test was chosen as being a well established technique on which many results were available for analysis. That the distribution of standard errors of log-potency in this test is not normal will be seen from the histogram shown in Figure 1. While some of the

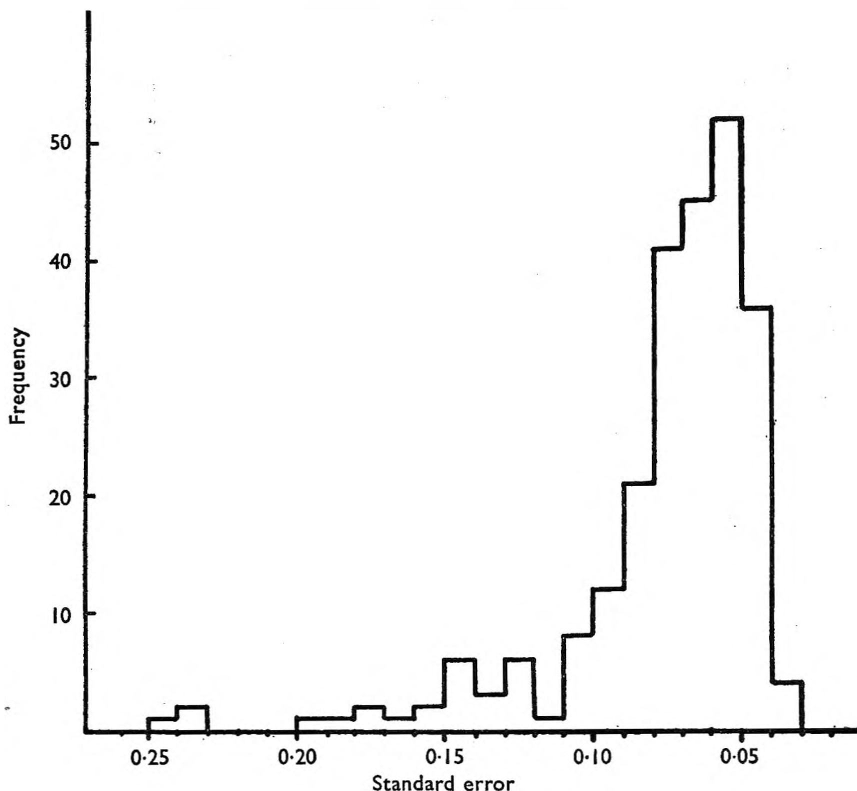

Fig. 1. Histogram showing the distribution of standard errors of log-potency for 257 assays.

conclusions reached in this study are peculiar to the insulin test, some of the more general findings may possibly be applicable to many other types of probit assay.

The Test

A standard 4-point design was employed throughout, with a dose ratio high: low of 2:1·2, i.e., a log dose interval of 0·2219. A total of 96 mice were used for each assay, their extreme weights varying by not more than 1·5 g. The same mice were sometimes used up to 4 times, with at least 1 week between repetitions, but in one assay all the mice would have been employed the same number of times. The animals used for assays on one day would have been deprived of food since the previous afternoon, their normal diet consisting of an adequate supply of bread and milk. The criterion of response in the test depends on the production of hypoglycæmic convulsions by sufficiently large doses of insulin. To observe these the injected mice are placed in jars (3 mice in a jar) in a constant temperature cabinet (34° C.), and observed up to 1¼ hours after injection. Mice convulsing, or showing symptoms of convulsions, are removed and given an injection of glucose (they are not returned to the jars). After 1¼ hours the total responses to each treatment are counted up, and the computation of relative potency then made by a standard type of probit analysis.

Results

The results took the form of data taken from the records of routine assays of crystalline insulin (a mixture of ox, pig and sheep insulins) carried out under the direction of one of the authors (G.A.S.). The first set of figures so obtained concerned 257 assays carried out between September, 1949, and February, 1950. These covered in all 28 samples of insulin, the number of tests per sample varying considerably. The values examined were:—(1) the responses out of 24 for each treatment, i.e., 257 values each for standard high and low, test high and low (SH, SL, TH, and TL respectively), (2) the weighted mean slope of standard and test for each assay, and (3) the weight assigned to the log potency estimate for each assay, this weight being the reciprocal of the variance of the log potency. Some weight values were accidentally omitted from this series, leaving 249 estimates.

Two further sets of values were later extracted from the records. These will be described in the appropriate sections below.

The distribution of responses. Under ideal conditions the responses to high and low doses would be distributed independently according to the expansion of the binomial $(p + q)^{24}$, where q is the true proportion reacting to the dose, and 24 the number of mice per treatment group. In practice, however, many uncontrolled factors influence the responses, and the distributions found (Table I) have variances much greater than binomial distributions with the same means. For example, the mean response to SH was 13·7 out of 24 (*ca.* 57 per cent.), the variance being 22·2. The variance of a binomial distribution with $q = 0{\cdot}57$ would be $24 \times 0{\cdot}43 \times 0{\cdot}57$, or 5·9. With the higher doses of both standard and test the responses out of 24 and the probits of these values could be fitted by a normal distribution (Table II). Since the responses to the low doses were

TABLE I

FREQUENCY DISTRIBUTION OF RESPONSES TO HIGH AND LOW DOSES OF INSULIN IN 257 MOUSE ASSAYS

Ratio of high dose to low dose = 5 : 3

Response		Standard		Unknown	
Out of 24	Probit	High	Low	High	Low
0	2·579	0	7	1	14
1	3·268	2	28	0	15
2	3·617	2	23	1	29
3	3·850	3	24	1	32
4	4·033	3	23	2	23
5	4·188	6	19	3	21
6	4·326	6	28	6	24
7	4·451	5	21	6	17
8	4·569	10	18	7	26
9	4·681	11	13	14	11
10	4·790	12	10	18	7
11	4·895	17	5	18	8
12	5·000	15	14	16	5
13	5·105	15	4	26	6
14	5·210	30	8	20	5
15	5·319	24	7	22	8
16	5·431	20	2	21	4
17	5·549	21	1	13	1
18	5·675	15	1	21	0
19	5·812	15	1	10	1
20	5·967	8	0	11	0
21	6·150	7	0	12	0
22	6·383	8	0	7	0
23	6·732	1	0	1	0
24	7·421	1	0	0	0

TABLE II

THE NORMAL DISTRIBUTION FITTED TO FREQUENCIES OF RESPONSES AND PROBITS OF RESPONSES TO HIGH DOSES OF STANDARD AND UNKNOWN INSULINS

obs. = observed; $Exp._r$ = expected from distribution of responses; $Exp._p$ = expected from distribution of probits of responses

Response /24	Frequencies					
	Standard			Unknown		
	Obs.	$Exp._r$	$Exp._p$	Obs.	$Exp._r$	$Exp._p$
0	0			1		
1	2			0		
2	2		8·3	1		
3	3	10·3		1	12·2	10·1
4	3			2		
5	6		5·8	3		
6	6	5·6	7·5	6		6·5
7	5	7·7	9·6	6	6·7	8·4
8	10	10·7	11·0	7	9·7	10·8
9	11	13·0	13·2	14	12·2	12·3
10	12	15·7	14·4	18	15·9	14·2
11	17	18·1	15·2	18	18·1	16·2
12	15	21·1	17·4	16	21·4	17·8
13	15	21·2	18·1	26	22·0	19·0
14	30	21·4	18·4	20	23·5	20·5
15	24	20·8	19·1	22	21·9	20·2
16	20	20·1	18·1	21	21·1	20·1
17	21	16·9	18·1	13	17·8	18·1
18	15	14·3	16·4	21	15·5	17·7
19	15	11·6	14·5	10	11·8	15·7
20	8	9·3	12·9	11	9·3	12·4
21	7	6·6	9·6	12	6·6	9·3
22	8			7		
23	1	12·7	9·7	1	11·2	7·9
24	1			0		
χ^2	—	15·8	16·6	—	13·6	11·9
(d.f.)		(17)	(18)		(16)	(17)
p		0·60	0·60		0·56	0·80

truncated at the zero response level, the estimation of mean and standard deviation was carried out by fitting a linear regression to the probits of cumulative frequencies at successive response levels. The "standard low" points are shown graphically in Figure 2. Using the parameters so obtained, the probits of responses gave a considerably better fit to a normal distribution than did the responses alone (see Table III). With the probit values significant deviations only occurred at one or other of the extremes, and it was therefore decided to carry out subsequent analyses on probits of responses. In this connection the empirical probits used in the original computation of results for 0 and 100 per cent. responses correspond to ½ and 23½ responses out of 24, that is, to the interval boundaries in a frequency table. We have therefore transformed these values arbitrarily to those shown in Table I, by the use of the table of working probits given by Finney.[1]

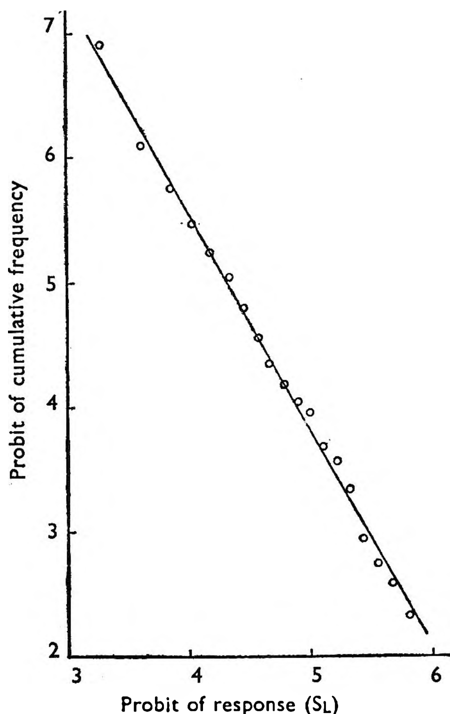

FIG. 2. Graphical method of estimating the mean and variance of the truncated distributions of responses to low doses of standard. The figure shows the plot for probit of response against probit of cumulative frequency.

To establish rather more firmly the normality of the probit responses, two further distributions were investigated:—(1) the 514 values of b', the probit difference between the responses to high and low doses of each preparation, and (2) the 257 estimates of the difference in probits between the unweighted mean response to standard and that of test, that is ½ (SH + SL − TH − TL), where SH, SL are the probit responses to high and low doses of standard, TH, TL to the test sample. Both these distributions could be fitted by the normal distribution with a probability of the order of 0·20. With this general conformity of the probit response to a normal variate in mind, an analysis of variance was carried out on the unweighted probit responses of the 257 assays (Table IV). There it will be seen that only the linear regression and "between assays" mean square are significant against the residual mean square. Thus in the overall picture, there is no detectable difference between standard and test solutions, nor is their interaction with assays (5) significant. The mean square indicating departure from parallelism (4) is large but not significant, nor is the doses × assays interaction. These two observations suggest

TABLE III

THE NORMAL DISTRIBUTION APPLIED TO RESULTS WITH LOW DOSES OF STANDARD AND UNKNOWN

Coding as in Table II, means and variances estimated graphically by the method shown in Figure 2

Response /24	Frequencies					
	Standard			Unknown		
	Obs.	Exp.r	Exp.p	Obs.	Exp.r	Exp.p
0	7	21·2	17·5*	14	12·5	30·6
1	28	10·0		15	7·9	
2	23	12·9	22·7	29	10·7	33·2
3	24	15·8	25·3	32	14·9	33·4
4	23	19·5	26·9	23	18·7	30·3
5	19	20·9	24·9	21	22·1	26·4
6	28	22·2	23·5	24	23·5	22·9
7	21	22·5	21·0	17	25·5	18·8
8	18	21·7	19·6	26	25·1	14·8
9	13	20·0	15·9	11	23·2	12·3
10	10	17·5	13·9	7	19·4	9·4
11	5	14·6	11·1	8	16·6	7·4
12	14	11·6	9·1	5	12·7	5·6
13	4	8·8	7·2	6	9·2	12·0*
14	8	6·4	5·8	5	6·0	
15	7	11·5	12·7	8	9·3	
16	2			4		
17	1			1		
18	1			0		
19	1			1		
χ^2		72·9	30·3		88·5	27·3
d.f		15	14		15	12
p		$<$·001	·007		$<$·001	·007

* χ^2 values omitting these entries:—
Standard $\chi^2 = 12{\cdot}8$, 13 d.f., $p = 0{\cdot}50$.
Unknown $\chi^2 = 13{\cdot}1$, 11 d.f., $p = 0{\cdot}30$.

TABLE IV

ANALYSIS OF VARIANCE OF UNWEIGHTED PROBIT RESPONSES IN 257 ASSAYS

Source of variation	d.f.	Mean square	p
1. Between std. and test (S–T)	1	0·0266	$>$0·20
2. Between doses (H–L)	1	265·6719	$<$0·001
3. Between assays	256	1·1360	$<$0·001
4. (S–T) X (H–L)	1	0·2783	0·20–0·05
5. (S–T) X Assays	256	0·1222	$>$0·20
6. (H–L) X Assays	256	0·1299	$>$0·20
7. Residual inter-action (S–T) (H–L) (Assays)	256	0·1147	—
8. Pooled error, items 4–7	769	0·1225	—

that the standard and test preparations produce parallel regressions of probit response on dose, and that their common slope does not vary significantly from assay to assay. From these findings, it may be inferred that the four responses in any one assay, are mutually correlated. The correlation coefficients were therefore computed between

(*a*) the pairs of values (SH + SL) and (TH + TL), and
(*b*) the pairs of values (SH + TH) and (SL + TL),

the first giving the correlation between the sums of standard and test probit responses, the second between the sums of high and low dose responses. Both values for r were highly significant, that for (*a*) being +0·8058, and for (*b*) +0·8003, each based on 257 pairs of results.

Using the values of b', the probit differences of high and low dose responses, as estimates of the slopes, it was found that there was no correlation between the slope of standard and that of test within individual assays. For 257 pairs of values r was found to be $+0{\cdot}0380$.

Thus in comparing the responses obtained in different assays only one factor appears to vary. That is the absolute sensitivity of the mice to the treatments; this is confounded with variations of the absolute doses administered. In a given group of mice, assuming uniform sensitivity throughout the group, the absolute probit responses are correlated equally between standard and test as between high and low doses, the order of their values being determined by the sensitivity of the mice (and by the dose given), the slopes being governed by the intrinsic regression coefficient of the technique. The variance of any one probit response under these conditions would be given by the error mean square of Table IV.

TABLE V

DISTRIBUTION OF 257 ESTIMATES OF THE WEIGHTED MEAN SLOPE FOR STANDARD AND UNKNOWN COMPARED WITH A NORMAL FITTED CURVE

Slope intervals	Frequency	
	Observed	Expected
$<1{\cdot}8$	9	10·3
1·8–2·2	8	7·5
2·2–2·6	5	11·2
2·6–3·0	15	14·9
3·0–3·4	24	19·0
3·4–3·8	25	23·7
3·8–4·2	35	25·5
4·2–4·6	22	27·7
4·6–5·0	25	25·8
5·0–5·4	19	23·5
5·4–5·8	24	20·6
5·8–6·2	14	15·7
6·2–6·6	9	11·6
6·6–7·0	10	8·3
7·0–7·4	10	5·1
$\geqslant 7{\cdot}4$	3	6·6

χ^2 for deviations 19·0, 15 d.f., $p = 0{\cdot}20$

In practice, of course, the evaluation of individual tests is complicated by the introduction of weighting coefficients, which dismisses the possibility of the use of a common error variance for the probit responses. How the weighting of responses is reflected in the results obtained is indicated below.

The slope distribution. The slope of any pair of responses is given by b', their probit difference, divided by 0·2219, the constant log dose interval. The variance of the b' values was 0·2449, equivalent to twice the error variance for a single estimate, and the mean b' was 1·017 probits. Since there was no correlation between the slope of standard and test in any one assay, the variance of their unweighted means would be half the overall variance. Thus in true slope units the overall mean value would be $\dfrac{1{\cdot}017}{0{\cdot}2219} = 4{\cdot}583$ with a variance between assays of $\dfrac{0{\cdot}2449}{(0{\cdot}2219)^2} \times \frac{1}{2} = 2{\cdot}487$.

To compare with these estimates we have the 257 values of the weighted mean slope of standard and test, the frequency distribution of which is given in Table V. The unweighted mean of this series was 4·439, with a variance between assays of 2·300, both these values being less than those derived from the previous unweighted data.

The frequencies of Table V may be fitted to a normal distribution with a probability of about 0·20, and although this fit may be rather fortuitous in the light of further evidence (see below), it may be assumed that the weighted mean slopes are distributed approximately normally.

The standard error of log potency. The standard error of a single

estimate of log-potency in these assays in its uncorrected form is given by:

$$s.e. = \pm \frac{1}{b}\sqrt{\left(\frac{1}{nSw_S} + \frac{1}{nSw_T}\right)}$$

Where b is the weighted mean slope of standard and test; $n = 24$, the number of animals per treatment group; and Sw_S, Sw_T, the sums of the weighting coefficients for high and low dose response probits for standard and test respectively. If it be assumed that standard and test in any one assay are administered in equipotent doses, producing equal responses, then their weighting coefficients will also be equal. The standard error may then be written in the form $\pm \frac{1}{b}\sqrt{\left(\frac{2}{nSw}\right)}$. The reciprocal of the standard error will therefore be $\pm b\sqrt{(Sw)} \times \sqrt{(12)}$; from the point of view of investigating the nature of the distribution of this expression we may further eliminate the constants to reduce it to $b'\sqrt{(Sw)}$ where b' is the probit difference equivalent to the slope b. It has been shown that b and b' are normally distributed, hence the distribution of $b'\sqrt{(Sw)}$ will only be normal if it is linearly related to b'. Any given value of b' however, may be associated with a range of values of Sw according to the degree of asymmetry of the responses relative to the 50 per cent. point. Thus Sw is maximal when the responses are symmetrically disposed, and minimal when they are least symmetrical, i.e., when either response is 0 or 100 per cent. Figure 3 shows these limiting values of $b'\sqrt{(Sw)}$ over the range of positive values of b' encountered in the observations. The values of $b'\sqrt{(Sw)}$ actually obtained were distributed over the area between these limiting curves, and their regression analysis on b', Table VI, shows that the linear regression term is by far the greatest. We could therefore infer that the relationship over the whole range is sufficiently near to

TABLE VI

THE REGRESSION ANALYSIS OF $b'\sqrt{(Sw)}$ ON b' (GROUPED DATA)

b' = probit difference of high and low dose responses
Sw = sum of the weighting coefficients corresponding to high and low responses

Source of variation	d.f.	Mean square
Linear regression	1	86·190
Deviations from linearity	12	0·186
Residual error	500	0·021

Linear regression coefficient = 0·807

direct proportionality for the distribution of $b'\sqrt{(Sw)}$ not to deviate significantly from the normal. Since this function was derived from the reciprocal of the standard error by the elimination of constants and the assumption only of equal potency of standard and test, which we know to be the case in the over-all picture, it may be inferred that the reciprocals of the standard errors will be normally distributed. A histogram of these values, which correspond to the square roots of the weights assigned to log potencies, is shown in Figure 4 together with a fitted normal curve.

Deviations from the normal produce a value for χ^2 of 23·01 which with 19 degrees of freedom corresponds to a probability of 0·24. The mean value was 15·0, standard deviation $\pm 5 \cdot 085$.

To confirm this distribution a further series of 424 values were extracted from the records. Deviations from normality gave $\chi^2 = 19 \cdot 63$, which with 19 degrees of freedom, gave $p = 0 \cdot 40$. On the other hand while the variance in this second series of $\sqrt{}$(weight) values was not significantly

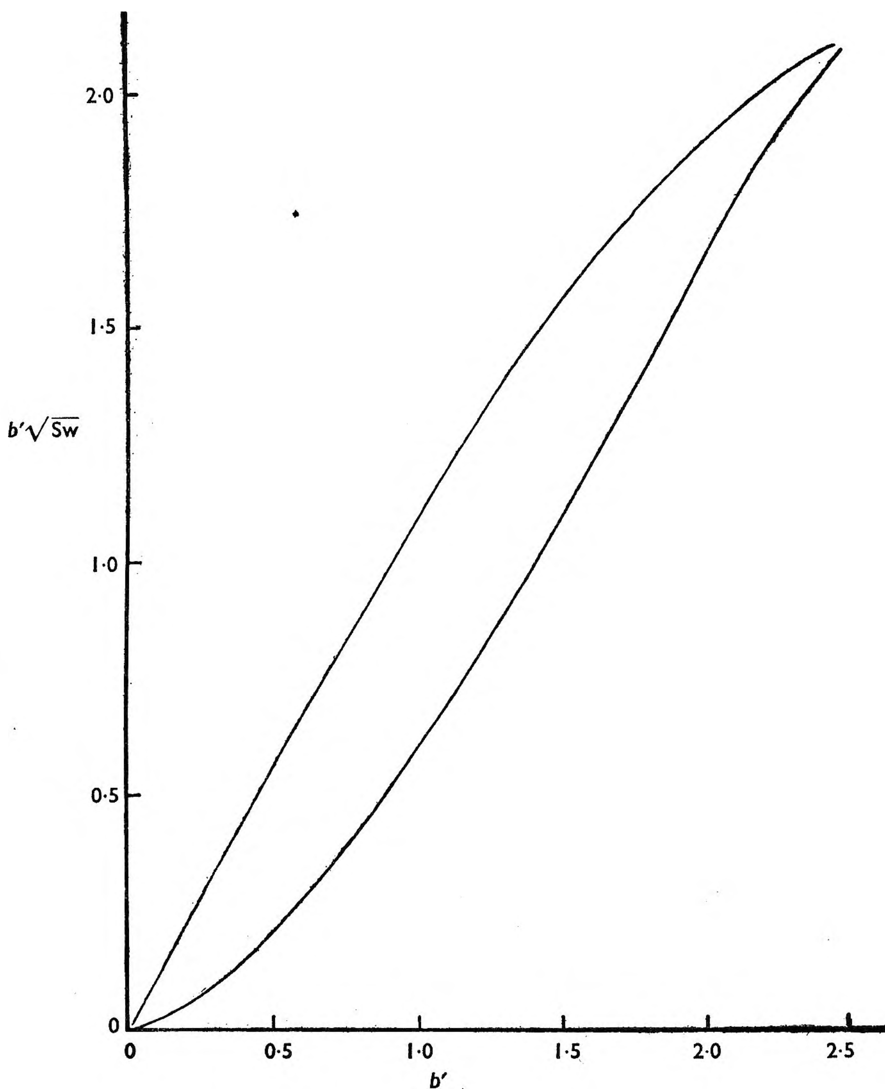

FIG. 3. Limiting values of $b'\sqrt{(Sw)}$ related to b'. The upper line of maximum values corresponds to pairs of responses symmetrical about the 50 per cent. point, the lower line of minimum values to pairs of responses, one of each pair being 0/24 or 24/24. In practice of course the relationship is discontinuous.

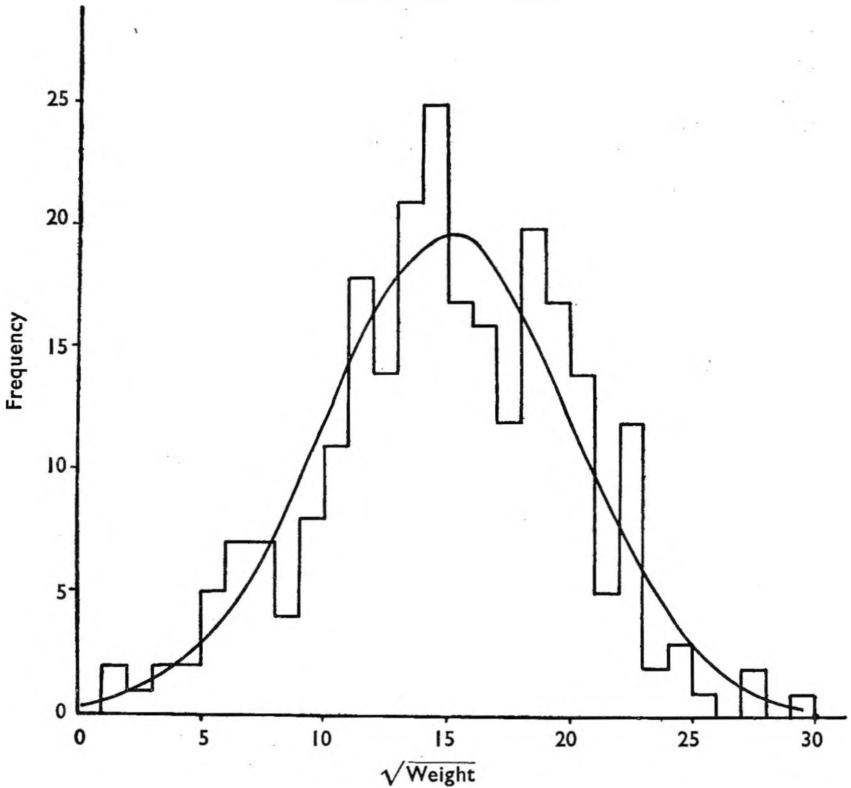

FIG. 4. Histogram of the distribution of the reciprocals of standard errors of log potency ($\sqrt{\text{weight}}$), with a normal curve with the same mean and variance.

reduced (19·66 compared with 25·86), the mean was significantly greater at 16·92. Thus it appeared that the internal accuracy of the second series of tests was greater than that of the first series. Since the internal error might be reduced either by more symmetrical responses about the 50 per cent. point, or by an increase of the regression coefficient, it seemed desirable to decide in which manner the change had been produced.

Further inspection of results obtained over the same period as the second series described above showed that the mean slope of 464 assays was 4·906 compared with the mean slope of the original series of 4·439. Thus the higher mean of the $\sqrt{(\text{weight})}$ values of the second series may be accounted for by an increase in the mean slope. Since this was the first indication in the data that the slopes were not simple estimates of some true mean value, it became necessary to investigate the variables which might effect such a change.

The only factors which could be demonstrated to influence the slope were the weight of mice used and their previous usage. Table VII gives the frequencies of different slope values according to the weight range and previous history of the mice. In the case of normal mice, i.e., those used

TABLE VII

THE FREQUENCY DISTRIBUTION OF SLOPE VALUES RELATED TO BODY WEIGHT OF MICE AND PREVIOUS USAGE

Key to weight groups: 1 14·5–16·0
2 16·0–17·5
3 17·5–19·0
4 19·0–20·5
5 20·5–22·0 g.

	Number of times previously used:														
	NONE					ONCE					TWICE or more				
Wt. group:	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Slope values:															
1·8–2·1			1					1							
2·1–2·4		3	1						1	1					
2·4–2·7	5	2	2				1		1	1					
2·7–3·0	5	8	1	1			1			1					
3·0–3·3	3	3	5	3				1	2	1					
3·3–3·6	3	3	6	3				2	4					1	
3·6–3·9	5	8	10	4	2		3							2	1
3·9–4·2	3	5	11	10	2		2	5	4	4				1	
4·2–4·5	2	6	7	11	1		3	2	3	1					1
4·5–4·8	5	10	4	5	2	1		3	1	2			1		
4·8–5·1	6	4	3	5	2	2		4	1	5					
5·1–5·4	3	7	11	8					3	2		1	1		1
5·4–5·7	6	8	7	8			1	1	1	1					1
5·7–6·0	1	5	11	11	2			3	1	1			1	1	1
6·0–6·3	1	4	5	7	3			1	2				1		1
6·3–6·6	5	3	6	3	1					1					
6·6–6·9		3	2	2	1		1		2					1	1
6·9–7·2	2	1	2	4		1			1					1	
7·2–7·5		2	2	4				1							
7·5–7·8			2	1											
7·8–8·1		2		1										1	
8·1–8·4			1		1				1						
⩾8·4			2	2	1										
Total frequencies ..	55	87	102	93	18	4	12	24	28	21	0	1	4	8	7
Mean slopes	4·49	4·71	5·01	5·30	5·55	5·40	4·17	4·64	4·71	4·46	—	5·25	5·48	5·33	5·38

for the first time, a significant regression of slope on mouse weight may be demonstrated (Fig. 5). This does not hold however in the case of mice which have undergone previous testing, although admittedly the numbers of assays are somewhat lower in these groups. The mean slope given by mice previously used once is considerably less than would be given by normal mice of the same average weight, yet that for mice previously used twice or more, while significantly greater than the mean of the once used group, does not differ significantly from the slope for normal mice of the same average weight.

DISCUSSION

It has been shown that the reciprocals of standard errors of log potency obtained in a number of assays are distributed approximately normally. Some factors which make it impossible for the distribution to be truly normal have been described. It is interesting to note that Hemmingsen[2] reported that the variation of slope values from test to test exceeded that to be expected from consideration of the binomial distribution of responses. Thus in his data either the slope did in fact vary, or the mice within each assay were so heterogeneous as to render the application of the binomial sampling rule invalid. It is likely that both of these

conditions should equally well apply to his results as well as to our own. On the other hand, Smith[3] could not detect significant differences of slope associated with the weight of mice or their previous usage. His examination of the results of 231 assays was made by isolating each variable in turn, however. Thus the results obtained in the different weight groups were confounded with the previous usage of the mice, and *vice versa*. Both Hemmingsen and Smith employed a far coarser range of weight for the mice included in any one test, while the latter author used mice up to 30 g. in weight, 8 g. higher than our maximum. The limitation we have imposed on the weight of mice used for testing, particularly with regard

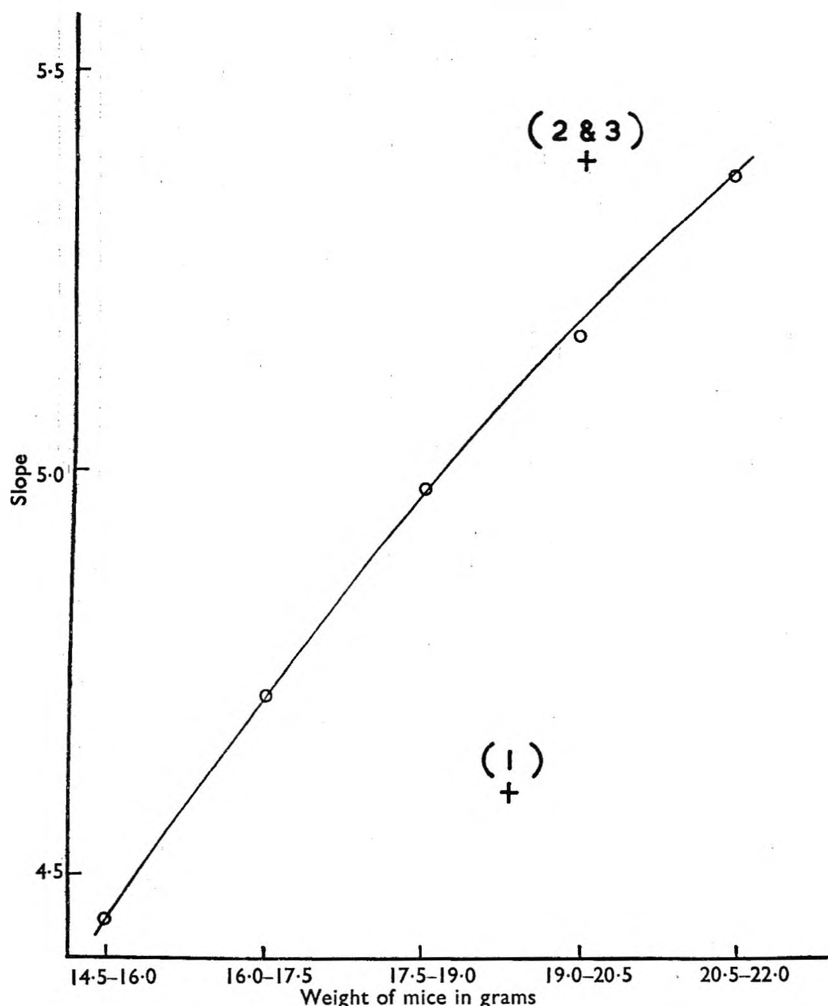

FIG. 5. The relationship between the slope of the log. dose-response line and body weight of normal mice, i.e., those used for the first time. The mean slopes for mice used once (1), and two or more times (2 and 3) previously are shown plotted against their respective mean weights.

to the upper limit of 22 g., entails a complicated system of selection for mice used more than once. Thus those which fell in the heavier groups at their first usage, and subsequently gained weight at the normal rate, might be too heavy for further use, while similar animals failing to gain in weight would be pooled for assay purposes with previous "lightweights" which had grown normally. Further experimental work would be necessary to investigate this problem satisfactorily, but so far as the theme of this paper is concerned it is sufficient to record that the slope of the assays cannot be considered constant.

Summary

1. The distribution of probits of responses out of 24 to high and low doses of insulin in routine mouse assays is approximately normal.

2. Equally significant positive correlations hold between the responses to standard and unknown samples as between high and low doses. No significant correlation could be detected between the slopes of standard and test within assays, although in the overall picture the two were parallel.

3. The mean slopes for standard and test within assays were normally distributed.

4. The reciprocals of the standard errors of log-potency do not differ significantly in their distribution from the normal, but the effect of using weighting coefficients for different responses is to make this distribution approximate if the slopes are distributed in a truly normal manner.

5. The slope of the assays has been shown to be directly proportional to the body weight of the mice, when these have not been used previously. This relationship did not appear to apply to animals used for the second, third, or fourth time.

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AN APPLICATION OF THE VITALI-MORIN REACTION TO THE DETERMINATION OF SMALL QUANTITIES OF HYOSCINE HYDROBROMIDE IN SOME PHARMACEUTICAL PREPARATIONS

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Several methods for the determination of hyoscine hydrobromide in pharmaceutical preparations have been described in the literature.[1,2,3] In general, however, these methods are not suitable for the determination of very low proportions of the alkaloid, particularly in the presence of interfering substances, and the object of the work described here was to devise a method capable of determining amounts of hyoscine hydrobromide of the order of 0·005 per cent. w/v of solution.

Kirkpatrick[4] has studied the catalytic reduction of a number of alkaloids, including hyoscine at the dropping mercury electrode. A method based on this principle has been found to give quantitative results when applied to solutions of hyoscine hydrobromide but the presence of electrolytes (other than those specified for the base solution) and of traces of other alkaloids renders the method unsuitable for routine application. Allport and Wilson[5] have studied Morin's[6] modification of Vitali's test for solanaceous alkaloids and have applied it successfully to belladonna, stramonium and their galenical preparations, whilst Allport and Jones[7] have extended the application of this reaction to the determination of atropine, hyoscyamine and hyoscine in hypodermic tablets and injection solutions. Other workers[8] have reported that this method gives accurate and reproducible results only when the reagents, the reaction times and the water content of the acetone are rigidly controlled. Preliminary experiments in these laboratories on hyoscine hydrobromide solutions have confirmed these findings.

Attention was, therefore, directed to investigating the factors affecting Allport and Wilson's method and to applying the method to the determination of hyoscine hydrobromide (*a*) in official preparations and (*b*) in solutions of pethidine hydrochloride containing hyoscine: such solutions are commercially available as injection preparations containing usually 0·0108 per cent. w/v or 0·0216 per cent. w/v hyoscine hydrobromide in a 5·0 per cent. w/v solution of pethidine hydrochloride containing a preservative.

The sample of hyoscine hydrobromide used throughout the investigation conformed to the requirements of the British Pharmacopœia 1948 and except where otherwise stated other reagents were of "Analar" quality.

INVESTIGATION OF THE ORIGINAL* METHOD USING HYOSCINE HYDROBROMIDE

(*a*) *Effect of solvent.* James and Roberts[9] have reported that pyridine, malonic ester, acetone and methyl ethyl ketone produce strong colours when used as solvents in the Vitali reaction with atropine. Tests carried out using Allport's method on an aqueous solution of hyoscine hydrobromide indicated that of these solvents only pyridine and acetone gave

TABLE I

EFFECT AFTER 6 MINUTES OF DIFFERENT AMOUNTS OF POTASSIUM HYDROXIDE ADDED TO PYRIDINE AND ACETONE (10 ML. QUANTITIES) OF VARIOUS WATER CONTENTS

Water content per cent. w/v	Pyridine		Acetone	
	0·1 ml. 3·0 per cent. potassium hydroxide solution	0·1 ml. 0·5 per cent. potassium hydroxide solution	0·1 ml. 3·0 per cent. potassium hydroxide solution	0·1 ml. 0·5 per cent. potassium hydroxide solution
0·075	flocculent crystalline precipitate	clear	clear	clear
0·095	flocculent crystalline precipitate	clear	clear	clear
0·15	flocculent crystalline precipitate	clear	clear	clear
0·225	strongly opalescent	clear	clear	clear
0·30	strongly opalescent	very slightly opalescent almost clear	very slightly opalescent	clear
0·45	strongly opalescent	very slightly opalescent	slightly opalescent	very slightly opalescent
0·60	strongly opalescent	slightly opalescent	opalescent	very slightly opalescent
0·75	turbid	slightly opalescent	opalescent	slightly opalescent
1·00	turbid	opalescent	clear	clear

strong purple colours. A range of other solvents were tested but none produced a purple colour as intense as that produced with pyridine or acetone. In view of these findings it was decided to investigate quantitatively the possibilities of pyridine as solvent and to determine whether it offered any advantage over acetone as used hitherto.

(*b*) *Effect of potassium hydroxide concentration and water content of solvent.* Preliminary tests with pyridine as solvent showed that the water content of the pyridine and the concentration of caustic alkali used had an important bearing on the results. Addition of 0·1 ml. of a 3 per cent. solution of potassium hydroxide in methanol to 10 ml. quantities of

* The essential details of Allport and Wilson's method are as follows. Hyoscine alkaloid is extracted by means of chloroform from ammoniacal solution, converted into its water-soluble acetate by acetic acid and an aliquot, evaporated to dryness, is nitrated by treatment with fuming nitric acid. The dry nitrated base is dissolved in acetone and the intensity of the purple colour produced on the addition of potassium hydroxide solution is measured in a Lovibond tintometer.

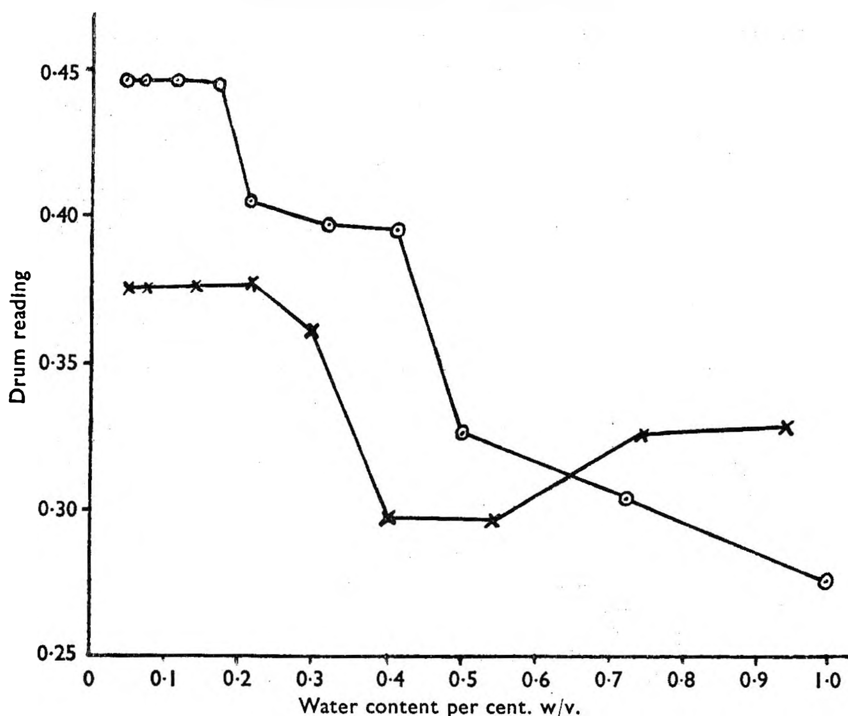

FIG. 1. Effect of water content of the solvent on the intensity of the purple colour developed in ⊙ pyridine and × acetone.

pyridine containing known amounts of water produced an opalescence or a crystalline precipitate according to the water content of the pyridine, after about 5 to 6 minutes. A similar effect, though to a less marked extent, was observed with acetone. Tests were, therefore, carried out to ascertain the maximum amount of potassium hydroxide which could be added to 10 ml. quantities of either solvent without the formation of a precipitate or turbidity which would interfere with the subsequent colorimetric comparison. It was found that the addition of 0·1 ml. of a 0·5 per cent. solution of potassium hydroxide in methanol to 10 ml. of solvent produced no precipitate or turbidity after 6 minutes standing in either acetone or pyridine containing less than 0·3 per cent. w/v of water. Comparison of the results using 3·0 per cent. and 0·5 per cent. solution of potassium hydroxide and solvent of varying water contents is shown in Table I.

The effect of the water content* of the solvent on the intensity and stability of the colour produced in the presence of hyoscine was next investigated, the lower concentration of potash being used in view of the foregoing results.

To a series of 10 ml. quantities of a 0·02 per cent. w/v aqueous solution of hyoscine hydrobromide, 2 ml. of dilute solution of ammonia (10 per

* The maximum permitted water content of "Analar" acetone is 1·0 per cent. w/v whilst that of "Analar" pyridine is 0·25 per cent. w/v.

cent. w/w) was added and the hyoscine base extracted from each solution with 3 quantities, each of 3 ml., of chloroform, the subsequent procedure being as in the original method. Each portion of nitrated base was dissolved in 10 ml. of acetone of various contents up to 1·0 per cent. w/v and 0·1 ml. of 0·5 per cent. potassium hydroxide solution added to each solution. The solutions were well mixed and the intensity of the purple colours produced measured in a 1 cm. cell using a Spekker photoelectric absorptiometer, exactly 5 minutes after the addition of the potassium hydroxide reagent. Ilford 605 gelatin filters were used.

TABLE II

HYOSCINE HYDROBROMIDE IN AQUEOUS SOLUTION. REPRODUCIBILITY OF RESULTS BY THE MODIFIED METHOD USING PYRIDINE (WATER CONTENT < 0·17 PER CENT. W/V)

	Hyoscine hydrobromide	
	Amount added mg.	Amount found* mg.
A	0·50	0·52 0·51
B	0·937	0·945 0·940
C	1·50	1·52 1·51
D	2·125	2·160 2·115
E	2·811	2·74 2·76
		2·74 2·78

* Results in brackets indicate duplicate results on the same acetate solution.

This series of tests was repeated using 10 ml. quantities of pyridine as solvent for colour development, the water content of each portion having previously been adjusted to give a range up to 1·0 per cent. w/v. The results are shown in Figure 1.

It is evident from the results that: (i) the intensity of the colour produced using pyridine as solvent is approximately 20 per cent. greater than that produced using acetone as solvent, provided the water content of the solvent does not exceed 0·17 per cent. w/v. Furthermore, with this limiting water content reproducible results are obtained; (ii) water contents of each solvent in excess of 0·17 per cent. w/v adversely affect the intensity of the colours produced, giving erratic results.

In view of these findings it is apparent that under the prescribed conditions pyridine is superior to acetone as solvent and in view of the low water content of "Analar" pyridine it was decided to use this solvent in all subsequent work. The water content of "Analar" pyridine can be reduced, if necessary, to well below the suggested upper limit of 0·17 per cent. w/v by allowing to stand over barium oxide (which has been previously heated to 200° C. for 4 hours and allowed to cool) with occasional shaking over a period of 2 to 3 days. The clear layer can be decanted off as required. It has been found, however, that of the

numerous batches of "Analar" pyridine used during routine testing, the water content is frequently below 0·17 per cent. and further dehydration is unnecessary.

The upper limit (1·0 per cent. w/v) of "Analar" acetone on the other hand allows for a considerably wider variation in water content between batches of solvent. Furthermore, it has been found that the production of acetone containing not more than 0·17 per cent. of water is not an operation applicable to routine use—the usual drying reagents do not reduce the water content sufficiently even after prolonged standing.

(*c*) *Effect of light.* It was found that the purple colour produced both in pyridine and acetone faded rapidly when placed in strong sunlight. Similar solutions kept in the dark faded less rapidly. Such solutions (both in acetone or pyridine) fade fairly rapidly for the first 2 to 3 minutes following the addition of the potassium hydroxide solution, but after this period the rate of fading of the colour is appreciably diminished.

It is essential, therefore, that the solution be kept in the dark prior to measurement of the colour and that the actual measurement be carried out as rapidly as possible.

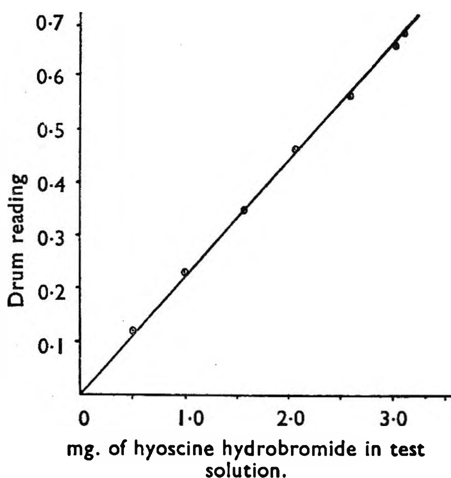

FIG. 2. Calibration curve of hyoscine hydrobromide.

(*d*) *Results.* A typical calibration graph, using aliquots of a solution containing 2·0 mg. of hyoscine hydrobromide per 10 ml. by the general method given below is shown in Figure 2 while the reproducibility on a series of test solutions is given in Table II.

APPLICATION TO PHARMACEUTICAL PREPARATIONS

The general method quoted below is essentially that as described by Allport *et al.* but has been modified in addition to slight differences in technique by: (*a*) replacing "Analar" acetone with "Analar" pyridine of a water content less than 0·17 per cent. w/v, (*b*) reducing the concentration of the potassium hydroxide solution from 3·0 to 0·5 per cent. w/v, (*c*) using the Spekker photoelectric absorptiometer in preference to the Lovibond tintometer as a means of recording the colour intensity.

(A) *General Method*

Special reagents. (*a*) Acetic acid: 6 per cent. w/v acetic acid containing approximately 5 per cent. of absolute ethanol.

(*b*) "Analar" pyridine containing less than 0·17 per cent. w/v of water.

(*c*) Potassium hydroxide: 0·5 per cent. w/v of "Analar" potassium hydroxide in "Analar" methanol. This reagent must be freshly prepared.

Procedure. To 10 ml. of the solution under test or a suitable volume containing up to 3·0 mg. of hyoscine hydrobromide add 3 ml. of dilute solution of ammonia (10 per cent. w/w) and 3 ml. of chloroform. Shake the mixture for 2 minutes, allow to separate and run off the chloroform layer. Repeat the extraction with 2 further 3 ml. quantities of chloroform shaking each for 2 minutes, allow to separate and mix each with the first chloroform extract. Wash the mixed chloroform solution once with 3 ml. of water, shaking for 2 minutes. Allow to separate, run off the chloroform layer into a 50 ml. stoppered cylinder and wash the aqueous portion with a further 1 ml. of chloroform which is added to the chloroform solution in the cylinder. Add exactly 20 ml. of 6 per cent. acetic acid reagent and shake the mixture for 1 minute. After allowing to stand until the immiscible liquids have separated, transfer 1·0 ml. of the acid layer (accurately measured by means of a micro-burette) to a Pyrex evaporating dish and evaporate just to dryness on a water-bath. Add 0·2 ml. of "Analar" fuming nitric acid (ensure that the acid comes into contact with the whole of the residue) and evaporate until the nitrated base is just dry. Dissolve the residue in 3 ml. of pyridine and transfer to a 10 ml. dry stoppered cylinder. Wash the dish separately with further quantities of pyridine adding each washing to the contents of the cylinder and adjust the total volume to 10 ml. with pyridine. Add 0·1 ml. of potassium hydroxide solution and thoroughly mix the contents by inverting several times. Transfer the solution to a 1 cm. cell and keep in the dark. Exactly 5 minutes after the addition of the potassium hydroxide solution measure the intensity of the purple colour as rapidly as possible in a Spekker photoelectric absorptiometer using Ilford 605 gelatin filters and a solution blank prepared by the addition of 0·1 ml. of potassium hydroxide solution to 10 ml. of pyridine.

Prepare a calibration graph in the same manner using suitable dilutions of a standard hyoscine hydrobromide solution covering the required range. Read off from the graph thus prepared the amount of hyoscine hydrobromide contained in the test solution.

(B) *Applications*

The method has been successfully applied to aqueous solutions of hyoscyamine sulphate B.P.C. and of atropine sulphate B.P. The intensity of the colours produced using equal weights of hyoscyamine and atropine sulphates are inversely proportional to the molecular weights of these compounds.

(*a*) *Official preparations.* The following preparations have been studied in detail and the results obtained are shown in Table III.

(i) *Injectio Hyoscinæ Hydrobromidi B.P.* Take 5 ml. for assay and proceed as by the general method. The presence of chlorocresol does not interfere with the determination.

(ii) *Guttæ Hyoscinæ B.P.C.* Dilute 10 ml. to 100 ml. with water. Take a 10 ml. aliquot and proceed as detailed in the general method.

The presence of methyl and propyl hydroxybenzoates does not interfere with the determination.

(iii) *Tabellæ Hyoscinæ Hydrobromidi B.P.C.* Take an appropriate number of tablets dependent on the active agent content, disintegrate by the addition of a few ml. of water and wash into a separator. Dilute to approximately 10 ml. with water and proceed as before.

(iv) *Oculentum Hyoscinæ B.P.* Consistently low results were obtained with this preparation. This was found to be due to incomplete extraction of the active agent from the ointment base. Several different methods of extraction were attempted but all yielded results approximately 10 per

TABLE III

OFFICIAL PREPARATIONS. RESULTS OBTAINED USING THE MODIFIED METHOD

Official preparation	Amount taken for test	Hyoscine hydrobromide	
		Added mg.	Found mg.
Injectio Hyoscinæ Hydrobromidi B.P. 1948	5 ml.	2·06	2·09 2·05
			2·11 2·11
			2·04 2·00
Guttæ Hyoscinæ B.P.C. 1949	1 ml. (by aliquot)	2·29	2·29 2·21
			2·25 2·29
			2·29 3·00
Tabellæ Hyoscinæ Hydrobromidi 1/100 gr. (≡ 0·648 mg.)	0·2579 g.	2·592	2·63 2·60
			2·60 2·52
	0·1935 g.	1·944	1·94 1·91

cent. low. No suitable method giving accurate and reproducible results has been found up to the present.

(*b*) *Solutions containing pethidine hydrochloride.* Preliminary experiments indicated that pethidine interferes with the measurement of the final colour by producing a turbidity in the pyridine solution and inducing rapid fading of the purple colour. Application of the general method without modification was, therefore, useless and an investigation was undertaken to evolve a suitable method whereby the pethidine could be removed from the solution without reducing the hyoscine content. The solubility of hyoscine alkaloid in light petroleum (b.pt. 60° to 80° C.) has been reported[10] as 1 in 510 and the solubility in water[11] as 1 in 9·5. Pethidine base, on the other hand, is miscible with light petroleum (b.pt. 60° to 80° C.) but relatively insoluble in water. This offered a convenient method of separating the alkaloids.

Preliminary experiments indicated that no detectable amount of hyoscine was lost when 4 ml. of dilute solution of ammonia was added

to 10 ml. of a solution containing 0·03 per cent. w/v of hyoscine hydrobromide and 5·0 per cent. w/v of pethidine hydrochloride and the precipitated pethidine base extracted separately 6 times with 3 ml. quantities of light petroleum (b.pt. 60° to 80° C.), the mixed petroleum extracts being washed with 3 ml. of water which was returned to the ammoniacal hyoscine solution.

Subsequent tests indicated that 4 extractions (each of 3 ml.) with light petroleum was sufficient to remove most of the pethidine base from the aqueous phase using the above conditions. The small amount of pethidine remaining in the ammoniacal solution did not interfere with the subsequent determination of hyoscine. The proposed method quoted below was applied to a series of pethidine hydrochloride solutions 5·0 per cent. w/v containing varying amounts of hyoscine hydrobromide. The results obtained are given in Table IV.

TABLE IV

RESULTS OBTAINED USING THE LIGHT PETROLEUM EXTRACTION PROCEDURE ON SOLUTIONS OF HYOSCINE HYDROBROMIDE CONTAINING 5 PER CENT. W/V OF PETHIDINE HYDROCHLORIDE

Solution (volume = 10 ml.)	Hyoscine hydrobromide added mg.	Hyoscine hydrobromide found mg.
A	1·00	1·02, 0·99
B	2·00	1·99, 1·98
C	3·00	3·05, 3·05
D	0·50	0·49, 0·48
E	1·08	1·05, 1·08
F	2·16	2·12, 2·15

Proposed method. To 10 ml. of the test solution containing 5 per cent. w/v of pethidine hydrochloride contained in a separator add 4 ml. of dilute solution of ammonia (10 per cent. w/w) and 3 ml. of light petroleum (b.pt. 60° to 80° C.). Shake the mixture for 2 minutes, allow to separate and run off the ammoniacal layer into a second separator and shake for 2 minutes separately with 3 further 3 ml. quantities of light petroleum (b.pt. 60° to 80° C.) allowing each to separate. Add the light petroleum extracts in turn to the first light petroleum solution contained in the first separator and wash with 3 ml. of distilled water. Run off the lower aqueous layer into the ammoniacal solution containing the hyoscine base and reject the light petroleum solution. Add 3 ml. of chloroform and continue as described in the general method with the exception that prior to taking the 1 ml. aliquot of acetic acid solution for evaporation the solution is filtered through a small pleated filter-paper rejecting the first few ml. of filtrate. The latter precaution was adopted due to the fact that the acetic acid solution was invariably turbid (in contrast to

the clear solution obtained in the absence of pethidine hydrochloride). Filtration yielded a clear filtrate. The presence of chlorocresol in the test solution does not affect the results.

During the extraction process both with light petroleum and chloroform low results were obtained if the hyoscine was allowed to remain in contact with the alkaline solution for a longer time than necessary to complete the extraction procedure. This is apparently due to the unstable nature of hyoscine alkaloid under such conditions of alkalinity. It was also found that the hyoscine present in the acetic acid solution is not very stable. All tests carried out on the same acetic acid solution should be done within 1 to 2 hours of preparation.

Attempts were made to extract the pethidine base from alkaline solutions with the aid of a continuous extraction apparatus using light petroleum (b.pt. 30° to 40° C.) as the refluxing solvent, but the results were low and in some instances this procedure resulted in total destruction of the hyoscine alkaloid present.

Summary

1. The colorimetric method described by Allport *et al.* for the estimation of hyoscine hydrobromide has been shown to give variable results if the water content of the solvent in which the colour develops is not controlled. "Analar" pyridine of low water content has been shown to be a more suitable solvent than "Analar" acetone in the original method.

2. The modified method has been shown to give accurate and reproducible results using official preparations containing hyoscine hydrobromide, except in the case of Oculentum Hyoscinæ B.P., for which a satisfactory extraction procedure has still to be devised.

3. The method has been successfully adapted to the determination of hyoscine hydrobromide in the presence of pethidine hydrochloride.

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THE CHROMATOGRAPHIC PURIFICATION AND ULTRA-VIOLET SPECTROPHOTOMETRIC ASSAY OF STRYCHNINE IN GALENICALS

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The British Pharmacopœia method for the assay of strychnine in galenicals comprises: (1) Purification of the galenical by extracting with chloroform in an acid medium to remove the chloroform-soluble substances; liberation of the alkaloid by alkali and extraction with chloroform; elimination of brucine, without destruction of strychnine, by oxidation with nitric acid in presence of 3 per cent. sulphuric acid; extraction of the relatively pure strychnine with chloroform, after rendering alkaline with sodium hydroxide. (2) Estimation of the strychnine by acid titration.

The methods of assay of different national Pharmacopœias are in principle similar but differ in detail.

The Expert Committee on the Unification of Pharmacopœia, World Health Organisation, recently recommended in connection with the proposed aim of an acceptable International Pharmacopœia that more simple methods of assay of vegetable drugs should be sought. The Committee further recommended that if such methods are found to be satisfactory they should be included in the International Pharmacopœia.

The following procedure differs in principle from the B.P. method. The chromatographic adsorption technique as described by Brownlee,[1] modified to suit the use of smaller quantities, is substituted for the B.P. method of purification of the galenical containing strychnine (see also Reimers, Gottlieb and Christensen[2]). The strychnine is estimated in the purified product directly by ultra-violet spectrophotometry. This technique has the advantage over the titration method of being directly applicable without the further solvent extractions made necessary by the presence of titratable impurities.

Spectrophotometric Examination of Strychnine and Brucine

In galenical preparations the undesired alkaloid brucine always accompanies the strychnine. A chromatographic separation of brucine and strychnine does not appear to have been effected. Brucine will interfere with the direct spectrophotometric estimation of strychnine.

Strychnine absorbs in the ultra-violet only with maximum absorption at 255μ (Brustier and Blanc,[3] Elvidge[4]).

We have examined the ultra-violet absorption spectra of strychnine and brucine using absolute ethanol as solvent by the Unicam spectrophotometer. Strychnine shows a maximum absorption at 254 mμ; brucine shows a maximum absorption at 264 mμ and another less distinct band at 301 mμ.

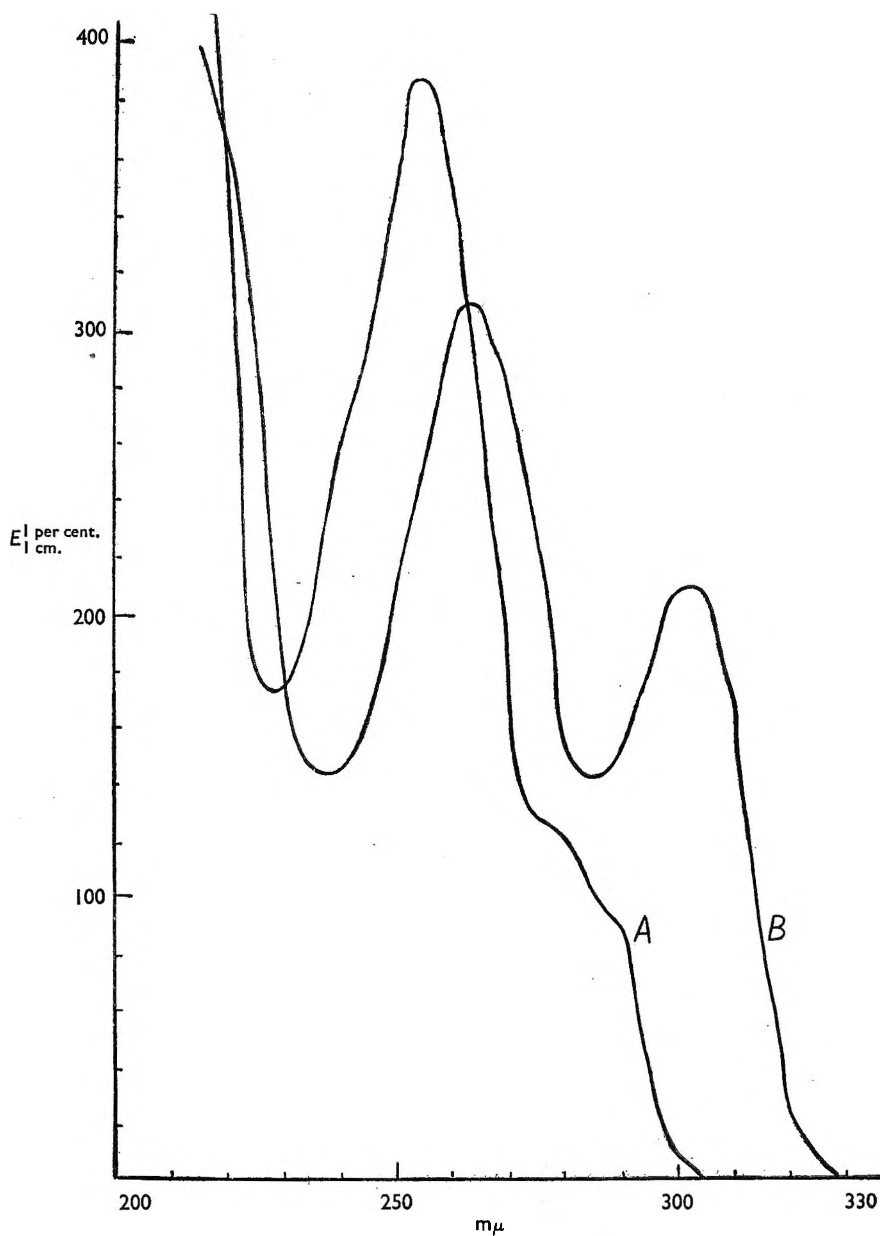

FIG. 1. Showing absorption curves and $E^{1\text{ per cent.}}_{1\text{ cm.}}$ of (A) strychnine (m.pt. 270° to 280° C.) in absolute ethanol, and (B) anhydrous brucine (m.pt. 178° C.) in absolute ethanol.

Experimental

Using pure strychnine (m.pt. 270° to 280° C.) and pure anhydrous brucine (m.pt. 178° C.) in absolute ethanol, the absorption curves were determined from the spectra taken with the Unicam instrument. Figure 1 shows the $E_{1\,cm.}^{1\,per\,cent.}$ of each alkaloid is plotted against wavelength. It is clear that the absorption at 301 mμ is due only to brucine. Therefore, in a pure mixture of strychnine and brucine, only the amount of brucine can be calculated from the extinction measured at 301 mμ and the strychnine obtained by the extinction difference at 254 mμ. In galenical preparations other unknown ultra-violet-absorbing impurities interfere with these direct calculations. However, the effect of the other impurities may be estimated by eliminating the spectrum of brucine which is readily oxidised to a non-absorbing product without destruction of strychnine.

Oxidation of Brucine

Since the nitrate ion absorbs at 302 mμ, oxidation with nitric acid cannot be used in the spectrographic assay. It was found that when pure brucine is treated with potassium persulphate at 60° to 70° C. in presence of 3 per cent. sulphuric acid for 1 hour, oxidation takes place with complete disappearance of the brucine absorption bands at 264 mμ and at 301 mμ. This was confirmed by making the oxidised solution alkaline and extracting with chloroform; no brucine was obtained.

When strychnine is treated by the prescribed potassium persulphate procedure it remains unchanged. To confirm this, different weights of each of strychnine and of brucine in different proportions were dissolved in 10 ml. of 3 per cent. sulphuric acid, 0·5 g. of potassium persulphate was added and dissolved by shaking and the solution was kept at 60° to 70° C. for 1 hour. The solution was then completed to 100 ml. with distilled water and the amount of strychnine determined by measuring the E 254 mμ value. Another portion was rendered alkaline with sodium hydroxide and the unchanged strychnine was extracted with chloroform. The residue left after distillation of the chloroform was dissolved in absolute ethanol, made up to a convenient volume with the same solvent, and the amount of strychnine determined in the same way. The same weight of the mixture of strychnine and brucine was analysed for its strychnine content by the B.P. procedure for comparison. Results of a series of determinations are shown in Table I.

Determination of Strychnine in Galenicals

Liquid Extract of Nux Vomica

Assay procedure. Into a glass tube 25 to 30 cm. long, 1·3 cm. in diameter, with a constricted end, 15 g. of active alumina was packed dry in portions forming an adsorption column 14 cm. long. The column was connected to a suction apparatus, 2 ml. of the liquid extract was poured on and gentle suction was applied. Before the liquid began to disappear from above the adsorption column, 86 per cent. ethanol was added little by little to wash down the alkaloids on the sides of the tube.

TABLE I

COMPARISON OF RECOVERY OF PURE STRYCHNINE FROM STRYCHNINE–BRUCINE MIXTURES BY THE B.P. METHOD AND SPECTROPHOTOMETRIC ANALYSIS

Prepared amounts of alkaloid mixture		Pure strychnine recovered from the mixture					
				Spectrophotometric method after potassium persulphate treatment			
Brucine g.	Strychnine g.	B.P. method g.	Error per cent.	Direct measurement in 0·3 per cent. sulphuric acid g.	Error per cent.	After chloroform extraction measured in absolute ethanol g.	Error per cent.
0·1942	0·2512	0·2420	−3·0	0·2562	+1·9	0·2390	−4·8
0·1001	0·1230	0·1180	−4·0	0·12314	+0·2	0·1225	−0·4
0·1001	0·1845	0·1790	−2·0	0·1790	−2·0	0·1760	−4·5
0·0515	0·0610	0·0542	−1·1	0·0624	+2·0	0·0605	−0·8
0·0734	0·0861	0·0829	−3·0	0·0869	+0·9	0·0880	+2·2
0·0212	0·0245	0·0234	−5·0	0·0250	+1·0	0·0236	−3·5
0·0432	0·0492	0·0460	−6·0	0·0500	+1·0	0·0480	−2·4
0·0683	0·0608	0·0576	−5·0	0·0576	−5·0	0·0600	−1·3
0·0121	0·0608	0·0559	−8·0	0·0605	−0·4	0·0576	−5·2
Average error per cent.			−4·7		−0·04		−2·3

The adsorption column was then washed with greater amounts of 86 per cent. ethanol until the percolate was alkaloid free.

Usually about 50 ml. of 86 per cent. ethanol was sufficient for complete washing. The clear percolate was transferred quantitatively to a 100-ml. flask and completed to volume with 96 per cent. ethanol. An aliquot portion, usually 20 to 30 ml., of this was distilled, the residue was dissolved in 10 ml. of 3 per cent. sulphuric acid, 0·5 g. of potassium persulphate was dissolved in the solution by shaking and the solution was kept in a water-bath at 60° to 70° C. for 1 hour. After cooling, the solution was transferred quantitatively to a 100-ml. flask with distilled water. The solution was mixed by shaking, filtered through a dry filter-paper and dry funnel into a dry flask. The strychnine was then determined by measuring the E 254 mμ value of the solution in a Unicam spectrophotometer. Strychnine could be calculated according to the well-known relationship

$$C = E/E_{1\,\text{cm.}}^{1\,\text{per cent}}$$

where C = concentration in g. per cent., $E_{1\,\text{cm.}}^{1\,\text{per cent.}} = 390$ at 254 mμ, E = measured extinction at 254 mμ.

Tincture of Nux Vomica

10 ml. of the tincture were allowed to flow through an adsorption column of 10 g. of active alumina packed dry in the glass tube already described, and the assay completed as for the liquid extract.

To measure the sensitivity of the described procedure it was carried out on the galenicals with and without addition of known volumes of standard solution of pure strychnine in 86 per cent. ethanol. At the same time the galenical was assayed by the B.P. procedure with and without the addition of known quantities of strychnine for comparison. The results are shown in Tables II and III.

TABLE II

COMPARISON OF SENSITIVITY OF THE B.P. METHOD AND THE SPECTROPHOTOMETRIC PROCEDURE FOR THE ESTIMATION OF STRYCHNINE IN LIQUID EXTRACT OF NUX VOMICA

B.P. procedure				Spectrographic method after potassium persulphate treatment			
Strychnine in liquid extract per cent.	Strychnine Added g.	Strychnine Recovered g.	Error per cent.	Strychnine in liquid extract per cent.	Strychnine Added g.	Strychnine Recovered g.	Error per cent.
1·40	0·0608	0·0580	−3	1·51	0·02430	0·02320	−4
1·43	0·1210	0·1160	−4	1·47	0·00488	0·00466	−4
1·41	0·0912	0·0850	−6	1·49	0·00365	0·00357	−2
1·46	0·0860	0·0820	−4	1·47	0·00681	0·00710	+4
1·40	0·0950	0·0900	−5	1·52	0·00852	0·00834	−2
1·45	0·1220	0·1190	−3	1·45	0·00511	0·00513	+0·3
1·42	0·1300	0·1250	−4	1·46	0·00340	0·00323	−3
1·42	0·0980	0·0930	−5	1·42	0·00272	0·00256	−4
Average: 1·42			−4	1·47			−1·8

TABLE III

COMPARISON OF THE SENSITIVITY OF THE B.P. METHOD AND THE SPECTROPHOTOMETRIC PROCEDURE FOR THE ESTIMATION OF STRYCHNINE IN TINCTURE OF NUX VOMICA

B.P. procedure				Spectrophotometric method after potassium persulphate treatment			
Strychnine in tincture per cent.	Strychnine Added g.	Strychnine Recovered g.	Error per cent.	Strychnine in tincture per cent.	Strychnine Added g.	Strychnine Recovered g.	Error per cent.
0·119	0·060	0·054	−10	0·130	0·00243	0·00251	+3
0·122	0·085	0·075	−11	0·125	0·00292	0·00275	−5
0·119	0·098	0·092	−6	0·121	0·00339	0·00334	−1
0·126	0·101	0·093	−8	0·125	0·00584	0·00609	+4
0·121	0·076	0·073	−4	0·120	0·00438	0·00440	+0·5
0·119	0·082	0·078	−5	0·122	0·00365	0·00359	−1
0·122	0·086	0·082	−4	0·127	0·00486	0·00490	+0·8
0·119	0·112	0·107	−4	0·130	0·00365	0·00359	−1
0·122	0·094	0·088	−6	0·126	0·00242	0·00236	−1
Average: 0·121			−6	0·125			−0·07

DISCUSSION

The accuracy of estimation of strychnine spectrophotometrically depends largely upon the success of elimination of the interfering ultra-violet-absorbing impurities from the galenical preparation.

This in turn depends upon the method of purification of the galenical. Using chromatographic purification the kind and nature of the adsorbing agent was important in achieving an approximate degree of purification. Pure active alumina was tried and on spectrophotometric examination of the purified galenical after potassium persulphate treatment the spectrum of pure strychnine was obtained. This indicates that the absorbing impurities were completely eliminated. Using activated recovered alumina the absorbing impurities were not completely removed. The lack of absorption of the impurities at 254 mμ is the critical factor in determining the accuracy of the method.

The absorption spectrum of the interfering impurities may be estimated

by subtracting the absorption of pure strychnine from the total absorption of strychnine and impurities in the galenical (see Fig. 2).

The curves A and B were obtained using the average of the experimental data in Table II. The 8 times repeated average with the B.P. method gave 1·42 per cent. of strychnine (the manufacturers order single determination method has 1·50 per cent. of strychnine ± 0·05). That average with spectrophotometric procedure was 1·47 per cent. of

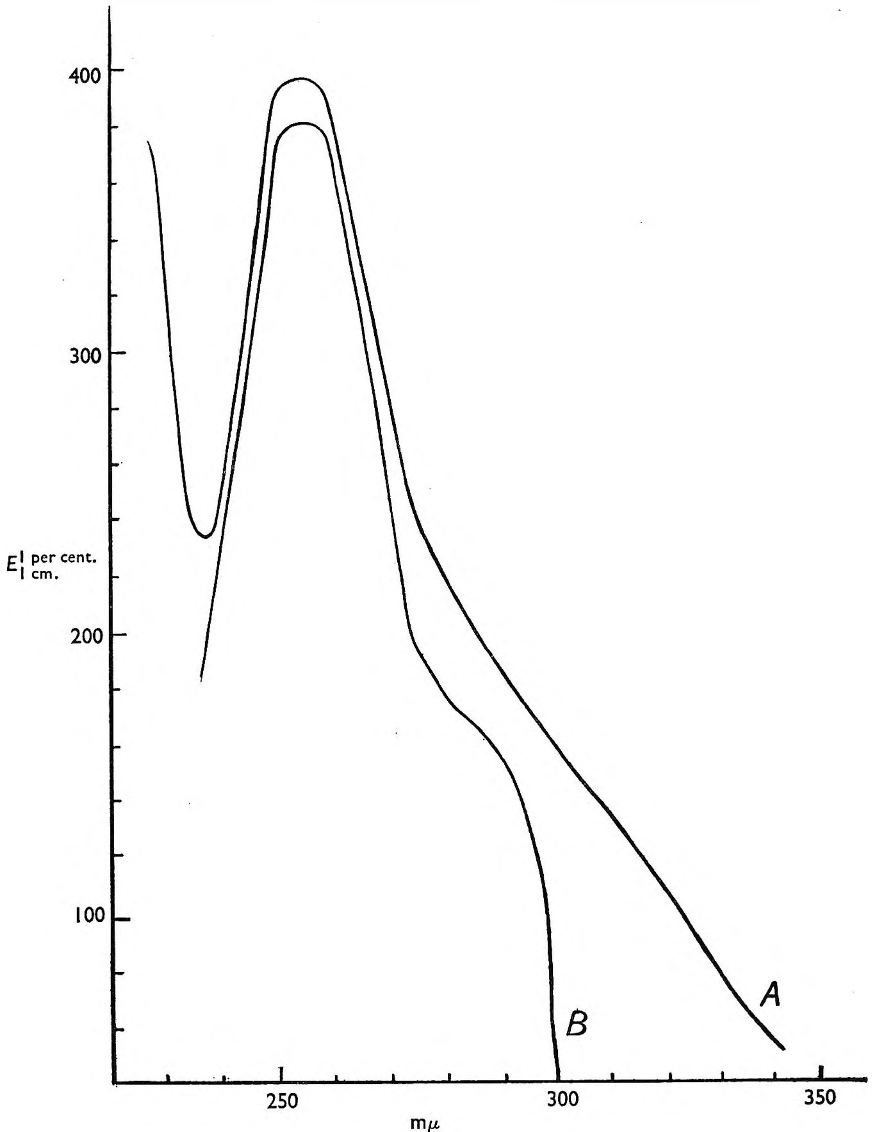

FIG. 2. Absorption curves of strychnine in liquid extract of nux vomica: (A) after chromatographic purification and potassium persulphate treatment; (B) calculated from the B.P. procedure.

strychnine. Taking the average of the B.P. procedure as the minimum value for strychnine, the spectrophotometric method gives 0·05 per cent. higher and will not exceed 2 per cent. error as shown in Table II.

The average extinction at each wav-length for the 8 samples as found by the spectroscopic process after potassium persulphate treatment is plotted in curve A, Figure 2. For curve B the B.P. method was employed to obtain a standard minimum value of the stry-hnine concentration. The difference of absorption between curves A and B (due to ultraviolet absorbing impurities) is plotted against wavelengths as in Figure 3. From the curve it is seen that the amount of absorption due to these impurities is at a minimum at 250 to 260 mμ at which the absorption is maximal.

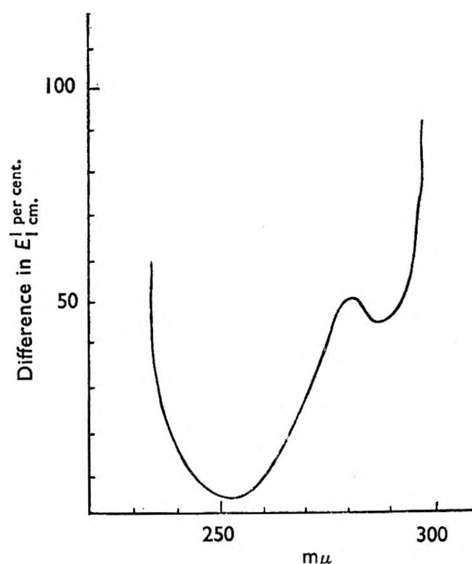

FIG. 3. Absorption spectra of impurities in liquid extract of nux vomica obtained by difference between the average spectrophotometric values and the minimum value by the B.P. procedure.

This means that the ultraviolet-absorbing impurities have a negligible amount of absorption in the region of maximal absorption of strychnine and do not interfere in spectrophotometric determinations of strychnine in galenical preparations.

SUMMARY

1. A modified chromatographic purification of nux vomica galenicals from pigments and resinous substances is described.

2. A new oxidising agent for brucine is recommended.

3. A spectrophotometric procedure for the estimation of strychnine in galenicals is described with an accuracy of not less than 98 per cent.

4. The method is comparatively rapid, and avoids difficulties, such as emulsification, which occur during extraction procedures.

Our thanks are due to Prof. I. R. Fahmy for suggestions and advice, to Prof. R. A. Morton, F.R.S., of Liverpool University, for editorial assistance, and to H. Shaker for his assistance in reading and checking the absorption curves and data.

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THE EFFECT OF BARBITURATE ANÆSTHESIA ON THE BLOOD α-KETO ACID LEVELS IN RATS AND RABBITS

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During a recent investigation[1] into an alleged vitamin A-aneurine synergism[2] it was necessary to determine the blood α-keto-acid (pyruvate and α-ketoglutarate) levels in rats. It is not easy to obtain true resting values because the slightest degree of struggling of the animal results in a considerable increase in these acids.[3,4] To overcome this difficulty cord resection[5] and light barbiturate anæsthesia[6] have been recommended. The latter procedure appeared most suitable for our purpose, but before it was adopted the effect of the barbiturate to be used (pentobarbitone, nembutal) on blood keto-acid levels was investigated in detail. It was found that anæsthesia is accompanied by a considerable decrease in these blood constituents.

Experimental

Animals. (*a*) *Rats.* Adult rats (about 250 g.), previously deprived of food for 18 hr., were injected intraperitoneally with 0·1 ml. of a solution of pentobarbitone containing 1 grain (65 mg.) in 1 ml and held firmly to prevent struggling until anæsthesia was complete. Blood samples were drawn at appropriate times by opening up the thorax, cutting the aorta, and allowing the blood to flow into a small (10 ml.) beaker which had been previously moistened with citrate; this anti-coagulant is recommended because it tends to stabilise the keto-acids.[7] As soon as possible after drawing, aliquots of the blood were delivered into a known volume of trichloracetic acid and the pyruvate and α-ketoglutarate levels determined by a modification[1] of the Friedemann-Haugen method.[8]

(*b*) *Rabbits.* Adult rabbits (about 2·5 kg.), previously deprived of food for 18 hr., were injected intraperitoneally with 1·2 ml. of the solution of pentobarbitone and blood samples drawn at the appropriate time from a marginal ear vein. The blood keto-acid levels were examined in the manner just referred to.[1]

Results

(*a*) *Rats.* Figure 1 records the results obtained in an experiment involving 5 rats. As 2 ml. of blood is required for a duplicate determination of keto-acids, one animal has to be sacrificed for each determination; it is thus not possible to follow the change in levels in a single animal during narcosis; animals were therefore killed at various times after injection of the anæsthetic and the values obtained plotted on the curve reproduced in Figure 1. The resting levels were taken as those obtained as soon as anæsthesia was sufficiently advanced to allow the body wall to be opened; this was generally within 2 to 3 minutes of injection. The assumption

that this gives a true picture of the resting values 9·14 μg./ml. (S.D. ± 2·31) for pyruvate and 5·76 μg./ml. (S.D. ± 2·70) for α-ketoglutarate is justified because these mean values, obtained on 20 rats drawn at random from a stock colony, approximate very closely to the values reported by other workers[9,10] using different methods for obtaining a basal value.

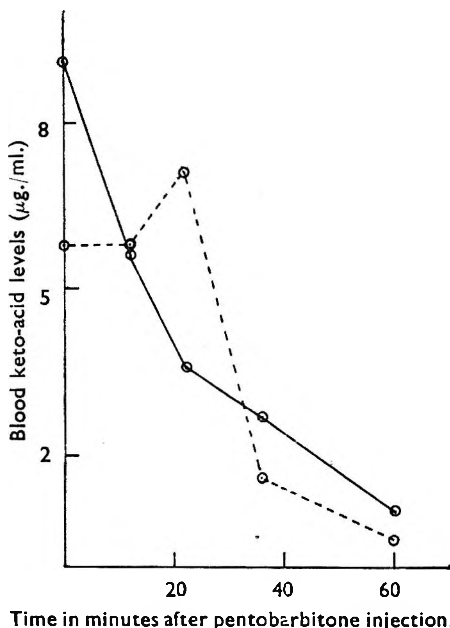

FIG. 1. The fall in the blood pyruvate and α-keto-glutarate levels of rats after intraperitoneal injection of pentobarbitone (0·1 ml.).
——— Pyruvate levels.
– – – – – α-Ketoglutarate levels.

It will be seen from Figure 1 that there is a steady fall in pyruvate levels until, 60 minutes after injection, there is almost no pyruvate in the blood. The α-keto-glutarate follows the same pathway; the slight increase noted 22 minutes after injection cannot be considered significant, when the normal variations (S.D. ± 2·70) are taken into account together with the fact that the accuracy of the determination of this keto-acid is less than that of pyruvate.

(*b*) *Rabbits.* The results obtained for the pyruvate levels of rabbits during anæsthesia are recorded in Figure 2. In this experiment the change in levels could be followed in the same animal, and it will be seen that the same general picture is obtained as with rats. The fall in pyruvate levels continues for 60 to 75 minutes after narcotisation, and 2 hr. afterwards the normal levels had not been restored, although the rabbits were recovering from the anæsthetic. It should be pointed out that the baseline levels for the rabbits were obtained before injecting the barbiturate; the values are somewhat higher than those previously recorded,[9] but in the present experiments no particular efforts were made to prevent the rabbits from struggling. The changes in the α-keto-glutaric levels of the rabbits are not recorded here, but, as in rats, they showed the same general downward tendency.

One experiment was carried out in which a rabbit was first injected with pentobarbitone and immediately afterwards with adrenaline (1 ml. of 1 in 500), which is known to raise blood keto-acid levels considerably.[11,12] 30 minutes later the blood levels of both pyruvate and α-keto-glutarate were about 75 per cent. above their original values; this was in spite of the fact that the animal was deeply anæsthetised.

Discussion

The results presented show quite clearly for the first time that blood pyruvate and α-keto-glutarate levels of rats and rabbits fall rapidly during anæsthesia produced by a typical barbiturate. This is the reverse of the effect observed in the case of blood lactate levels which have been found to rise on the administration of hexobarbitone[13] and thiopentone.[14] Until much more is known concerning the mode of action of barbiturates in tissues other than brain, especially on carbohydrate metabolism, neither the importance nor the significance of this drop in keto-acids can be fully assessed. It does seem, however, that as (*a*) levels are still normal immediately after narcotisation is complete and (*b*) anæsthesia can continue although the levels are above normal (as after the simultaneous administration of the narcotic and adrenaline), the fall in blood keto-acid levels is not directly concerned with either the onset or the maintenance of anæsthesia.

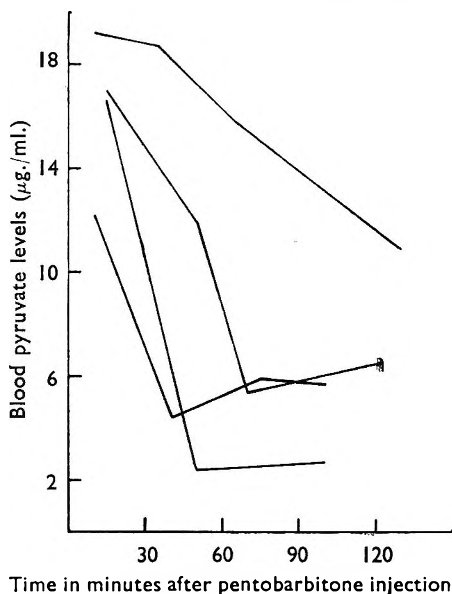

Fig. 2. The fall in the blood pyruvate levels of rabbits after intraperitoneal injection of pentobarbitone (1·2 ml.). Each curve represents a different animal.

The drop in pyruvate levels during narcosis may be caused by the existence of a sub-basal metabolic condition rather than by direct inhibition of glycolysis; the concomitant increase in lactate levels is probably due to inhibition of respiration, the smaller amount of pyruvate formed being reduced to lactate under the increased degree of anærobiosis prevailing, instead of being oxidised aerobically to carbon dioxide and water.

It may be presumed that the reduced levels of α-keto-glutarate in the blood are also caused by the lowered rate of carbohydrate catabolism which must accompany the general functional depression caused by the barbiturate.

Summary

1. The blood pyruvate and α-keto-glutarate levels of rats and rabbits fall rapidly during pentobarbitone-induced anæsthesia.

2. The fall can be counteracted by the simultaneous injection of adrenaline.

Our thanks are due to Prof. R. A. Morton, F.R.S., for his interest in this work, and to the Medical Research Council for a personal grant to one of us (G. R. W.) and for a grant towards laboratory expenses.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Mescaline, New Synthesis of. M. U. Tsao. (*J. Amer. chem. Soc.*, 1951, **73**, 5495.) The cactus alkaloid, mescaline, β-(3:4:5-trimethoxyphenyl)-ethylamine was synthesised by methylating gallic acid to produce the methyl ester of 3:4:5-trimethoxybenzoic acid, which was then reduced to 3:4:5-trimethoxybenzyl alcohol by means of lithium aluminium hydride. The –OH group was replaced by –Cl and then –CN to produce 3:4:5-trimethoxyphenyl-acetonitrile, the reduction of which by lithium aluminium hydride gave mescaline. A. H. B.

ANALYTICAL

Antioxidants in Lard, Estimation of. J. H. Mahon and R. A. Chapman. (*Anal. Chem.*, 1951, **23**, 1116.) Preliminary experiments indicated that a ferrous tartrate reagent for propyl gallate and a ferric chloride plus 1:1′-bipyrine reagent for butylated hydroxyanisole, nordihydroguaiaretic acid, and tocopherol were satisfactory and these reagents were studied more intensively. Procedures were developed for the extraction and spectrophotometric determination of propyl gallate using the absorption at 540 mμ after treatment with ferrous tartrate; butylated hydroxyanisole, nordihydroguaiaretic acid, and tocopherol were determined from the colour produced (at 515 mμ) with ferric chloride 1:1′-bi-pyrine reagent; the methods permitted the determination of all combinations of these four antioxidants except when propyl gallate and nordihydroguaiaretic acid were present in the same sample. Results are given for the extraction and colorimetric procedures recommended; the lower limits of the determinations, were propyl gallate 0·003 per cent., butylated hydroxyanisole 0·005 per cent., nordihydroguaiaretic acid 0·005 per cent., and tocopherol 0·015 per cent.; recoveries ranged from 96·4 per cent. for nordihydroguaiaretic acid in combination with butylated hydroxyanisole, to 98·4 per cent. for nordihydroguaiaretic acid alone. R. E. S.

Ascorbic Acid in Pharmaceutical Preparations, Estimation of. D. G. Chapman, O. Rochon and J. A. Campbell. (*Anal. Chem.*, 1951, **23**, 1113.) Several methods for the estimation of ascorbic acid in the presence of interfering materials such as iron and copper salts have been investigated with special reference to the limitations of these procedures as applied to preparations which may contain other interfering substances. The method of Roe *et al.* (*J. biol. Chem.*, 1948, **174**, 201) based on the reaction between dehydroascorbic acid and 2:4-dinitrophenyl-hydrazine gave a measure of the reduced ascorbic, dehydroascorbic acid, and diketo-l-gulonic acid; results obtained indicated that none of the added materials present in commercial multivitamin products caused interference in the recovery of ascorbic acid, the precision of the method as shown by the small standard deviation being good. The U.S.P. method gave an extremely high recovery of ascorbic acid when ferrous iron was present; cuprous chloride resulted in a low recovery, whereas cupric sulphate caused no interference. The method of Gawron and Berg (*Ind. Engng. Chem., Anal. Ed.*, 1944, **16**, 757) using 8 per cent. acetic acid to extract the ascorbic acid was not affected by the presence of iron but gave low recoveries in the presence of cuprous chloride, cupric sulphate, and

ferrous sulphate plus cupric sulphate. The procedure of Brown and Adam (*J. Sci. Food Agr.*, 1950, **1**, 51) who used a sodium acetate-hydrochloric acid mixture buffered to *p*H 0·65 to overcome the interference due to ferrous iron was satisfactory in the presence of ferrous sulphate and cupric sulphate separately but not if these substances were present together. In the presence of ferrous iron the method of Brown and Adam proved to be most suitable for routine analyses; if both ferrous iron and copper were present, the method of Roe *et al.* was most reliable. R. E. S.

Cadmium Iodide Linear Starch Reagents. J. L. Lambert. (*Anal. Chem.*, 1951, **23**, 1247.) The cadmium iodide-linear starch reagent reported previously was studied to determine its usefulness and potentialities for the colorimetric determination of trace amounts of oxidising substances. The intensity of the starch-iodide blue colour developed in solution was found to be directly proportional to the concentration of the oxidising agent over relatively wide ranges. Potassium bromate was selected as the oxidising agent and the rate of colour development was studied and the extinction coefficient determined. The blue linear starch-iodide colloid was little affected by weak acids, and by strong acids only at high concentrations. High concentrations of polyvalent cations tended to precipitate the blue colloid except in the presence of complexing agents such as tartrates and phosphates. In applying the reagent to a particular procedure it is suggested that the rate of colour development in the solution used should be determined by using the extinction coefficient as described, whereby the straight-line plot of optical density versus concentration can be obtained without redetermining a number of points. The reagent is colourless, unaffected by age, and capable of giving highly reproducible results. This reagent makes possible the first satisfactory utilisation of the extremely delicate starch-iodine reaction for colorimetric work, and has the added convenience of stability. It should make possible the development of a number of new analytical procedures.

R. E. S.

Calcium, Magnesium and Mercury Compounds, Application of the Schwarzenbach Method to Analysis of. H. R. Hernandez, U. Biermacher and A. M. Mattocks. (*Bull. nat. form. Comm.*, 1950, **18**, 145.) Ethylenediaminetetraacetic acid reacts with bivalent metals to give a complex with a low degree of ionisation. Magnesium, however, has less affinity for the reagent than other metals. Magnesium also forms a stable red complex with a dye, Eriochrome black-T, at *p*H 8 to 9 while other polyvalent metallic ions do not affect the blue colour of the dye. In the assay of calcium and magnesium salts the equivalents of about 40 to 50 mg. of Ca. and 30 mg. of Mg. are required. The salts are brought into solution, if necessary with dilute hydrochloric acid. An excess (40 ml.) of a standardised 1 per cent. solution of the disodium derivative of the reagent is then added to the solution and the *p*H is adjusted to about 9 with 10 ml. of an ammonium chloride 6·75 per cent. and concentrated ammonium hydroxide 57 per cent. buffer solution. 6 drops of a methanolic solution containing 5 per cent. of a mixture of the dye (1 part) and hydroxylamine hydrochloride (9 parts) are then added and the excess of reagent titrated with a standardised 0·75 per cent. solution of magnesium chloride. Combination with the dye to give a sharp blue/red colour change occurs when all the reagent is used. For mercury compounds it is more convenient to use reagent and magnesium chloride solution of twice the strength described, using the equivalent of about 40 to 50 mg. of Hg. Digestion of these compounds to obtain solubility can be carried out in most cases in a 250 ml. Erlenmeyer flask, but it was found more convenient to use a 250 ml. Kjeldahl flask with some compounds. In either

case the subsequent titration was performed as described above, in the flask used, about 15 ml. more of buffer solution being required to neutralise any excess of acid used in digestion. J. R. F.

Chloride in Presence of Iodate, Determination of. L. S. Stanton. (*Anal. Chem.*, 1951, **23**, 1331.) The determination of chloride by the Volhard method was found to be unsatisfactory in the presence of iodate, apparently because of the progress of a slow side reaction between iodic acid and sodium thiocyanate. An experimental study was carried out which showed that accurate results were obtained by the Volhard method if iodate was previously removed by the use of barium nitrate. Barium iodate was free filtering, but carbonates and sulphates decreased the filtration rate; more rapid filtration was obtained if carbonates were destroyed by acidification prior to barium iodate precipitation and if the presence of sulphates was avoided. R. E. S.

Chromium, Separation of, from Vanadium. R. K. Brookshier and H. Freund. (*Anal. Chem.*, 1951, **23**, 1110.) The determination of small amounts of chromium in vanadic oxide was investigated; titration procedures were found to be inaccurate and the perchromic acid extraction method gave erratic results. A study of the extraction procedure using ethyl acetate was made and gave the following optimum conditions: pH at equilibrium $1{\cdot}7 \pm 0{\cdot}2$, concentration of hydrogen peroxide 0·02 mole/l., temperature 20° C. or less, and number of extractions 3. The blue perchromic acid decomposed rapidly in aqueous solution and its immediate extraction with ethyl acetate was necessary; the blue substance was stable in ethyl acetate solution for 30 minutes. The following elements did not interfere: iron, mercury, vanadium, titanium, nickel, molybdenum. A standard sample containing 0·68 per cent. of chromium was examined by this procedure; parallel analyses using 0·10 and 0·25 g. samples gave a value of 0·65 per cent. chromium. R. E. S.

Citric Acid and Acetone, New Reaction for. A. di Giacomo and G. Rispoldi. (*Boll. chim.-farm.*, 1951, **90**, 311.) If to a solution of citric acid a few crystals of anthraquinone and twice its volume of concentrated sulphuric acid are added and the whole heated, the anthraquinone dissolves with the production of a yellow colour. On further heating, gas is evolved and then a yellowish-orange to blood-red colour, according to the amount of citric acid present, is formed. As this colour is due to acetone derived from the citric acid, acetone will also give it. A yellowish-orange colour can be obtained with 0·05 mg. and a blood-red colour with 5 mg. Using the dry acid a blood-red colour can be obtained with 1 mg. Tartaric, malic, lactic, benzoic and salicylic acids do not give the reaction. It is given by 0·5 mg. of acetone. H. D.

Methanol in Distilled Spirits, Spectrophotometric Estimation of. G. F. Beyer. (*J. Ass. off. agric. Chem.*, 1951, **34**, 745.) The method of Boos (*Anal. Chem.*, 1948, 20, 964) using chromotropic acid has been investigated. Previously no effort has been made to regulate the amount of ethanol that should be present in order to produce the maximum amount of colour; this amount was found to be 22·0 to 24·0 per cent. in the sample to be oxidised. It was found that heating the solution after the addition of sulphuric acid tended to destroy the violet colour. Spectrophotometric curves are given for the colour produced, the maximum being at 580 mμ. It is claimed that the proposed method is at least as accurate as the modified Denigè's method, and the colour is more stable. R. E. S.

Nitroglycerin Tablets, Rapid Assay of. J. H. Cannon and R. F. Heuermann. (*J. Assoc. off. agric. Chem.*, 1951, **34,** 716.) The assay is a small scale operation with simple apparatus which gives quick results. Powdered tablets are mixed with water and extracted with ether, the ether extract being evaporated after the addition of ethanolic potassium hydroxide solution. Reduction is accomplished with Devarda's alloy and the resulting ammonia is distilled off and determined by titration against 0·01N sulphuric acid. Advantages claimed for the assay are the use of ethanol in the ammonia distillation to prevent frothing, and the absorption of the distilled ammonia in ice-water. The results obtained in 18 assays showed a mean of 6·83, standard deviation from the mean 0·011 or 0·16 per cent. R. E. S.

Phenacetin, Colorimetric Determination of. D. Horn. (*Pharm. Zentralh.*, 1951, **90**, 296.) About 0·1 g. of phenacetin is heated on the water-bath for 5 minutes with 50 ml. of 10 per cent. nitric acid. After cooling, and possibly filtering, the colour is determined at 465 mμ (filter S47). The presence of acetylsalicylic acid, quinine, caffeine, starch or sugar does not interfere with the colour production. Amidopyrine and phenazone give interfering colours, and it is necessary to heat the mixture until the blue colour produced disappears. In this case the solution becomes turbid on cooling, and must be cleared by centrifuging. If the proportion of phenazone is greater than 50 per cent. a preliminary separation is necessary; the mixture being treated with 20 ml. of water and 0·2 g. of tartaric acid and the phenacetin shaken into ether. Although some phenazone also goes into the ether solution, it is not sufficient to interfere with the reaction. G. M.

Phenacetin, Estimation of. A. Casini. (*Annali. Chim.*, 1951, **41**, 611.) Although phenacetin is included in most pharmacopœias none of them gives any method of estimation and even where, as in the U.S.P. and B.P., tablets are tested, this is only done by means of solvents, with no test to show that the residue obtained is really phenacetin. The author therefore investigated various published methods. He found that Weismann's method of determining the acetic acid set free on hydrolysis with hydrochloric acid failed to work, as even after 6 hours boiling only 74 per cent. of the phenacetin was saponified. Miller's colorimetric method using the colour produced by nitric acid in a solution in methanol was neither sensitive nor reliable. Degner and Johnson's colorimetric method, using the colour produced by the addition of chromic acid after boiling with concentrated hydrochloric acid, reading at the wavelength of 543 mμ in a Bechman spectrophotometer gave results within 1 per cent., but spectrophotometers are not usually available in pharmacies. He therefore recommends the use of tetra-iodophenacetin hydriodide, $(C_{10}H_{13}O_2N)_2I_4,HI$. Place 0·3 g. of phenacetin, 6 ml. of glacial acetic acid and 80 ml. of water in a 200 ml. stoppered graduated flask. Warm to about, but not above, 70° C. to get complete solution. Add, with continuous shaking, 50 ml. of 0·25N iodine and then 6 ml. of concentrated hydrochloric acid. Stopper immediately and shake vigorously to encourage the separation of the tetraiodophenacetin. After cooling, make up to the mark with water, mix well and allow to stand for 30 minutes. Filter off 50 ml. through a sintered glass filter and determine the excess of iodine with 0·1N thiosulphate. H. D.

Phenylmercuric or Ethylmercuric Compounds, Determination of. V. L. Miller, D. Polley and C. J. Gould. (*Anal. Chem.*, 1951, **23,** 1286.) A method is given for the direct determination of these compounds in aqueous solution without prior wet oxidation. A solution containing between 50 and

100 μg. of the compound in 0·5 to 20 ml. of water or very dilute acid or alkali is accurately measured into a separating funnel and shaken with 3·5N hydrochloric acid containing hydroxylamine hydrochloride and a definite volume of dilute dithizone solution in chloroform; the chloroform layer is separated and washed by shaking with 3N hydrochloric acid followed by shaking with water. The light absorption of the resulting solution is then determined photoelectrically. The green colour of the unreacted dithizone is determined rather than the yellow of the organic mercury dithizonate; the values for unknown samples are determined by comparison with a standard curve. Details of the procedure are given together with an adaptation for the determination of quantities of organic mercury compound as low as 1 μg. The possibility of interference from copper ions was investigated and is discussed. R. E. S.

Rotenone, Determination of, Using Mercury Acetate. I. Hornstein. (*Anal. Chem.*, 1951, **23**, 1329.) It has been found possible to determine rotenone quantitatively by adding an excess of mercuric acetate in methanol to a solution of rotenone; the *iso*propenyl double bond in the rotenone molecule reacts quantitatively with the mercuric acetate-methanol reagent and for each mole of rotenone 1 mole of acetic acid is formed. Titration with 0·1N sodium hydroxide gives a direct measure of the rotenone present. In the procedure developed, sodium chloride was used to convert excess of mercuric acetate to the chloride and thus permit direct titration of the acid with standard alkali using an excess of phenolphthalein as indicator. Results given showed that the reaction between mercuric acetate and the double bond could be carried out at room temperature and was virtually complete after 15 minutes; the volumetric method gave slightly higher results than the gravimetric method which has been shown to yield results generally about 1 per cent. lower than the correct value. The volumetric procedure appears to be as accurate as the gravimetric method. R. E. S.

Starch-Iodate Reagent, Linear. J. L. Lambert (*Anal. Chem.*, 1951, **23**, 1251.) An examination was made of a colorimetric reagent containing linear A-fraction potato starch, iodate ion, and cadmium ion which, at the proper *p*H showed a selectivity for iodide ion. The reaction of iodate ion and iodide ion to produce triiodide ion which reacts with the linear starch to form the well-known blue complex, gave reproducible and quantitative results. Details are given of the light absorption curves of the linear starch-triiodide ion blue complex and studies were made of the rate of development of the blue colour, and the effect of temperature and of various acids on the colour density. The colourless reagent prepared proved to be stable for periods up to 6 weeks and to be selective for iodide ion when used in a solution of a weak acid such as formic. The presence of small amounts of many common inorganic and organic substances can be tolerated but the peculiar interference of the bromide ion was such that its concentration must be known before using this reagent; large concentrations of bromide ion produced an orange or purplish colour. R. E. S.

Sulphate in Presence of Iron, Determination of. N. Gandolfo. (*Annali. Chim.*, 1951, **41**, 600.) For the accurate determination of sulphate by precipitation with barium, if iron is present it must be removed or some of it will be precipitated with the barium sulphate, but if the iron is precipitated as ferric oxide this is difficult to filter and may carry down some sulphate. If however the iron is precipitated as ferroso-ferric hydroxide, filtration is easy and the precipitate does not carry down sulphate. The solution containing the ferric and sulphate ions is put in a 100-ml. beaker with 2 or 3 ml. of 10 per cent. potassium iodide solution and boiled to drive off part of the liberated iodine, but leaving sufficient

to oxidise part of the reduced iron. This can be done by stopping the boiling as soon as the first violet fumes come off. If it is wished to do this quite accurately, the iron should be determined in a separate sample, and after boiling off the liberated iodine, sufficient 0·1N iodine added to convert two-thirds of the iron to the ferric condition. A freshly prepared 10 per cent. solution of sodium hydroxide is added until a slight turbidity is formed and then an excess of 10 ml. and the mixture is boiled for 2 to 3 minutes, allowed to settle and filtered while still hot. The precipitate is washed and the sulphate determined in the filtrate in the usual way. H. D.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Barley Starch, Constitution of. I. C. MacWilliam and E. G. V. Percival. (*J. chem. Soc.*, 1951, 2259.) The structure of the starch from the barley grain was investigated and found to contain 19 per cent. of amylose. By repeated precipitations with butanol highly purified amylose was obtained. It was methylated by repeated treatments with methyl sulphate and sodium hydroxide in nitrogen, the product was hydrolysed, and the resulting mixture separated on a cellulose column. The main product was 2:3:6-trimethyl-D-glucose, which was accompanied by small quantities of tetramethylglucose and a mixture of dimethylglucoses. The amount of tetramethylglucose was estimated to be 0·24 to 0·29 per cent. of the methylated glucoses produced on hydrolysis, and, corresponds to the presence of one non-reducing terminal group per 400 $\pm$ 40 glucose residues. The fractionation by precipitation with aqueous pyridine saturated with butanol to obtain barley starch amylopectin did not yield a product containing less than 2 per cent. of amylose. Methylation studies, involving the application of paper chromatography, indicated one non-reducing terminal group for 26 $\pm$ 2 non-terminal glucose residues. The application of periodate oxidation confirmed this result and indicated that over 86 per cent. of the branching linkages between the unit chains involved 1 : 6-linkages.

A. H. B.

Hecogenin, A Source of. R. E. Callow, J. W. Cornforth and P. C. Spensley. (*Chem. Ind.*, 1951, **33**, 699.) The steroidal sapogenin, hecogenin, has a 12-keto group in ring C and, as a starting-point for the partial synthesis of cortisone, would be comparable with the bile acids. The sisal plant, *Agave sisalana* Perrine, widely cultivated in East Africa to produce fibre, was examined and the whole leaves were found to contain hecogenin, which can be readily extracted. It was shown that hecogenin could also be extracted from sisal waste, consisting of short fibres and debris. The extraction process is described. Hecogenin was obtained from the crude sapogenin fraction by chromatography on alumina or more simply by removal of a low melting material with hot light petroleum, followed by a Girard separation of the residue. The yield of hecogenin varied from 0·04 to 0·1 per cent. on the air-dry weight of sisal waste.

A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Enzymes, Separation of, by Paper Chromatography. K. V. Giri and A. L. N. Prasad. (*Nature, Lond.*, 1951, **168**, 786.) By the application of the usual paper chromatographic technique, and using aqueous acetone or sodium chloride or suitable combinations of these two as developing solvents, the separation of individual enzymes from mixtures prepared artificially, or obtained from natural sources, such as mould or tissue extracts, was accomplished. The

details of the method for the separation of amylase from the proteinase, trypsin, are described and a photograph of the chromatogram recorded. Neither aqueous acetone nor water alone was able to bring about any appreciable movement of trypsin, but the addition of sodium chloride in concentrations as little as 0·5 per cent. to the aqueous acetone or water caused considerable movement of the trypsin from the starting line. Saline solutions also arrested tailing. A. H. B.

Paper Chromatography on Borate-impregnated Paper. C. A. Wachtmeister. (*Acta chem. scand.*, 1951, **5**, 976.) The possibility of using paper impregnated with borate for the separation of phenols, phenolic aldehydes, phenolic acids and certain sugars was investigated. With any substance, or group of isomeric substances, parallel chromatograms were run using paper impregnated with a sodium borate buffer and, for comparison, a sodium phosphate buffer of approximately the same *p*H 8·7. The buffers were 0·1 M with respect to boric or phosphoric acid. Organic substances capable of forming strongly acidic complexes with boric acid would be expected to show decreased R_F values on borate-impregnated paper as compared with the values on unimpregnated or phosphate-impregnated paper. This proved to be the case, because there was a considerable decrease in the R_F values of phenols and phenolic aldehydes containing two adjacent hydroxyl groups, whereas, on the other hand, compounds with isolated hydroxyl groups as well as *o*-hydroxyaldehydes moved at approximately the same rate on phosphate- and borate-impregnated paper. Phenolic acids containing two adjacent hydroxyl groups or a hydroxyl group in *ortho*-position to a free carboxyl group also had smaller R_F values on borate- than on phosphate-impregnated paper. A good separation of glucose and sorbose was achieved using borate-impregnated paper, although these two sugars apparently move at the same rate in the systems ordinarily used. The preparation of parallel chromatograms on borate- and phosphate-impregnated paper affords a simple micromethod for the detection of borate reactive groupings. A. H. B.

BIOCHEMICAL ANALYSIS

Œstrogens, Chromatographic Separation of. J. F. Nyc, D. M. Maron, J. B. Gorst and H. B. Friedgood. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **77**, 466.) A column of pulverised rubber (vulcanised rubber powder) has been successfully adapted to the quantitatively accurate chromatographic separation of a mixture of crystalline œstrone, œstradiol and œstriol. When 20 ml. each of 20, 40 and 60 per cent. aqueous methanol (v/v) are passed successively through the column, œstriol is eluted by the 20 per cent., œstradiol by the 40 per cent. and œstrone by the 60 per cent. concentration of methanol. Known amounts of a mixture of the three natural œstrogens can be separated sharply from each other and estimated quantitatively with an experimental error of approximately ± 10 per cent. Moreover, it is possible to determine the identity of an unknown œstrogen by the concentration of methanol which will remove it from a rubber column. The method of preparation of the column and the process of selective elution are described. S. L. W.

Vitamin B_{12} and the Animal Protein Factor, Chick Assay of. M. E. Coates, G. F. Harrison and S. K. Kon. (*Analyst*, 1951, **76**, 146.) A method of assay with chicks for vitamin B_{12} and the animal protein factor is described and its accuracy discussed. Comparison of values found by chick and microbiological assay of crude materials containing vitamin B_{12} showed very clearly that the two techniques were not measuring the same thing, it being evident that

values found by the chick tests represented other growth factors as well. Difficulties in obtaining satisfactory responses to crystalline vitamin B_{12} are reported.

R. E. S.

Vitamin B_{12}, Microbiological Estimation of, using the *Lactobacillus lactis* Dorner Cup-Plate Method. W. F. J. Cuthbertson, H. F. Pegler and J. T. Lloyd. (*Analyst*, 1951, **76**, 133.) The cup-plate method for the rapid microbiological assay of aneurine and riboflavine has been found applicable to the estimation of vitamin B_{12} with a suitable strain of *Lactobacillus lactis* Dorner. The procedure involves the use of a medium similar to those found necessary for other strains of lactobacillus; ascorbic acid is needed in the assay medium but not in that for preparation of the inoculum. The effect of a number of factors on the assay have been studied including the maintenance of the organism, the preparation of the medium and the assay plates, the time of incubation, the concentration of vitamin B_{12} in the test samples, and the inoculum density. Methods for the calculation of the results are given together with typical assay figures; on average, fiducial limits of ± 15 per cent. ($P = 0{\cdot}05$) may be expected in assays employing six plates, although narrower limits can be obtained with a greater number of plates. Assays by the plate method and by a tube technique employing *L. leichmannii* 313 have given comparable results. Vitamin B_{12c} and the deoxyribosides interfere with the procedure; the effect of the latter, but not the former, can be eliminated by combining the method with paper chromatography, the presence of deoxyribosides in addition being generally apparent from the type of zone produced. The combined chromatographic and microbiological procedures make it possible, with a $(2 + 2)$ assay design, dose ratios of 10 to 1 and incubation overnight, to attain a satisfactory degree of precision on a few μl. of vitamin B_{12} solution. R. E. S.

Vitamin B_{12}, Microbiological Estimation with *Lactobacillus leichmannii* 313 by the Turbidimetric Procedure. W. B. Emery, K. A. Lees and J. P. R. Tootill. (*Analyst*, 1951, **76**, 141.) Details are presented of a microbiological tube assay for vitamin B_{12} with *Lactobacillus leichmannii* 313 as test organism. The assay method depends on the inability of *L. leichmannii* 313 to synthesise vitamin B_{12} under defined conditions, so that there is a direct relationship between the growth of this organism and the concentration of vitamin B_{12} in the test medium over a certain range. Details of the media required, involving a modification of the process of Snell, Kitay and McNutt (*J. biol. Chem.*, 1948, **175**, 473) are given. Statistical analyses of a $(3 + 3)$ assay and a standard response curve are given and show that the method is sufficiently sensitive and accurate for routine use. R. E. S.

Vitamin D, Analytical Purification by Differential Solubility, Precipitation and Chromatography. J. Green. (*Biochem. J.*, 1951, **49**, 45.) Methods for the quantitative separation of vitamins D from interfering sterols and vitamin A have been developed. The vitamins D could be quantitatively separated from their provitamins and other precipitable sterols by differential solubility in 72 per cent. ethanol followed by digitonin precipitation. Chromatography on floridin earth, under the conditions described, produced an efficient separation of tachysterol and vitamin A from the vitamins D. An examination of acid-washed floridin earth columns showed that, provided the columns contained no free hydrochloric acid, vitamin D could always be recovered quantitatively; tachysterol was destroyed to a large extent by single passage through a column washed with 90 per cent. ethanol, but not if the column was washed with pure ethanol. The complex destruction of vitamin A was conditioned by the acidity

and the moisture content of the column the destruction being probably due to polymerisation. For analytical purposes, the 90 per cent. ethanol-washed column was the most useful, since with one liquid chromatogram, vitamin D could be separated from tachysterol and vitamin A; β-carotene was destroyed on the column and did not interfere. R. E. S.

Vitamin D, Reaction with Iodine Trichloride. J. Green. (*Biochem. J.*, 1951, **49**, 36.) It was found that iodine trichloride in carbon tetrachloride solution reacted with substances containing systems of three or more conjugated double bonds and, in addition, a group of sterols related to the vitamins D, involving chlorination and liberation of iodine. For quantitative studies the vitamin D solution in carbon tetrachloride is placed in a spectrophotometric cell, a specially prepared solution of iodine trichloride is added from a burette followed by carbon tetrachloride to volume, when the resulting colour is measured; this procedure is continued until the addition of more iodine trichloride solution produces no increase in the colour of the resulting reaction mixture. Details of the method and of the precautions necessary to ensure accuracy are given. A number of substances related to vitamin D were subjected to the reaction with iodine trichloride; it was found that although in the case of calciferol only three bonds usually reacted and the side-chain double bond was preferentially unreactive, in ergosterol all three bonds reacted; with lumisterol, two double bonds reacted; the reagent also reacted quantitatively with vitamin A and β-carotene. The titration of many samples of calciferol and vitamin D_3 showed an overall accuracy of ± 5 per cent. R. E. S.

CHEMOTHERAPY

Thyroxine, Biological Action of Substances Related to. J. H. Wilkinson, M. M. Sheanan and W. F. Maclagan. (*Biochem. J.*, 1951, **49**, 710.) It has been shown (Sheanan, Wilkinson and Maclagan, *Biochem. J.*, 1951, **48**, 188) that 3:5-diiodo-4-hydroxybenzoic acid shows a slight antithyroxine activity when tested in mice by the oxygen consumption method. Esterification produced a considerable enhancement of activity which was maximal when the *n*-butyl ester was used. A series of 15 3:5-diiodo-4-alkoxybenzoic acids and their esters have now been tested similarly, but all were markedly inferior to *n*-butyl-3:5-diiodo-4-hydroxy benzoate. 4 branched-chain alkyl esters of 3:5-diiodo-4-hydroxy benzoic acid were much less active than straight chain esters. 3:5-diiodo-4-methoxybenzonitrile showed slight antithyroxine activity, but 3:5-diiodo-4-*n*-propoxybenzonitrile and the corresponding benzamide were inactive. J. R. F.

Tubercle Bacilli, Bacteriostatic Action of Certain Compounds on. R. de Fazi and G. Berti. (*Annali Chim.*, 1951, **41**, 621.) The authors give details of the methods of preparation of a large number of esters of *p*-aminobenzoic acid and of *cyclo*hexyl esters of various aromatic acids and results of tests of their action *in vitro* on *Myco. tuberculosis.* The most active compound studied was cinnamyl *p*-aminobenzoate, next phenylethyl *p*-aminobenzoate and β-decahydronaphthyl-*p*-aminobenzoate. Then *cyclo*hexyl *p*-aminobenzoate, benzyl-*p*-aminobenzoate and *cyclo*hexyl *p*-aminocinnamate. *Cyclo*pentyl *p*-aminobenzoate and *cyclo*hexyl salicylate were next and then the di*cyclo*hexyl ester of *N′* (*p*-carboxyphenyl) glycine and the *cyclo*hexyl ester of *N* (*p*-carboxyphenyl) glycine. *p*-Amino*cyclo*hexane carboxylic acid and the amide of hexahydrobenzoic acid had slight activity and *N*-dodecyl *p*-aminobenzoate none. H. D.

Tuberculosis, Chemotherapy of. *N*-Substituted 4-Aminodiphenyls. L. Bauer, J. Cymerman and W. J. Sheldon. (*J. chem. Soc.*, 1951, 2342.) The aim of the investigation was to attempt to discover any correlation between basicity and antibacterial activity in a series of *N*-substituted derivatives of 4-aminodiphenyl and β-naphthylamine. Concurrently two other effects were to be examined: (*a*) variation in lipoid solubility, and (*b*) possession of optimal flat surface area. The compounds, *N'*-4'-diphenyl-*N N*-diethylethylenediamine, *N*-4'-diphenylyl-2-morpholinoethylamine, 4-2'-diethylaminoethoxydiphenyl, and the highly basic 4-diphenylylguanidine and -diguanide, were prepared. Other compounds were obtained by the condensation of 4-aminodiphenyl with *p*-hydroxy-*p*-dimethylamino, *p*-nitro, and *p*-methoxy-benzaldehyde, and these Schiff's bases were hydrogenated at atmospheric pressure in the presence of Adam's catalyst to give the corresponding substituted benzylamino-compounds.

A. H. B.

PHARMACY

NOTES AND FORMULÆ

Methaphenilene Hydrochloride (Diatrine Hydrochloride). (*New and Non-official Remedies: J. Amer. med. Ass.*, 1951, **147**, 862.) Methaphenilene hydrochloride is *N*: *N*-dimethyl-*N'*-(α-thenyl)-*N'*-phenylethylenediamine hydrochloride. It is a white or pale yellow, crystalline powder with a faint odour, m.pt. 184° to 189° C., soluble in water, sparingly soluble in ethanol and chloroform, and almost insoluble in ether. A 2 per cent. aqueous solution has *p*H 4·8 to 5·6 and yields the free base as an oil on the addition of alkali and a pink precipitate with Reinecke's salt. A solution in sulphuric acid is light yellow at first but becomes light orange on standing and cherry red on the addition of a 1 per cent. solution of isatin in sulphuric acid. Methaphenilene hydrochloride loses not more than 0·5 per cent. of its weight when dried at 110° C. for 4 hours, yields not more than 0·15 per cent. of sulphated ash, and complies with a limit test for heavy metals. It contains 9·2 to 9·6 per cent. of nitrogen (determined by the Kjeldahl method), and 99 to 101 per cent. of methaphenilene hydrochloride when assayed by adding an excess of silver nitrate and titrating with ammonium thiocyanate. It is also assayed by precipitating the dipicrate by the addition of a saturated solution of trinitrophenol containing sulphuric acid and contains 98 to 102 per cent. of methaphenilene hydrochloride. A 0·001 per cent. solution in ethanol exhibits ultra-violet absorption maxima at 2450 Å ($E^{1\text{ per cent.}}_{1\text{ cm.}}$, 537 ± 5), and 2900 Å, and a minimum at 2840 Å. Methaphenilene hydrochloride is a histamine antagonist. The average adult dose is 50 mg.

G. R. K.

PHARMACOGNOSY

Ergot Mycelium, Effects of Nitrogen compounds on Growth and Alkaloid Biosynthesis in. S. K. Sim and H. W. Youngken. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 434.) A study has been made of the growth and alkaloidal production (as ergotoxine) in the mycelial tissues of *Claviceps purpurea*. When grown in modified culture media ergot alkaloids were found in small amounts in all the mycelial cultures examined. When these cultures were agitated mechanically the dried weight of the mycelia increased by some eightfold, but this increase in growth was accompanied by a marked decrease in alkaloidal content. On the other hand the addition of small quantities of indole, ornithine and tryptophane to a standard culture medium caused a decrease in mycelial growth with some increase in alkaloidal content. The addition of arginine, and of ammonium sulphate, caused no significant responses in either growth or alkaloidal content in mycelial tissues.

S. L. W.

PHARMACOLOGY AND THERAPEUTICS

Antidiuretic Activity: A Method of Assay. M. Ginsberg. (*Brit. J. Pharmacol.*, 1951, **6**, 411.) Groups of 18 rats, previously conditioned to water administration by stomach tube, after being deprived of food for 18 hours but denied free access to water for the last hour only, were placed in metabolism cages, and urine collected in graduated cylinders. Each rat was then given 2 doses of tepid water by stomach tube (each dose = 5 ml./100 g. of bodyweight), with 1 hour between each dose. 1 hour after the second dose the total volume of urine excreted in the preceding 2 hours was measured (V_2), and a third dose of water was then given and the test material injected subcutaneously. The urine output was then measured at intervals of 30 minutes for 120 or 150 minutes. The volume of urine, V_2 was expressed as a percentage of the amount of water administered in the first 2 doses. Those rats from which this value deviated from the mean for the whole group by more than 33 per cent. of the mean were rejected. 30 minutes was allowed for complete absorption of the material injected. The following formula was used:

$$\text{Percentage water excretion} = \frac{V_t - V_{30}}{3V_1 - (V_2 + V_{30})} \times \frac{100}{1}$$

where V_1 = the volume of water administered in each dose.
V_2 = the volume of urine excreted in the 2 hours before injection.
V_{30} = the volume of urine excreted in the first 30 minutes after injection.
V_t = the volume of urine excreted during the period of "t" minutes after injection (t = 60, 90, 120 or 150 minutes).

That is, the percentage water excretion at a given time is the volume of urine passed from 30 minutes after injection expressed as a percentage of the water administered but not excreted 30 minutes after injection. Solutions for injection are prepared in 0·9 per cent. sodium chloride solution, from 0·2 to 0·4 ml. being the amount usually injected. In each assay two dose levels of standard (vasopressin) and unknown are used; the ratio, high to low dose, being the same for standard and unknown. The error may be estimated from the results and the assay can be completed in 4 to 5 hours. "Unknown" amounts of vasopressin have been assayed against vasopressin standards; the mean standard error of 12 assays was 8·9 per cent. A total "unknown" activity equivalent to 10 milliunits is sufficient for a satisfactory assay. S. L. W.

Bacitracins A, B and C, Biological Properties of. G. G. F. Newton, E. P. Abraham, H. W. Florey, N. Smith and J. Ross. (*Brit. J. Pharmacol.*, 1951, **6**, 417.) It has been shown that crude preparations of bacitracin contain at least three polypeptides which are active against certain bacteria, as well as a number of other polypeptides showing no antibacterial activity. It has been hoped that the nephrotoxicity of commercial bacitracin might be due to impurities rather than to the antibiotic itself. The observations recorded in this paper, however, make it reasonably certain that this nephrotoxicity is due, at least in part, to the antibacterially active constituents, and that there is little hope of obtaining a preparation of bacitracin which does not damage the kidneys. For therapeutic purposes it does not appear to be worth trying to separate bacitracin A from bacitracin B, since although the latter is somewhat less toxic than the former it is less potent against certain bacteria. The data at present available suggest that bacitracin C has a greater acute toxicity than the other two bacitracins. S. L. W.

Cation Exchange Resins: Optimal Potassium Content for Clinical Use. E. W. McChesney. (*J. Lab. clin. Med.*, 1951, **38**, 199.) It has been substantiated that each g. of resin ingested by a human subject prevents the absorption of from 1 to 1·5 meq. of both sodium and potassium. The dose of resin generally used is about 50 g. per day, and when taken in conjunction with a diet of moderate sodium content this dosage usually gives prompt relief of œdema. The principal complications resulting are acidosis and potassium deficiency. The acidosis is not considered undesirable *per se*, but the potassium deficiency, which occurs quite frequently, leads to general weakness. This is particularly likely to occur on low sodium diets since the cation exchange resins have greater affinities for potassium than sodium, and large fæcal losses of potassium may occur. *In vivo* studies on rats indicate that, for clinical use, the optimal potassium content of both carboxylic and sulphonic types of resins lies between 1 and 2 meq./g. A daily intake of 12·5 g. of K-form and 37·5 g. of NH_4-form carboxylic resin (containing a total of 90 meq. of potassium) would be capable of binding about 1 g. of sodium in the intestine, would assure a positive potassium balance and would result in very little net change in acid-base balance. Such a combination would neutralise about 4 l. of gastric juice of normal acidity. A daily intake of 12·5 g. of K-form and 37·5 g. of NH_4-form sulphonic resin (containing a total of 52 meq. of potassium) would bind about 1·3 g. of sodium in the intestine, would assure a positive potassium balance and would give a small net alkali loss. Such a combination would neutralise about 1600 ml. of gastric juice of normal acidity.

S. L. W.

Cephalosporin P_1, Biological Properties of. A. C. Ritchie, N. Smith and H. W. Florey. (*Brit. J. Pharmacol.*, 1951, **6**, 430.) Cephalosporin P_1 is an antibiotic from a species of *Cephalosporium*. The cephalosporin used in this investigation was lyophil-dried material prepared by chromatography and countercurrent distribution between solvents. Cephalosporin P_1 inhibits the growth *in vitro* of staphylococci, corynebacteria and *Cl. tetani* at considerable dilution, but has little effect on streptococci, Gram-negative organisms or tubercle bacilli. Penicillin-resistant staphylococci are sensitive to the drug. Serum reduces but does not abolish its action. By repeated culture staphylococci are made resistant to its action *in vitro*. In concentrations of more than 1:200,000 it is bactericidal; in weaker concentrations it is bacteriostatic. It has the same order of activity against staphylococci as aureomycin and terramycin. Intravenously in mice it is less toxic than aureomycin or terramycin. No gross or microscopic lesions were found *post mortem* in mice given 5 mg. 12-hourly by mouth for 5½ days. The drug did not affect the blood pressure of a decerebrate cat but modified the contractions of isolated guinea-pig uterine muscle. It is well absorbed by mouth or by subcutaneous injection. It disappears from the blood rather quickly, though little is excreted in either urine or bile. After oral administration serum levels are reached and maintained greater than those necessary to inhibit the staphylococcus *in vitro*, yet mouse experiments show that while it has some protective actions against staphylococcal infections this action is much weaker than that of aureomycin or terramycin.

S. L. W.

Chloramphenicol, Serum Concentrations following Intravenous and Intramuscular Injections. J. J. Burnell and W. M. M. Kirby. (*J. Lab. clin. Med.*, 1951, **38**, 234.) This study was undertaken to test the toxicity of a new preparation of chloramphenicol (chloramphenicol 25 per cent. in aqueous acetyldimethylamine 50 per cent.) and to measure serum concentrations following

parenteral administration. When administered intravenously in the concentrated form (0·5 g. of chloramphenicol in 2 ml.), 5 of 10 patients complained of pain along the course of the vein, and in 6 patients the veins subsequently thrombosed. A fall in blood pressure occurred in 3 of the patients. When the concentrated solution was diluted in 100 ml. of saline or glucose solution, and administered by intravenous drip over a period of 15 to 30 minutes to 45 patients very few toxic reactions were observed, but in 2 patients receiving intravenous injections twice daily the antecubital veins thrombosed after 6 days. 6 patients receiving 1 g. intramuscularly tolerated the preparation well and did not complain of pain. Following a single intravenous injection of 1 g. of chloramphenicol serum concentrations averaged 20 μg./ml. at 1 hour, and gradually declined to an average of 2·4 μg./ml. at 24 hours. Following 0·5 g. intravenously concentrations fell from 10 μg./ml. at 1 hour to 3·8 μg./ml. at 12 hours. Comparing levels following oral and intravenous administration, serum concentrations were slightly higher with the intravenous route at 3 hours, but were twice as high at 12 hours, and persisted for a longer period than with the oral route. Intramuscular injections did not give adequate serum levels for therapeutic purposes. It would appear that when oral administration of chloramphenicol is not possible, 1 g. every 12 hours intravenously will provide effective therapy.

S. L. W.

Hyoscine and Anti-histamine Compounds in the Prevention of Seasickness. E. M. Glaser and G. R. Hervey. (*Lancet*, 1951, **256**, 749.) A controlled and crossed over experiment was made at sea in which 68 healthy volunteers (soldiers) were in turn given 1 mg. of hyoscine hydrobromide, 25 mg. of diphenhydramine hydrochloride (benadryl), 25 mg. of promethazine hydrochloride (phenergan), and a placebo. Of those who might otherwise have vomited 96 per cent. were protected by hyoscine, 61 per cent. by promethazine and 30 per cent. by diphenhydramine. If nausea and vomiting are considered together, the figures were 77, 65 and 30 per cent. respectively. All the drugs were remarkably free from side-effects in the doses given. These findings strongly confirm previous observations that hyoscine hydrobromide effectually prevents seasickness in a large number of people and that 1 mg. is a safe dose. Hyoscine did not prevent nausea with equal success, but since it was unquestionably more effective in preventing vomiting there can be no doubt that it would be the preferred drug. Hyoscine caused a feeling of dryness in the mouth in most subjects, but headaches, dry mouth, giddiness and drowsiness are all symptoms of seasickness which were present in a number of untreated men.

S. L. W.

Procaine Amide and Dibenzylmethylamine in Experimentally Produced Ventricular Tachycardia. R. Charlier and A. Klutz. (*Arch. int. Pharmacodyn.*, 1951, **87**, 241.) Administration of 500 mg. of procaine amide to 4 anæsthetised dogs with aconitine-induced ventricular tachycardia restored the sinus rhythm, but the action, lasting for a maximum of 10 seconds before remission, was of a much shorter duration than that produced by *p*-oxyphenylethanol methylamine (sympatol) of 47 minutes and by 1 : 3-oxyphenyl-1-oxy-2-ethyl-aminoethane (M.I.36) of 145 minutes duration. Experiments using 6 dogs, with aconitine-induced ventricular tachycardia, injected intravenously with dibenzylmethylamine 5 to 10 mg./kg. of body weight showed that this drug had no effect on the tachycardia in 4 of the animals, gave activity in one, and doubtful results in the other animal.

J. R. F.

Procaine Amide in Cardiac Arrhythmias. H. Miller, M. H. Nathanson and G. C. Griffith. (*J. Amer. med. Ass.*, 1951, **146**, 1004.) Of 55 patients who received 250 to 500 mg. of the drug intravenously the cardiac arrhythmia was modified in 46. Of 31 with frequent ventricular systoles the ectopic beats were eliminated in 26, the effect normally occurring in 1 minute after administration. Chronic auricular fibrillation was not modified in 11 cases, in 6 of these ventricular premature beats also present, were eliminated. 3 out of 4 cases of supraventricular tachycardia were restored to sinus rhythm by the drug. The fact that the ventricular beats are more consistently affected suggests the drug has a sympathetic blocking action. No untoward effects occurred following rapid intravenous administration but no definite advantage was observed. It was demonstrated that arrhythmias could be controlled by oral administration. The drug was well tolerated and untoward reactions infrequent and of a mild nature. J. R. F.

BACTERIOLOGY AND CLINICAL TESTS

Hexachlorophen Soap, Bactericidal Properties of. H. C. Jacobsen. (*Pharm. Weekbl.*, 1951, **86**, 733.) Hexachlorophen (2:2′-dihydroxy-3:5:6 : 3′:5′ : 6′-hexachlorodiphenylmethane) satisfies all the requirements for use in the preparation of a bactericidal soap. It is stable under these conditions, non-toxic and non-irritating, and is colourless, odourless and non-volatile. At a concentration of 1 : 10^6, with an alkaline reaction, it is fatal to *Staphylococcus aureus* in 1 minute at 37° C. When soap is present, however, the action is much slower, concentrations of up to 0·5 per cent. of hexachlorophen requiring about 5 minutes in presence of appreciable quantities of soap. The lethal concentration of the compound, in water, towards *Bact. coli* is 1 : 330; and towards *Salm. typhi*, 1 : 500. In these latter cases the soap alone destroys the bacteria, and this is also the case for a number of other Gram-negative organisms. The value of this soap for disinfecting the hands thus appears somewhat dubious, since the "resident" flora of the hands, which is difficult to remove by soap, is protected by the soap from the action of the disinfectant. On the other hand, hexachlorphen is absorbed on the skin and may be detected some days after washing with liquid soap containing this compound. Regular use of the soap is therefore more effective and has been shown to reduce the number of bacteria on the skin. In carrying out culture tests with *Staph. aureus*, it is necessary to take into consideration the great sensitivity of this organism to hexachlorophen, and it is best to make an initial dilution of one drop of the solution under test with 0·2 to 0·5 ml. of sterile horse serum, which destroys the activity to a large extent. The bacteriostatic action of hexachlorophen is detectable, in ordinary bouillon, at 1 part in 8×10^6. G. M.

Octyl Cresol—A New Germicide. D. Boocock. (*Mfg. Chem.*, 1951, **22**, 308.) Octyl cresol is a mixture of octylated *meta*- and *para*-cresol with up to 10 per cent. of the *ortho* compound and containing traces of hydrocarbons and other alkyl phenols. It is a viscous transparent fluid with a faint odour, almost insoluble in water and sodium hydroxide solution, but miscible with most organic solvents. The bacteriostatic activity of octyl cresol against *Staph. aureus* and *Bact. coli* was compared with a number of other germicides. Its Rideal Walker coefficients against *Staph. aureus* and *Salmonella typhi* are 480 and 10 respectively. Its toxicity is low. Because of its powerful germicidal and fungicidal action, it has many potential uses, and its applications, in particular its uses as germicidal fogs or thermal aerosols, are described. A. H. B.

BOOK REVIEWS

THE PLANT GLYCOSIDES, by H. J. McIlroy. Pp. 125 and Index. Edward Arnold and Co., London. 1951. 18s.

The author presents an up-to-date, concise account of plant glycosides, which is not intended to be comprehensive. After a brief general treatment of the subject, the book is divided into sections dealing with natural glycosides of alcohols and phenols, cyanogenetic glycosides, thioglycosides, phenyl benzo-pyrone glycosides, anthocyanins, coumarin glycosides, saponins, phytosterol and solanum alkaloids, cardiac glycosides, anthraquinone glycosides and the nucleosides. A chapter on "Recent Advances" brings "the survey of available literature up to October, 1950." Each chapter records brief details of the glycosides and includes an extensive bibliography. The extensive use of structural formulae, especially in the description of the proof of structure and synthesis of a few types of glycoside, help to relieve the book from appearing as a catalogue of compounds. It is stated that "it is not intended that this work should be treated as more than an up-to-date summary for convenient reference," and if this is borne in mind the book can be said to fulfil the intentions of its author. A. H. BECKETT.

ENZYMATISCHE ANALYSE, by Hermann Stetter. Pp. 196 and Index. Verlag Chemie GMBH, Weinheim. 1951. DM.17.50.

Enzymatic analysis is becoming increasingly important in the investigation of materials of natural origin. The extremely high specificity of enzyme-substrate reactions, alone, enhances the value of this type of analytical procedure as compared with other, more general, analytical methods. This book presents for the first time a comprehensive account of this branch of analysis, covering the literature up to the beginning of 1950. The author has been careful to point out the distinction, which it is necessary to make, between microbiological analytical methods, involving the use of enzyme systems still within a living organism, and those methods in which an enzyme system is separated in solution from the living organism before use. It is only this latter class of analytical operation which falls within the scope of the book, though both qualitative and quantitative methods are described.

The subject matter has been divided into three sections according to the type of analytical procedure used. The first and largest section includes those methods which involve the use of substrate specific enzymes. Such methods depend upon the principle that the action of a specific enzyme on the substance under test results in the formation of some product, which can be estimated by a standard analytical procedure. Enzyme groups, the use of which are described in this section, include esterases, carbohydrases, aminases, proteases, dehydrases and certain oxidases. Methods are described for the estimation of such important biological materials as ascorbic acid, citric acid, co-carboxylase and various glycosides and amino acids. The utility of the book is much increased by the inclusion, in this and the other sections, of practical details for the conduct of all the more important estimations.

The second and much smaller group of methods includes all those in which the substance to be analysed either inhibits or activates enzyme action. Typical of these methods is the estimation of such alkaloids as eserine, morphine and diamorphine by means of the inhibitory action which they exert on the action of cholinesterase. The third section describes methods in which the enzyme is used as an indicator. The temperature, for example, at which a particular

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PHARMACOPŒIAS AND FORMULARIES

PHARMACOPŒA INTERNATIONALIS, Editio Prima, Volumen I.

By Professor D. M. Dunlop,
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The publication by the World Health Organisation of the Pharmacopœa Internationalis is a landmark in the progress of scientific international relations. It represents the fulfilment of long felt aspirations to unify the pharmacopœias of the world. It has been realised since about 1874 that the pharmaceutical Tower of Babel which existed as regards standards, terminology, strengths and composition of drugs was, as in other spheres, a source of confusion, misunderstanding and even of danger. A long step has been taken by the publication of the present volume to resolve these national differences though some still remain: for example, two strengths for the tinctures of digitalis and opium are recognised, probably the result of the impossibility of reconciling differences of long-established national habits in treating liquids by volume or by weight; and the limit test for lead and the limit test for heavy metals are both included in this pharmacopœia which thus perpetuates the differing analytical practices in this country and in the United States. The volume has a very British flavour about it, which is a tribute to the influence which must have been exerted by the very British Chairman of the Expert Committee which dictated its policy. Nevertheless, a considerable list could be made out of preparations which have different strengths in the International Pharmacopœia from those in the British Pharmacopœia. The book is beautifully produced and printed, though the typographical innovation which omits a full-point after "g" and "ml" but retains it after "cent." is, as Gibbon once said of Venice, "singular if not pleasing," as is the confusing habit of giving a dose as 0·0005 g instead of 0·5 mg. Incidentally, is there any point in giving an oral dose for adrenalinum? Since it is the stated object of the International Pharmacopœia only to include drugs of *established* therapeutic interest, as well as some substances used for diagnostic and pharmaceutical purposes, it is surprising that it should have been thought worth while to include aconitinum, bromoformium, and lobelini hydrochloridum. Hyoscyami mutici herba is also included which is not itself a medicinal agent but is only used as a source of hyoscyamine and atropine, but other monographs on the botanical material from which alkaloids, such as pilocarpine, are extracted, are not included. The title thiopentalum natricum cum natrii carbonate gives to thiopentone a pedantic accuracy which chiniofonum is perhaps fortunate to escape. Lastly, the simple doctor may find it difficult to recognise the familiar chloramine under the formidable title of tosylchloramidum natricum. These are, however, perhaps captious criticisms of what is an important and admirable work, the production of which can only have been achieved by patient compromise and general co-operation.

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enzyme becomes inactivated is characteristic, and measurement of the activity of an enzyme can be used to establish the temperature to which a particular product has been heating during the course of its preparation. The inclusion, in the preface, of a table of substances which may be estimated by methods of enzymatic analysis, is most useful. Numerous references to original papers are given throughout the text.

J. B. Stenlake.