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REVIEW ARTICLE

ANALGESICS: SOME DEVELOPMENTS

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Introduction

At a time which Dale has called the golden age of therapeutics, when chemotherapy is making one spectacular advance after another, it is still important to remember how much of medicine is concerned with the treatment of symptoms. The relief of pain will of course follow the cure of the disease, but this may be slow while the pain is urgent and the ætiology still obscure. Analgesics may be defined as drugs which reduce or relieve the sensation of pain without producing loss of consciousness or parallel depression of other senses. Thus general anæsthetics, while used at times, as in labour, for escape from pain are not true analgesics nor are they desirable in the everyday treatment of pain.

Local anæsthetics are also now of established value in the treatment of such localised trouble as fibrositis, and skilled and experienced practitioners can often give remarkable and sustained relief by such injections. There has been some use of local anæsthetics intravenously in the relief of pain. This is difficult to justify pharmacologically, for these drugs, apart from cocaine, which is manifestly unsuitable for such application, are rapidly cleared from the blood-stream and fail to follow the Hughlings Jackson " law " in their action. Instead of the usual descending paralysis produced by narcotics—descending in the sense that the most recently developed and highly specialised functions of the central nervous system are the first to be depressed—intravenous local anæsthetics are quite liable to paralyse respiration in doses which scarcely affect spinal reflexes. It is true that analgesics similarly break the " law." Adrian[1], while doubting whether pain is as much appreciated at the level of the thalamus as Head maintained, agrees that since . . . " Pain needs no learning to increase its potency. This must be due to a direct effect on the basal ganglia." Analgesics should therefore act at this level as on the cerebral cortex, but while there is presumptive evidence of selective effects, there is need for something like tracer technique to establish real localisation of drug action. Probably by introducing a radioactive isotope into the analgesic molecule, the concentration of the drug could be estimated in the tissues even in great dilution, and its fate followed.

Methods of testing the potency of analgesic drugs are numerous. Where long series of related compounds have to be compared, some animal screening is first necessary. The promising drugs may then be assessed comparatively on human volunteers, as in a recent paper by Prescott *et al*[2]. Probably even such results in man require checking by

experience in the actual relief of human suffering before much weight can be attached to them.

Screening tests have been carried out on all sorts of laboratory animals, and a variety of painful stimuli have been tried of which heat in one form or another has been most popular because of the precision with which it can be repeated and measured. It is important that the intensity and duration of the stimuli should be such as to cause no tissue damage, since such would inevitably lead to changes in threshold. Fortunately there is no need for stimuli of such severity. In assessing possible analgesic value the toxicity of the proposed remedy and any side-actions which it may elicit must be taken into account, but it may not be possible to appreciate such considerations till clinical trials are instituted. In man, heat may be applied, till pain is felt, by the use of a resistance coil or by focusing the light of a powerful lamp on a fixed area of blackened skin. Other methods preferred by some workers for the study of analgesic action consist of assessments of the modification of the pain which the drug affords when pain is elicited by injections of hypertonic saline or by muscular contraction in an ischæmic limb. These and other methods are listed with numerous references in the recent chemical review of the synthetic analgesics by Bergel and Morrison[3]. There is abundant evidence that there is liable to be a large psychological element and a substantial individual variation in all such assessments, so that rigorous controls are necessary.

While analgesics have been shown to be very active when applied to the mid-brain directly, in quite small doses, the precise mechanism of their action is still unknown. Unlike most narcotics, analgesics have little effect on the oxygen consumption of the brain slice and little effect on choline-esterase systems, but they may block the availability of amino acids or other essential metabolites to certain nerve-cells.

Opiates

Opium has been used in the relief of suffering for at least three thousand years. It is nearly 150 years since morphine was isolated from opium. Yet as recently as 1938 Fourneau[4] claimed that morphine and a few of its derivatives could alone be considered true analgesics. The coal-tar antipyretics, widely used for certain nerve and muscle pains, seemed so far behind opium in the relief of pain associated with organic disease that Fourneau suggested they be called "antalgics," while he called cannabis and the related tetrahydro-cannabinols "euphorigenics," euphoria being the most striking part of the effects they normally produce. The hemp drugs have a definite analgesic action in animals but, rather curiously, increase in dosage does not enhance this analgesic effect. (Davies, Raventos and Walpole[5].) They have a long history in therapeutics, and the synthetic compounds may bring them again to the fore (Macdonald[6], Avison, Morrison and Parkes[7]). While cannabis is scheduled with the dangerous drugs and is known to be a frequent drug of addiction it is claimed to be free from any such risk in therapeutics, but its applications there have been so wide that one hesitates

to accept them. Its value in the amelioration of mood in mental disorders appears to be established.

The literature of the opiates is enormous, and no attempt to review it can be included here. The United States Public Health Services cover it in two large volumes (Kreuger, Eddy and Sumwalt[8]). In experimental animals, the action of opium and of total opium alkaloids is substantially the action of the morphine they contain. Potentiation by the other alkaloids is hard to demonstrate, and so is potentiation by neostigmine, though this has been claimed. Such use of a choline-esterase inactivator is the more revolutionary in that for many years it has been customary to give atropine or hyoscine with morphine to reduce its side-actions if not to enhance its analgesic effects. Many experienced physicians use morphine almost exclusively.

Many workers have published tables in which the analgesic activity of a series of drugs is assessed numerically in terms of morphine. Such tables may be misleading. Here let it be stated that the ideal analgesic is not established by a claim that it is, say, six times stronger than morphine. Morphine is usually strong enough. What is wanted is a drug which has morphine's anxiety- and pain-relieving qualities together with less or none of its undesirable side actions. Morphine lies open to criticism in that it is liable :—

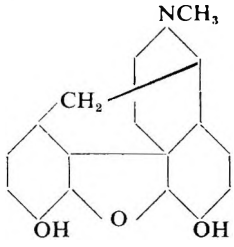
(1) to depress respiration.
(2) to produce nausea and vomiting.
(3) to be constipating.
(4) to produce tolerance and the chance of addiction.
(5) to increase itching and skin irritation.
(6) to be dangerous in susceptible subjects and young children.

A substance like codeine (methylmorphine) though it has only $\frac{1}{6}$ or less of the analgesic power of morphine, is often preferred because of its relative freedom from these side-actions. Diamorphine (diacetylmorphine) on the other hand, though a powerful and reliable analgesic, leads to habit formation so frequently and so quickly that its manufacture and importation are forbidden in the United States. There is a considerable movement to ban it similarly here, because of recent evidence of increased consumption and increasing addiction in various other countries. Mono-acetylmorphine is about four times as active as morphine when assessed by the methods of Hardy and Wolff (irradiation of blackened skin) or Smirk and Alam (pain produced by exercise of ischæmic limb), yet produces only " a moderate euphoria," much less nausea and vomiting, and allows of increased voluntary muscular effort in the presence of severe pain.

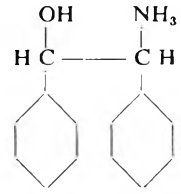
Dihydromorphinone (Dilaudid), and methyldihydromorphinone (Metopon), are in the diamorphine class or better as regards relieving pain. They are effective in the late stages of malignant disease, and good by mouth, but their actions are short and they may certainly provoke addiction.

The attempts to modify the structure of morphine in such a way as to relieve it of its side-actions but retain its analgesic value cannot be

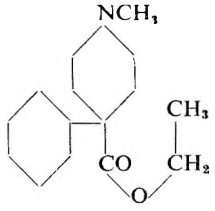
said to be strikingly successful though much research has been directed to this problem. Where the chemist has been successful in increasing the analgesic action by his manipulations, his product has usually shown increased toxicity, though not always a parallel increase in the two actions. As a rule the increase in potency is accompanied by a decrease in duration of action. Many regard the search for an analgesic which is not a potential drug of addiction as futile, but there is a sustained effort to find a morphine substitute which is less depressant to the respiratory centre. The failure so far to synthesise morphine in spite of attempts by many distinguished chemists has probably been a major difficulty but Gulland and Robinson[9] here and Grewe[10] in Germany have made marked progress. There is an alternative method of investigation—to try and identify the basic part of the morphine molecule with which analgesia is associated and then test various chemical modifications of this for potency.



Morphine.



β-Hydroxy-α:β-diphenylethylamine.



Pethidine.

This is the technique so successfully followed by Dodds[11] in the development of the synthetic œstrogens. In the case of morphine he believed that diphenylethylamine was the core of its efficiency, and tested 18 compounds by the Hardy-Wolff and other techniques. Peak activity was found in hydroxydiphenylethylamine, which gave "complete relief in doses of 200 to 400 mg. four hourly." At first this looked promising but later it appeared that many forms of pain were uninfluenced by these drugs though in malignant disease they were claimed to be of special value. In comparative tests in animals they show no significant activity.

Pethidine

Pethidine was introduced (Eisleb and Schaumann[12]), not as an analgesic but as a spasmolytic. Its original name was Dolantin, and it was introduced here as Dolantal, in America as Demerol. Its ability to

relieve pain was discovered later, and its rather remote chemical relationship to morphine suggested. Though as an analgesic it seemed to be in the codeine rather than in the morphine class, (Woolfe and Macdonald[14]), the facts that it had any real pain-depressing activity and that it provided a convenient molecule for modification by the synthetic chemist gave a fresh impetus to research in analgesia. Pethidine was the best of Eisleb's compounds as assessed by Schaumann[13]. A long series of related compounds synthesised by Bergel and his team were assessed pharmacologically by Macdonald and Woolfe[15]. Some had a longer action on mice than pethidine and some had a slightly stronger action—notably 2'-methylpethidine—but although certain of these derivatives received some clinical encouragement (Glazebrook and Brantwood[16]) the differences were not sufficiently great to be very important. Since then an ethyl ketone, Hoechst No. 10720 (ketobemidone) has been claimed to be ten times as active as pethidine, and has had a promising clinical trial (Kirchhof[17]).

Pethidine is of established value in relieving the pain of labour, and this may be related to its additional action as a spasmolytic. When combined with gas and air in doses of 100 mg, to 200 mg., however, it is reported to double the incidence of asphyxia neonatorum and the same risk is recorded on a much larger series when used with trichlorethylene[18].

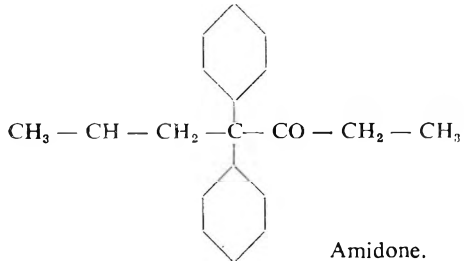
Is it therefore concluded that the routine use of pethidine by midwives cannot be approved. Whether the disadvantages of pethidine can be avoided in the new compound ketobemidone (Hoechst 10720) or in some of the heptanones or hexanones is still uncertain. The demand for a safe and reliable analgesic for use in the conduct of labour is insistent, and the use of inhalers for nitrous oxide or trilene without premedication seems to be reasonably satisfactory in some eighty per cent. of cases. This problem is now receiving widespread attention.

Amidone and Related Compounds

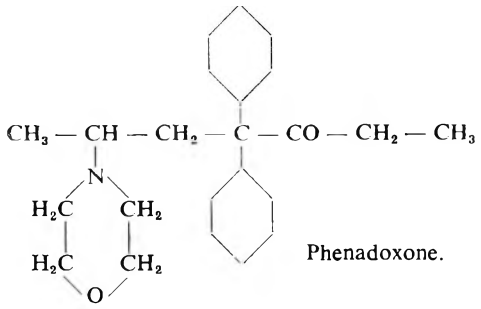
Amidone (Hoechst 10820) also known here as miadone and physeptone, and in America as methadon, adanon and dolophine, was revealed during the investigation of I. G. Farbenindustrie[19] at the end of the war. This compound again stimulated a fresh outburst of research. It was found to be at least as powerful an analgesic as morphine, yet less hypnotic. Its use however is often complicated by prolonged nausea and vomiting, and today it may be more important as a source of new compounds which may retain analgesic efficacy yet be free from these unpleasant side actions. Of such *iso*amidone, which was discarded by the original Hoechst workers has had favourable reports both here and in America[2,20] and 2-dimethylamino-5-acetoxy-4 : 4-diphenylheptane and the 2-morpholinopropyl compound (C.B. 11) are relatively free from unpleasant toxic effects.

It is a pity to find the latter (Phenadoxone, Heptalgin,) advertised as " activity six times that of morphine " even if certain animal tests confer such a ratio. It is much more important to be assured that the acute

toxicity is relatively much lower and that side effects apart from mild drowsiness with full dosage are negligible. Relative freedom from serious respiratory depression and constipating action is freedom indeed.



Amidone.



Phenadoxone.

Wilson and Hunter[21] comparing amidone, phenadoxone, and pethidine found that 5 mg. of amidone only relieved ischæmic pain in six of ten subjects, whereas 5 mg. of phenadoxone relieved nine of the ten. Both were better than 50 mg. of pethidine, but this was strikingly more euphorigenic than the newer drugs.

The optical isomers of amidone have been prepared (Thorp *et al.*[22]) and compared with the racemic forms (Thorp[23]). The lævo-isomer is responsible for the effects of amidone on the central nervous system, while the dextro compound shares equally in the spasmolytic, local anæsthetic and toxic actions on the circulation (but not on the respiration) in experimental animals.

No important recent developments have taken place in Fourneau's " antalgics." Amidopyrine is probably still the most potent but has lost favour because of its occasional effects on the bone-marrow. Aspirin is still the most widely used, yet phenacetin is regarded by critical observers as a more effective drug, though little used by itself. These two, in combination with a little codeine, at present enjoy an enormous vogue but there is some doubt whether the claimed " potentiation " in such mixtures will bear pharmacological scrutiny.

The barbiturates which were at one time claimed to be analgesic as well as hypnotic have failed to live up to any such claim except in anæsthetic doses—the use of the shorter-acting compounds as intravenous anæsthetics is undoubtedly a major advance. The barbiturate-antalgic combination, so bitterly opposed in the past by Willcox, has been

restricted by the inclusion of barbiturates in Schedule IV of the Poisons Act and by the wide publicity given to the toxic risks of amidopyrine which is similarly scheduled. Wayne[24] has recently emphasised the risks involved in the abuse of such drugs.

Most new analgesics, whether related to morphine or not, have been introduced as "free from morphine's tendencies to produce tolerance and addiction." None has seriously stood up to critical tests of such claims—perhaps it is too much to expect of an analgesic. But the advances in this field in the past ten years are full of hope and promise, and whether an approximately ideal drug will be provided by the acetylated alcohols corresponding to the ketones of the amidone group, by some other derivative or in some quite different way, it will surely be found in due course.

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RESEARCH PAPERS

THE ANTIDOTAL ACTIVITY OF SOME DITHIOLS AND ACETYLDITHIOLS IN MICE POISONED WITH OXOPHENARSINE

By M. Weatherall

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Received June 30, 1949

It is well known that dimercaprol (2:3-dimercaptopropanol, BAL) and other dithiols reduce the toxicity of compounds of arsenic to animals and enzyme systems (Peters, Stocken and Thompson[1]; Stocken and Thompson[2]; Fitzhugh, Woodard, Braun, Lusky and Calvery[3]; *et al.*). A number of 1:2-dithiols and three α:ω-dithiols not previously studied have been examined for toxicity and anti-arsenical activity, and the results are reported here. Some of the dithiols were prepared from the corresponding acetylated dithiols, and as it seemed possible that deacetylation might occur in the body, the activity of the acetylated dithiols was also examined. In order to have an adequate basis for this study, the activity of dimercaprol and dimercaprol glucoside (BAL-Intrav) was first explored in some detail.

The compound of dimercaprol and oxophenarsine is more toxic than oxophenarsine alone (Peters and Stocken[4]; Friedheim and Vogel[5]), and the toxicity of this compound has been examined further. Its high toxicity suggests that circumstances may exist in which dimercaprol potentiates oxophenarsine poisoning. This possibility has been examined, as has the possibility of potentiation by other dithiols.

Methods

Mice weighing 17 to 25 g. were used. They were kept at a temperature between 20° and 24°C. during and after experiments. They were fed on a standard diet of cubes of the following composition:—wheat bran, 19·2 per cent.; wheat ground middlings, 19·2 per cent.; Sussex ground oats, 19·2 per cent.; ground maize, 9·5 per cent.; ground barley, 9·5 per cent.; meat and bone meal, 9·5 per cent.; skim milk powder, 7·0 per cent.; fish meal, 4·8 per cent.; dried yeast, 1·3 per cent.; cod-liver oil 0·4 per cent.; salt mixture, 0·4 per cent. (manufacturer's figures). Food was withdrawn on the evening before experiments, and was restored immediately after treatment. Except where it is otherwise indicated, all poisons were injected into the muscles of the right hind leg, and all thiols into the muscles of the left hind leg. Doses were adjusted according to body-weight, and have been expressed in mg.-molecules/kg. (mM/kg.) to facilitate comparison between the amounts of poison and antidote used. The mice were observed at least 1, 2, 4, 8, 20 to 21, 28 to 32

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and 48 hours after injection in most experiments, and approximate estimates of the survival time were made from these observations. Deaths were rare after 48 hours, and were not included in estimates of the mean survival time or of the mortality. The mice were watched until four weeks after the day of injection. With one exception discussed below, late deaths occurred seldom and erratically, and have not been reported as their significance is doubtful. Post-mortem examinations and weighing of organs, when performed, were carried out within an hour of death. The preparation and chemical properties of the dithiols and acetylated dithiols used (Table V) have been or will be reported elsewhere (Evans and Owen[6]; Evans, Fraser and Owen[7]). Some of the free dithiols (dimercaprol glucoside, 0:16, 0:17, 0:19, 0:20 and 0:24) were prepared from their barium salts, and the solutions so obtained were standardised by titration in acid solutions at 0°C. with iodine (Weatherall and Weatherall[8], and *infra*). The other substances were received pure or nearly pure and were generally used freshly dissolved or suspended in olive oil or peanut oil. Dithiodulcitol was dissolved in aqueous sodium hydroxide, and the solutions were neutralised with boric acid. Certain acetylated dithiols (0:11, 0:14) which were difficult to dissolve in oil or in propylene glycol were dissolved in diethylene dioxide and the volumes injected were kept below 1·0 ml./kg. Otherwise the volumes injected were 10·0 ml./kg. in toxicity tests and 5·0 ml./kg. each of dithiol and arsenical in antidotal tests. The doses reported have been corrected for purity. The organic solvents in the volumes used were not lethal and had no detectable anti-arsenical activity.

Results

1. The toxicity of oxophenarsine.

The LD50 of oxophenarsine by subcutaneous injection in mice is given as 0·164 mM/kg. by Ercoli and Wilson[9]. Cranston, Clark and Strakosch[10] give the value 0·17 mM/kg. for intraperitoneal injection and cite the manufacturer's figures of 0·1 mM/kg. for intravenous and about 0·12 to 0·13 mM/kg. for subcutaneous injection. Eagle, Hogan, Doak and Steinman[11] found an LD50 of 0·165 mM/kg. intraperitoneally. Data obtained in the present series of experiments are collected in Table I and show that the mortality after intramuscular injection varied over the range 0·08 to 0·20 mM/kg. with an LD50 of about 0·14 mM/kg. In two experiments in which oxophenarsine was injected intraperitoneally in some mice and intramuscularly in others there was no significant difference in the mortality on any dose.

Death occurred more rapidly when the doses of oxophenarsine were large, and even with the rather crude measurements of survival time, in the range studied the means of the logarithms of the survival times were approximately linearly related to the logarithm of the dose in groups in which there were no survivors (Fig. 1 and Table I). When some animals survived, the mean survival time tended to be less than the value expected for a mortality of 100 per cent. An arbitrary correction for survivors

might be used, but it is doubtful whether such a procedure would be helpful in interpreting the results.

The weight of the lungs was measured in some of these mice. The lungs were substantially heavier than normal in mice poisoned with a small dose of oxophenarsine, and only slightly heavier when the dose was large (Table I). As the mice poisoned with a small dose lived longer, it seems probable that the development of heavy lungs depended

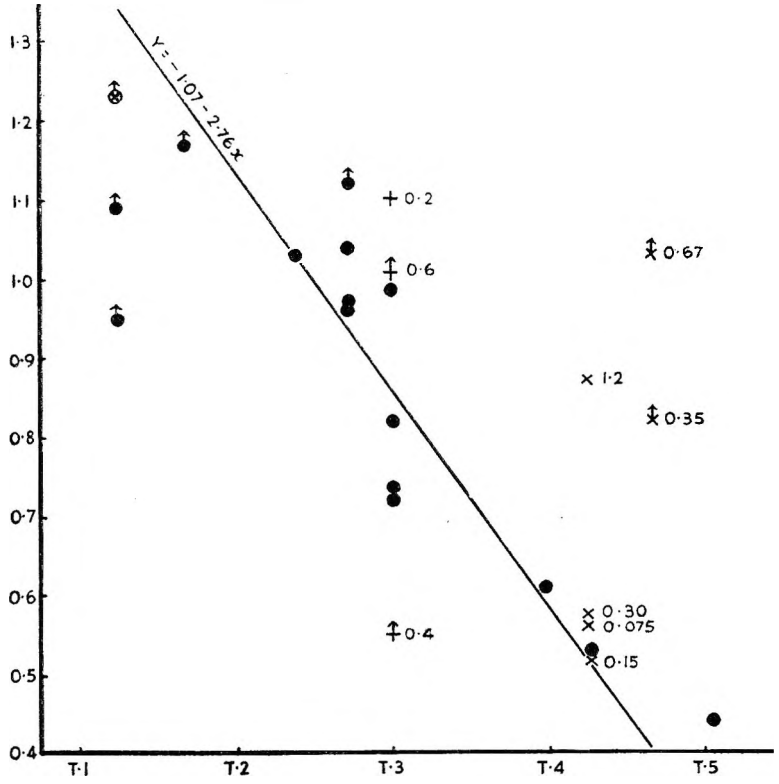


FIG. 1. The relation between log dose and log survival time in mice poisoned with oxophenarsine.

Ordinates—Survival time in log. hours.
Abscissae—Dose of oxophenarsine in log. mM/kg.

Each point represents a group of 5 to 10 mice, all of which died. Each arrow represents a group of 5 to 10 mice of which at least half died. No allowance has been made for survivors in calculating the mean survival time.

● No treatment
\+ Treated with dimercaprol
× Treated with dimercaprol glucoside
} The figure against the point gives the dose of dithiol in mM/kg.
⊗ Injected with an approximately equimolar mixture of oxophenarsine and dimercaprol glucoside

The regression line is that which fits best the points for oxophenarsine alone ($Y = -1{\cdot}07 - 2{\cdot}76x$).

The doses of dimercaprol glucoside are approximate.

TABLE I

THE TOXICITY OF OXOPHENARSINE GIVEN BY INTRAMUSCULAR INJECTION IN MICE

Dose of oxophenarsine	Total mortality No. dying/total No.		Log survival time of mice dying		Lung weight of mice dying	
			Mortality	Log Hours M±S.E.	Number of Mice	mg./g. body wt. M±S.E.
mM/kg.	Normal Mice*	per cent.				
0			—	—	10	6·6±0·32
0·067	0/15	0	—	—	—	—
0·080	4/9	44	4/9	1·25±0·19	2	12·2±2·46
0·093	1/5	20	—	—	—	—
0·107	2/8	25	—	—	—	—
0·113	4/19	21	3/10	0·94—	—	—
0·120	6/21	29	5/15	1·05±0·15	4	10·0
0·133	26/48	54	7/10	1·09±0·10	—	—
			16/20	0·95±0·07	—	—
			2/8	1·32	—	—
0·147	15/30	50	4/10	0·94±0·10	4	8·2±0·66
			8/10	1·17±0·10	—	—
0·153	8/20	40	5/10	1·23±0·13	—	—
0·160	15/18	83	—	—	—	—
0·173	15/17	88	10/10	1·03±0·08	—	—
0·178	6/15	40	5/10	0·95±0·11	—	—
0·187	38/43	89	6/6	0·96±0·10	—	—
			9/9	0·97±0·16	—	—
			10/10	1·04±0·09	10	6·6±0·42
			8/10	1·12±0·10	—	—
0·200	80/82	98	10/10	0·82±0·12	9	7·9±0·39
			10/10	1·00±0·09	8	7·1±0·37
			8/8	0·72±0·18	5	7·9±0·41
			10/10	0·73±0·16	—	—
0·250	34/34	100	10/10	0·61±0·10	—	—
0·267	16/16	100	10/10	0·53±0·03	—	—
0·320	10/10	100	10/10	0·44±0·01	—	—

* Killed by breaking neck.

on the length of life, but conclusive evidence on this point was not obtained.

II. The toxicity of the oxophenarsine-dimercaprol compound.

As was shown by Peters and Stocken[4], when oxophenarsine and dimercaprol are mixed in equimolar amounts before injection or given as the previously crystallised compound, the toxicity is substantially enhanced. This has been confirmed for the pure compound given by intramuscular injection (Table II), but it is interesting to note that in rats and mice it is not true when the compound is given intraperitoneally. In these circumstances the compound is actually less toxic than oxophenarsine, the toxicity of which is about the same by either route. After intramuscular injection of just lethal amounts of the compound, the mice died if anything more rapidly than mice poisoned with a (rather larger) just lethal amount of oxophenarsine. Their viscera were unusually congested and their lungs hæmorrhagic and substantially heavier than normal. After intraperitoneal injection some mice had convulsions and died in a few minutes: others survived for a few hours and at death tended to show less pulmonary congestion. Rats showed the same series of symptoms and difference in toxicity by intramuscular and intraperitoneal injection, and again the toxicity of oxophenarsine by either route was intermediate. Deaths and survivals observed after different dosages, in all in a dozen rats, do not suggest that the toxicity in rats is greatly different from that in mice.

III. The effect of dimercaprol in oxophenarsine poisoning.

The mortalities when various doses of dimercaprol have been given at various times after various doses of oxophenarsine are shown in Table III. In Figure 2 the dose of oxophenarsine has been plotted against the

TABLE II

THE TOXICITY OF THE OXOPHENARSINE-DIMERCAPROL COMPOUND AND OF MIXTURES OF OXOPHENARSINE AND DIMERCAPROL GLUCOSIDE IN MICE

Substance	Dose mM/kg.	Route of administration	Mortality No. dying/total No.	Log survival time of mice dying Log hours M ± S.E.	Lung weight of mice dying No. of Mice	Lung weight of mice dying mg./g. M ± S.E.
Oxophenarsine : dimercaprol compound	0·013	Intramuscular	0/5	—	—	—
	0·027		0/5	—	—	—
	0·040		2/20	—	2	14·2
	0·053		2/5	—	—	—
	0·067		16/20	0·87 ± 0·049	14	14·0 ± 0·80
	0·107		4/5	—	—	—
	LD50 = 0·055 mM/kg.					
	0·013	Intraperitoneal	0/5	—	—	—
	0·027		0/5	—	—	—
	0·053		0/5	—	—	—
	0·107		0/5	—	—	—
	0·120		1/5	—	1	11·6
	0·213		0/5	—	—	—
	0·233		2/9	—	2	14·7
	0·300		5/9	0·50 ± 0·186	5	12·0
	LD50 = 0·295 mM/kg.					
Oxophenarsine D.G. Mixture approx 3 : 1	0·133*	Intramuscular	16/20	1·15 ± 0·08	7	8·1 ± 0·59
Oxophenarsine D.G. Mixture approx. 3 : 4	0·133*		11/20	1·23 ± 0·09	7	8·5 ± 0·31
Oxophenarsine alone	0·133		16/20	0·95 ± 0·07	6	7·7 ± 0·53

* mM/kg. of oxophenarsine in mixture.

dose of dimercaprol with different symbols for mortalities below and above 50 per cent. A line has been drawn to separate the two sets of points, and therefore gives approximately the LD50 of combinations of the two substances. As the LD50 of dimercaprol alone is of the order of 1·0 mM/kg., 0·60 mM/kg. approaches the largest dose which can be used therapeutically without itself causing death. This dose is quite effective against 0·30 mM/kg. of oxophenarsine, and still has some effect even when it is given 80 minutes after a smaller dose of the poison; but these are about the limits of its activity. These findings are in reasonable agreement with those of Ercoli and Wilson[9] under slightly different conditions. Mice which were treated with dimercaprol but did not survive generally lived longer than untreated poisoned mice. This point has been explored further, as reported elsewhere (Weatherall and Weatherall[8]), and slight but just significant acceleration of death has been observed when very small doses of dimercaprol were given to mice

poisoned with very large doses of oxophenarsine. With equivalent quantities of poison and antidote, or an excess of antidote, only prolongation of life has been observed.

Observations have not been made on the mortality from sublethal doses of oxophenarsine treated with less than one equivalent of dimercaprol, but no potentiation was observed by one equivalent (Table III). Poisoned

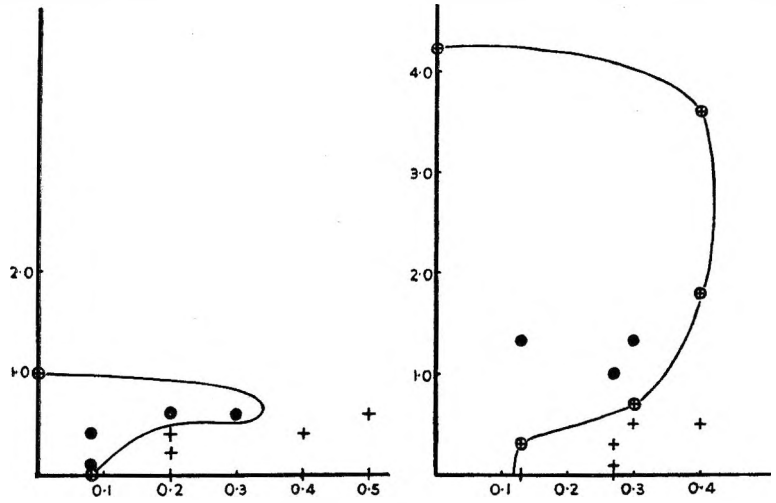


FIG. 2. Isobols for oxophenarsine and dimercaprol (left-hand graph) and for oxophenarsine and dimercaprol glucoside (right-hand graph).
Ordinates—Dose of dithiol in mM/kg.
Abscissae—Dose of oxophenarsine in mM/kg.
Each point represents the mortality for a group of mice.
● Less than 50 per cent. mortality.
⊕ Approximately 50 per cent. mortality.
\+ More than 50 per cent. mortality.
—— Line separating points where the mortality is less than and more than 50 per cent.

mice treated with dimercaprol had heavier lungs than had untreated mice poisoned with the same dose of oxophenarsine. Dimercaprol itself increases the lung weight slightly, and this increase may be an additive effect. The lungs were not heavier than those of untreated mice poisoned with less oxophenarsine and living about as long as the mice treated with dimercaprol: so if the increase in lung weight depends on the time of exposure rather than directly on the dose of oxophenarsine, the increase is not appreciably affected by dimercaprol. In any case, the great increase in lung weight observed in poisoning with oxophenarsine-dimercaprol was not observed when the two substances were given separately, and attempts to imitate its toxicity in this way have been practically unsuccessful.

IV. The toxicity of dimercaprol glucoside and its effects in oxophenarsine poisoning.

As dimercaprol glucoside has not been purified and isolated and the only materials available for biological work have been solutions pre-

TABLE III

THE MORTALITY OF MICE POISONED WITH OXOPHENARSINE AND TREATED WITH DIMERCAPROL OR DIMERCAPROL GLUCOSIDE

Treatment, minutes after oxophenarsine	Immediate							Im-med.	20 minutes	30 minutes	40 minutes	80 minutes	160 minutes
Dose of oxophenarsine mM/kg.	0·08	0·13	0·20	0·27	0·30	0·40	0·50			0·18—0·20			
No treatment	4/9	—	8/8	—	—	10/10	10/10			5/5			
Dimercaprol :—													
0·08 mM/kg.	2/9	—	—	—	—	—	—	—	—	—	—	—	—
0·20 ,, ...	—	—	8/8	—	—	—	—	—	—	—	—	—	—
0·40 ,, ...	0/9	—	6/9	—	—	10/10	—	—	—	—	—	—	—
0·60 ,, ...	—	—	1/8	—	2/9	—	10/10	3/5	2/5	—	2/5	4/5	5/5
No treatment	—	24/30	—	24/24	—	—	—			10/10			
Dimercaprol glucoside :—													
ca 0·08 mM/kg.	—	—	—	10/10	—	—	—	—	—	—	—	—	—
ca 0·30 ,, ...	—	10/20	—	10/10	—	—	—	—	—	—	—	—	—
ca 0·50 ,, ...	—	—	—	—	9/10	10/10	—	—	—	—	—	—	—
ca 0·70 ,, ...	—	—	—	—	4/9	—	—	—	—	—	—	—	—
ca 1·00 ,, ...	—	—	—	11/24	—	—	—	—	0/10	—	4/5	—	—
ca 1·33 ,, ...	—	7/20	—	—	0/10	—	—	0/10	2/10	6/9*	10/10	10/10	10/10
ca 1·80 ,, ...	—	—	—	—	—	5/10	—	—	—	—	—	—	—
ca 3·60 ,, ...	—	—	—	—	—	5/10	—	—	—	—	—	—	—

* Dose of oxophenarsine 0·33 mM/kg.

pared from the barium salt, itself of uncertain purity and always with less than the theoretical thiol content, figures for toxicity are necessarily approximations and depend on what criterion is used in calculating the concentration of the solution used. Danielli, Danielli, Mitchell, Owen and Shaw[12] and Danielli *et al.*[13] give figures based on the weight of the barium salt used per 100 ml. of solution prepared, and allow only for the replacement of the barium atom by two hydrogen atoms. In the present series of experiments it has been found that the toxicity of different batches, measured in these units, varies very widely, and that more constant values are obtained when the solutions are standardised by iodine titration at the time the toxicity is determined and the doses are measured in accordance with the titres. Iodine titration at room temperature gives a rapidly fading and rather arbitrary end-point, and better results are obtained in N hydrochloric acid at 0°C. This was not at first appreciated, and in early experiments solutions were standardised by a modification of Sampey and Reid's method[14] for monothiols, by adding excess of iodine, leaving overnight, and estimating the excess with sodium thiosulphate. This procedure gave a better defined end-point than direct titration at room temperature, but it subsequently appeared that with dithiols in these conditions the reaction proceeded beyond the disulphide stage, to a variable extent according to the amount of iodine present. In most of the indirect titrations, the excess of iodine was within the limits of 50 to 100 per cent. excess, and when further titrations were carried out under these conditions in parallel with direct titrations in N hydrochloric acid at 0°C., the indirect titre varied only between 2·5 and 3·5 times the direct titre. When only an indirect titration was performed and the excess of iodine was not outside the above limits, the strengths of solutions have been calculated on the assumption that the direct titre would have been one-third of the indirect, and are regarded as rough approximations. It has been assumed throughout that, in the direct titration, one gramme-molecule of iodine is equivalent to one gramme-molecule of dimercaprol glucoside.

Data about various batches of dimercaprol glucoside are given in Table IV, where the doses are expressed (A) as derived from the amount of barium salt used in making the solution and (B) from iodine titration. The dose-mortality curve was determined in one experiment with a large number of mice, and as the estimated slope of the line relating log dose to probit mortality was in good agreement with less accurate estimates obtained in other experiments, the LD50 was sometimes deduced from the mortality on a single dose by the use of this slope.

It will be seen that, whereas the most toxic solution (OB1) was about seven or eight times as lethal as the least toxic (142) when the concentrations were calculated from the amount of barium salt used, the difference was not more than two to threefold in terms of the iodine titre. If solutions prepared from specimen SO.1443 are not considered, the toxicity in terms of the iodine titre is reasonably constant. Specimens SO.1422 and SO.1443 were both somewhat unsatisfactory and de-

teriorated rapidly, possibly because they had not been freed sufficiently from traces of organic solvents (Owen, personal communication), and it seems reasonable to attribute the consistently higher toxicity of solutions prepared from SO.1443 to the presence of impurities. In the other solutions, toxicity is evidently attributable to the free thiol present, as might be expected; and the material which does not react with iodine, presumably chiefly products of oxidation, does not contribute much to the toxicity. The sulphydryl content may also be assumed to regulate the anti-arsenical activity, and so all doses in the present work have been

TABLE IV

THE TOXICITY OF SOLUTIONS OF DIMERCAPROL GLUCOSIDE

Specimen of barium salt used in preparation	Soln. No.	Dose mM/kg. A	Mortality No. dying/total No.	Slope of line relating log dose and probit	LD50 mM/kg. A	LD50 mM/kg. B
—	3	17·5 14·0 11·2	7/10 3/10 0/10	11·0	15·7	3·8
S0·1001	40	35·2 31·2 28·9	4/5 1/5 3/5	—	31·0	*ca* 4·7
S0·1001	48	29·5	10/10	—	<29·5	<*ca* 3·5
S0·1229	108	21·0 18·6 16·6 14·7 13·1 11·8 10·5 9·5	20/20 20/20 20/20 15/20 7/20 5/20 3/20 3/20	10·7	12·8	*ca* 4·4
S0·1422	142	46·7	8/15	—	45·0	4·5
S0·1443	0B1	10·5 7·9 5·2	8/8 8/8 2/16	—	5·9	2·1
S0·1443	143	10·1	8/15	—	9·7	2·6
S0·1443	144	23·8	5/15	—	25·6	2·1
9374A	241	14·0 12·6	14/15 9/15	12·5	11·9	—

(A) Calculated from the amount of barium salt used in preparing the solution.
(B) Calculated from the iodine titre of the solution (see text).

calculated from the results of iodine titration. As a result of doing so, a number of apparent irregularities in the behaviour of dimercaprol glucoside have disappeared. Another result of doing so is that the toxicity appears to be greater than the figures of Danielli *et al.*[14] suggest. As their doses include in the weight material which does not behave as a free thiol, they give an exaggerated suggestion of the harmlessness of the substance. Nevertheless its toxicity is undoubtedly much less than that of dimercaprol.

The activity of dimercaprol glucoside against oxophenarsine is shown in Table III and Figures 1 and 2. The activity is on the whole similar to that of dimercaprol itself. If anything the glucoside is a little less

active when given at the same time as the oxophenarsine. About 0·8 mM/kg. of the glucoside saved 5 of 9 mice poisoned with 0·30 mM/kg. of oxophenarsine, while 0·6 mM/kg. of dimercaprol was sufficient to save 7 of 9: but the difference is not significant. As the glucoside was less toxic, more could be given, and it was consequently superior against very large doses of oxophenarsine. Its much greater activity in these conditions is strikingly illustrated by the much larger area enclosed by its isobol than by that of dimercaprol (Figure 2). On the other hand, once poisoning was established, the glucoside was less effective. Dimercaprol still saved an occasional animal 80 minutes after oxophenarsine had been given, whereas no reduction in mortality was obtained with the glucoside after 40 minutes, even with large doses and in rather more extensive trials. As Danielli *et al.* suggest, the water-soluble thiol probably enters cells less readily, and so has less access to arsenic once the arsenic has been fixed by the tissues.

Mixtures of oxophenarsine and dimercaprol glucoside in proportions about a 1 to 1 molar ratio have been injected intramuscularly in mice, and no enhancement of toxicity of the arsenical has been found (Table II). Deaths were neither increased nor accelerated, and the lungs were, as usual in mice poisoned with oxophenarsine and treated with a dithiol, only moderately heavier than normal. Solutions probably containing less than one molecule of dimercaprol glucoside per molecule of oxophenarsine gave a negative nitroprusside reaction, whereas the reaction was positive when the dithiol was in excess; so it may be assumed that the two substances had combined. The evidence does not suggest that the compound has any enhanced toxicity.

IV. The toxicity of other dithiols and their effects in oxophenarsine poisoning.

The results of toxicity tests are summarised in Table V. The figures are sometimes very approximate, either because the amount of the material available was limited and the toxicity was low, or because preliminary tests indicated that the toxicity was too high for the substance, however active as an antidote, to be likely to be of any therapeutic use. The LD50s have been obtained either by injecting into one group of 15 to 20 mice a dose slightly larger than the LD50 and into another group a dose slightly smaller and estimating the LD50 by linear interpolation after transformation to log dose and probit mortality; or by the method of Kärber[15]. The efficacy against oxophenarsine was tested (a) by giving the antidote immediately after an LD95 to 99 (0·16 to 0·20 mM/kg.) to show whether any protection at all was obtained; (b) by giving the antidote 40 minutes, and sometimes at other times, after the oxophenarsine, to show whether protection could be obtained late in acute poisoning; (c) by giving a 50 per cent. larger dose of oxophenarsine and the thiol immediately afterwards, to show whether the thiol was effective against massive poisoning. The dose of thiol chosen was about one-third of its LD50, and tests (b) and (c) show roughly the maximal activity of dimercaprol and dimercaprol glucoside respectively at this level of

TABLE V

THE TOXICITY AND ANTI-ARSENICAL ACTIVITY OF SOME THIOLS AND ACETYLDITHIOLS

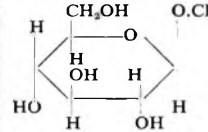
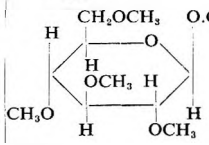
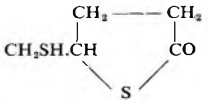
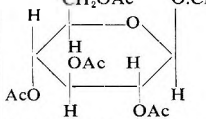
No.	Name	Formula	Mol. Wt.	Purity per cent.	LD50 mM/kg.	No. of mice in estimate of LD50	Activity against oxophenarsine	
							Immediate	After 40 minutes
—	Dimercaprol (BAL)	$CH_2SH.CHSH.CH_2OH$	124	100	1·0	99	†††	† †
0 : 13	1 : 2-dimercaptobutane-3 : 4-diol ...	$CH_3SH.CHSH.CHOH.CH_2OH$	154	80	1·0	20	†††	†††
0 : 17	1 : 2-dimercaptopentane-3 : 4 : 5-triol	$CH_3SH.CHSH.(CHOH)_2.CH_2OH$ ...	184	†	1·7–2·5	28	†††	†
0 : 24	1 : 2-dimercaptohexane-3 : 4 : 5 : 6-tetrol	$CH_3SH.CHSH.(CHOH)_3.CH_2OH$ ...	214	†	1·7	29	Not done	
0 : 8	2 : 3-dihydroxypropyl 2 : 3-dimercaptopropyl ether	$CH_2OH.CHOH.CH_2.O.CH_2.CHSH.CH_2SH$	198	78	1·3	55	†††	†
0 : 9	1 : 3-dihydroxy-2-propyl 2 : 3-dimercaptopropyl ether	$\begin{matrix}CH_2OH\\CH_2OH\end{matrix}\!\!>\!CH.O.CH_2.CHSH.CH_2SH$ ...	198	92	1·3	50	† †	†
—	(2 : 3-dimercaptopropyl) glucoside (dimercaprol glucoside, BAL-Intrav)		286	†	3·8–4·7	230	††††	†
0 : 19	3(2′ : 3′-dimercaptopropyl) mannitol...	$CH_2OH.CHOH.CH\!<\!\begin{matrix}O.CH_2.CHSH.CH_2SH\\CHOH.CHOH.CH_2OH\end{matrix}$	288	†	3·2	40	††††	0
0 : 16	6(2′ : 3′-dimercaptopropyl) mannitol...	$CH_2SH.CHSH.CH_2.O.CH_2.(CHOH)_4.CH_2OH$	288	†	2·6	40	†††	† †
0 : 20	6(2′ : 3′-dimercaptopropyl)sorbitol ...	$CH_2SH.CHSH.CH_2.O.CH_2.(CHOH)_4.CH_2OH$	288	†	2·6	29	†††	†††
0 : 23	2 : 3 : 4 : 6-tetramethyl 2′ : 3′-dimercaptopropyl glucoside		342	94	5·0–8·0	9	†††	†

TABLE V—continued

No.	Name	Formula	Mol. Wt.	Purity per cent.	LD50 mM/kg.	No. of mice in estimate of LD50	Activity against oxophenarsine	
							Immediate	After 40 minutes
0 : 15	δ-mercapto-γ-valerothiolactone ...		148	100	0·9	40	† †	† †
0 : 2	1 : 4-dithiothreitol	$CH_2SH.(CHOH)_2.CH_2SH$	154	100	0·7	25	——	Not done
0 : 22	1 : 4-dithioerythritol	$CH_2SH.(CHOH)_2.CH_2SH$	154	97	2·0–2·5	30	–	–
0 : 1	1 : 6-dithiodulcitol	$CH_2SH.(CHOH)_4.CH_2SH$	214	90	3·4, 2·2 *	55	† †	0
0 : 21	Diacetyl dimercaprol	(Probably) $CH_2SAc.CHSH.CH_2OAc$ ...	208	100	<2·5	18	–	–
0 : 12	3-acetoxy-1 : 2-bisacetylthiopropane (triacetyl dimercaprol)	$CH_2SAc.CHSAc.CH_2OAc.$	250	100	1·6	44	† †	† †
0 : 14	3 : 4-diacetoxy-1 : 2-bisacetylthiobutane	$CH_2SAc.CHSAc.CHOAc.CH_2OAc.$ ...	322	100	*ca* 3·0	12	† †	†
0 : 18	3 : 4 : 5-triacetoxy-1 : 2-bisacetylthiopentane	$CH_2SAc.CHSAc.(CHOAc)_2.CH_2OAc.$ ...	394	100	> 10·0	16	0	Not done
0 : 5	2 : 3-diacetoxypropyl 2 : 3-bisacetylthiopropyl ether	$CH_2SAc.CHSAc.CH_2.O.CH_2.CHOAc.$ $CH_2OAc.$	366	76	2·9	40	†††	0
0 : 6	1 : 3-diacetoxy-2-propyl 2 : 3-bisacetylthiopropyl ether	CH_2OAc CH_2OAc $CH.O.CH_2.CHSAc.CH_2SAc.$ ...	366	95	3·2	24	†††	Not done
0 : 11	Hexa-acetyl β-(2 : 3-dimercaptopropyl) glucoside		538	100	> 5·0	10	†	0
0 : 10	Diethyl 3 : 4-bisacetylthiobutane-1 : 1-dicarboxylate	$CH_2SAc.CHSAc.CH_2.CH(CO.O.C_2H_5)(CO.O.C_2H_5)$	302	70	*ca* 10·0	9	0	0
0 : 7	Ethyl 2 : 3-bisacetylthiopropoxyacetate	$CH_2SAc.CHSAc.CH_2.O.CH_2.CO.O.C_2H_5$	294	77	2·8	15	†	Not done
0 : 4	Tetra-acetyl 1 : 4-dithiothreitol ...	$CH_2SAc.(CHOAc)_2.CH_2SAc.$	322	100	> 1·3	10	–	Not done
—	Cysteine	$CH_2SH.CH(NH_2).COOH$	121	100	–	–	†	0

Ac $-CO.CH_3$.
* From mortality at 28 days.
† Prepared from barium salt : doses derived from iodine titre.

† Delayed death.
† † Saved some lives against *ca.* 1·5 LD50
††† Saved all lives against *ca.* 1·5 LD50
†††† Saved all lives against *ca.* 2·2 LD50

– Accelerated death.
—— Killed all mice on *ca* 0·67 LD50 of oxophenarsine.

dosage. The quantitative method of assessing anti-arsenical activity by the increment produced in survival time (Weatherall and Weatherall[8]) had not been devised when the majority of these dithiols were tested, and so the foregoing procedure was used. Some of the substances were later evaluated by the more precise method, with results as reported in a separate paper and briefly indicated below.

Brief comments can be made on the individual substances.

A. 1:2-dithiols derived from polyhydric alcohols. The higher analogues of dimercaprol tended to be less toxic and less active against oxophenarsine. 1:2-dimercaptobutane-3:4-diol (0:13) was pharmacologically indistinguishable from dimercaprol. 1:2-dimercaptopentane-3:4:5-triol (0:17) was more water-soluble, less toxic, and less efficient when given late in oxophenarsine poisoning. 1:2-dimercaptohexane-3:4:5:6-tetrol (0:24) was about as toxic as the pentane derivative. It was not tested against oxophenarsine, but Weatherall and Weatherall[8] found that it had no activity at all against phenylarsenoxide.

B. O-Ethers of dimercaprol. As with the dithiols derived from polyhydric alcohols, the more water-soluble substances with a longer carbon chain and more hydroxyl groups were less toxic, but here there was more loss of toxicity and less loss of therapeutic activity. The glucoside appeared to be the best of this group, but the sorbitol ether (0:20) was surprisingly active in established oxophenarsine poisoning and still gave 100 per cent. protection after 40 minutes. 1:3-dihydroxy-2-propyl 2:3-dimercaptopropyl ether (0:8) and the sugar ethers contain the grouping $-O-CH_2-CROH-CR_2OH$, to which Bradley and Berger[16] attribute the ability of causing paralysis as does myanesin. Berger and Bradley[17] describe the paralysis as not accompanied by excitement, tremors, twitchings or convulsions at any time. None of these ethers produced exactly such a paralysis. An apparent flaccid paralysis was observed after nearly lethal doses of 6(2′:3′-dimercaptopropyl) mannitol (0:16), but when disturbed the mice made quite powerful movements, and lethal doses of this substance, like most of the other dithiols, produced convulsions.

C. Other 1:2-dithiols. The γ-lactone of γ:δ-dimercaptovaleric acid (0·15) was a little more toxic than dimercaprol. Deaths occurred more quickly, usually within 90 minutes of injection, and were preceded by very vigorous convulsions. Congestion of the lungs was more conspicuous post-mortem than with most dithiols. Anti-arsenical activity was a little less than that of dimercaprol.

D. α:ω-Dithiols. 1:4-dithiothreitol (0:2) behaved quite differently from its 1:2-isomer, 0:13. It was about 30 per cent. more toxic, and caused violent convulsions and death within 5 or 10 minutes of intramuscular injection. Post-mortem examination of mice killed by the drug and of survivors killed 28 days later, showed nothing macroscopically abnormal. When injected in doses by themselves innocuous, 0:2 greatly accelerated death after oxophenarsine and increased the mortality from sublethal doses. Death in animals so treated was preceded by convulsions, and at post-mortem examination gross pulmonary congestion

and hæmorrhages were observed, with a highly significant increase in lung weight (11·0±0·53 mg/g. compared with 7·9±0·20 mg./g. in mice poisoned with the same dose of oxophenarsine alone). In the occurrence of convulsions, the rapidity of death and the increase in lung weight, poisoning with oxophenarsine and 1 : 4-dithiothreitol resembles poisoning with the oxophenarsine-dimercaprol compound, and it seems possible that a similar mechanism may underlie both phenomena. 1 : 4-dithioerythritol (0 : 22) was considerably less toxic than its stereo-isomer, and caused death less rapidly : but it also accelerated death in oxophenarsine poisoning, though less dramatically. 1 : 6-dithiodulcitol (0 : 1) had a particularly low acute toxicity, of about 3·4 mM/kg. But mice receiving doses over about 2 mM/kg. developed a remarkable disease at some time between 3 and 20 days after injection. The hind legs became paralysed and the posterior part of the body slowly wasted until the body weight fell sometimes by as much as 50 per cent. Diarrhoea was common, and a crust of dirt and excreta was usually present at the base of the tail. There was often dermatitis of the tail, and a number of mice developed periorbital abscesses. Death occurred at any time up to at least 28 days after injection and 14 days after the onset of paralysis, but a proportion of mice receiving doses of 2·5 mM/kg. or less recovered and appeared normal 28 days after injection. The latter were killed and were found to have paler and firmer lungs than normal, but no other gross abnormality. Two paralysed mice, killed at the same time, showed gross wasting and contractures, generalised loss of size and weight of the viscera proportional to the body-weight, and no macroscopic lesions of the central nervous system. This dithiol gave slight protection against oxophenarsine poisoning, but some protected mice subsequently developed the paralytic syndrome. The dithiodulcitol was dissolved in a solution containing sodium borate, but no such syndrome developed in mice treated with sodium borate in amounts corresponding to the amounts used in dissolving the dithiodulcitol and no other mice under experiment at the same time developed the same syndrome. Sodium borate gave no protection against oxophenarsine poisoning. A rabbit which received 2 mM/kg. of dithiodulcitol intraperitoneally had much diarrhoea and died within 24 hours with gross lung œdema and congestion. Another rabbit into which 0·2 mM/kg. was injected showed no ill-effects in the next three weeks.

E. Acetyldithiols. The acetyldithiols were all less toxic, on a molecular basis, than the corresponding free dithiols. Some, particularly triacetyl dimercaprol (0 : 12), had considerable activity against oxophenarsine. Both toxicity and anti-arsenical activity diminished as the molecular weight increased. The lethal doses of 3 : 4 : 5-triacetoxy-1 : 2-bisacetylthiopentane (0 : 18) and hexa-acetyl β-(2 : 3-dimercaptopropyl) glucoside (0 : 11) were too large to be dissolved in a harmless quantity of any solvent tried, and 0 : 11 only increased slightly the survival time of oxophenarsine-poisoned mice without saving any lives, while 0 : 18 was inactive. Triacetyl dimercaprol (0 : 3) behaved very similarly to

dimercaprol itself. Diacetyl dimercaprol (0:21) was anomalous. The method of synthesis was directed to producing the di-S-acetyl compound, but by iodine titration it appeared to be a monothiol, and one acetyl group had therefore presumably wandered to the oxygen atom. Its toxicity was low, though large doses produced symptoms like dimercaprol; but unlike either dimercaprol or triacetyl dimercaprol, it saved no lives and considerably accelerated death in mice poisoned with oxophenarsine. Tetra-acetyl 1:4-dithiothreitol (0:4) appeared to be less toxic than the free $\alpha:\omega$-dithiol and, like it, potentiated oxophenarsine poisoning.

Discussion

The main object of the work reported here was to examine the possibility that other dithiols might be more satisfactory therapeutic agents than dimercaprol. In order to do so, the toxicity of various dithiols was estimated and their anti-arsenical activity was compared with that of dimercaprol. The quantitative method of assessing anti-arsenical activity described by Weatherall and Weatherall[8] was not devised until most of the substances described here had been tested, and only approximate comparisons are afforded by the present data. Danielli *et al.*[13] found that the toxicity of dithiols was decreased by the introduction of hydrophilic groups into the molecule, and this is borne out by the present findings. Unfortunately, the diminution in toxicity has been accompanied by a loss of anti-arsenical activity, at least when oxophenarsine was used as the arsenical. Which loss preponderated depended on how the hydrophilic groups were introduced. Simple lengthening of the chain of a hydroxythiol by the introduction of -CHOH- groups resulted in more loss of activity than of toxicity. On the other hand, conjugation by means of an ether linkage with, for example, a sugar produced dithiols which were substantially less toxic than dimercaprol, but retained quite good activity against oxophenarsine. The most marked defect of the sugar ethers was their inability to save lives when they were given some time after the arsenical poison. Dimercaprol glucoside was clearly less efficient than dimercaprol in this respect, although the difference was less conspicuous with 6(2′:3′-dimercaptopropyl) sorbitol. Dimercaprol glucoside was the least toxic of all the dithiols tested, and had otherwise good anti-arsenical activity, and seemed clearly to deserve further investigation from the point of view of possible therapeutic value.

An alternative method of diminishing the toxicity of dithiols lay in masking the -SH groups by some combination which might be readily broken down in the body. As the acetyl derivatives of several of the dithiols studied were readily available, some were tried, and were found to possess moderately good activity against oxophenarsine. The acetylated dithiols of low molecular weight were more effective than larger molecules. The smaller molecules tend to be more soluble in water and may be expected to hydrolyse more readily to the free dithiols, so this difference was not surprising. It is possible that by slow hydrolysis suitable acetylated dithiols would liberate a moderate sustained concentration of

dithiol, which would be therapeutically more useful than the rather short action of dimercaprol. Experiments in which hexa-acetyl β-(2:3-dimercaptopropyl) glucoside (0:11) was given three hours before a dose of oxophenarsine indicated that no protective concentration of thiol had been liberated. Possibly some other acetyl dithiol would have been more effective.

Separation of the -SH groups of dithiols has been found in the three instances studied here to be undesirable. 1:6-dithiodulcitol was only weakly active against oxophenarsine and produced a peculiar wasting disease by some mechanism which has not been examined. Both 1:4-dithiols potentiated oxophenarsine poisoning, in a manner which resembled the toxic effects of the oxophenarsine-dimercaprol compound. The similarity suggested that a common mechanism might be involved. Peters and Stocken[4] suggested that the compound of oxophenarsine and dimercaprol possibly penetrated cells and there dissociated with the intracellular liberation of toxic arsenic. The 1:4-dithiols are likely to form a less stable ring with arsenic than the 1:2-compounds (Whittaker[18]), and possibly form such compounds *in vivo* which penetrate cells and then dissociate again with fatal results. Some support is lent to Peters and Stocken's concept by the harmlessness of the compound of oxophenarsine and dimercaprol glucoside, which is presumably more soluble in water and consequently less liable to enter cells. As indicated above, no evidence at all could be found of any enhancement of toxicity of oxophenarsine by such combination.

Summary

1. The toxicity in mice has been studied of oxophenarsine, the oxophenarsine-dimercaprol compound, 15 dithiols and 10 acetyl dithiols.

2. The LD50 of oxophenarsine given intramuscularly was about 0·14 mM/kg. Significant differences in mortality were not observed when oxophenarsine was given intraperitoneally.

3. Death occurred more rapidly after large than after small doses of oxophenarsine. The lungs of mice dying of oxophenarsine poisoning were heavier than normal, particularly when the doses were small and the time of survival long.

4. The LD50 of the oxophenarsine-dimercaprol compound given intramuscularly was about 0·06 mM/kg. and given intraperitoneally was about 0·30 mM/kg. Death occurred more rapidly than in oxophenarsine poisoning and the lungs were much heavier than either normally or in oxophenarsine poisoning.

5. The LD50 of dimercaprol glucoside was found to depend on the iodine titre of the solution used. Estimated on this basis, the least toxic samples examined had an LD50 of about 4·5 mM/kg.

6. Dimercaprol glucoside was effective against larger doses of oxophenarsine than was dimercaprol when the thiols were given immediately after oxophenarsine. The glucoside was less effective than dimercaprol when the thiols were given more than about 20 minutes after oxophenarsine.

7. Approximately equimolar mixtures of oxophenarsine and dimercaprol glucoside were not more toxic than oxophenarsine alone.

8. No other dithiols examined had as good a combination of low toxicity and high activity against oxophenarsine as dimercaprol glucoside. The nearest approach was made by other dimercaprol sugar ethers.

9. Acetylated dithiols of low molecular weight had quite good antiarsenical activity, but were not more active relative to their toxicity than free dithiols.

10. Acetylated dithiols with a molecular weight greater than about 300 had very low toxicity and little or no activity against oxophenarsine.

11. Three substances (diacetyl dimercaprol, 1:4-dithioerythritol and particularly 1:4-dithiothreitol) accelerated death and increased the mortality in mice poisoned with oxophenarsine.

The dimercaprol used in these experiments was a sample of water-purified BAL kindly presented by Professor R. A. Peters. Some of the dimercaprol glucoside was prepared by Dr. L. N. Owen, some was provided by the Ministry of Supply, and one sample was provided by Boots Pure Drug Co., Ltd. The other dithiols and all the acetylated dithiols were prepared by Dr. L. N. Owen and his associates. Oxophenarsine (mapharside, not diluted with sucrose as in commercial preparations) was generously presented by Dr. J. S. White, of Parke Davis and Co., Ltd. The oxophenarsine-dimercaprol compound was synthesised and kindly presented by Dr. L. A. Stocken. I am most grateful for all these gifts. I am much indebted also to Dr. L. N. Owen for numerous helpful discussions, to Mr. Leslie Angus, Miss Jean Tulloch and Miss Irene Munro for technical assistance, to Mrs. J. A. C. Weatherall for performing certain of the toxicity tests, and particularly to Professor J. H. Gaddum for his advice and criticism. The work was initiated during the tenure of a personal grant from the Medical Research Council and was supported by a grant for expenses from the Council.

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THE PARTITION CHROMATOGRAPHY OF ALKALOIDS

PART II.—THE ALKALOIDS OF AUSTRALIAN *DATURA FEROX* AND OF INDIAN HENBANE

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From the University, Nottingham

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IN Part I[1] attention was directed to the possibility that *Datura ferox* may yield different alkaloids when grown in different localities. Australian-grown *D. ferox* was found by Barnard and Finnemore[2] to contain hyoscyamine as the major alkaloid and material grown in the Crimea afforded, according to Libizov[3], hyoscyamine and hyoscine. We found that plants grown in this country produced hyoscine as the chief alkaloidal constituent and meteloidine as a relatively minor alkaloid. In view of the well authenticated cases of variation in the nature of the alkaloids within certain species of the Solanaceæ, viz., *Duboisia species* and *Datura metel*[4] it appeared of interest to accumulate more evidence on this point for *D. ferox*. Moreover, it would appear from the results reported in Part I and to be described in this communication that *D. ferox* may provide a convenient commercial source of hyoscine.

Samples of Indian henbane are from time to time offered for sale in this country and presumably are used for the manufacture of galenical preparations. As far as we are aware, no information is available either on the plant source of these materials[5] or on the nature of the alkaloids contained in them. Accordingly a comparison has been made of the alkaloids of Indian henbane with those of authentic *Hyoscyamus niger*.

EXPERIMENTAL

The Australian *D. ferox* available consisted of about 120 g. of whole plant grown at Canberra and seeds from plants grown at Shepparton, Victoria. The Indian henbane was a commercial sample purchased in this country. The botanical source of this material is doubtful but we are informed by Mr. J. L. Forsdike that it differs from *H. niger*.

Quantities of 100 g. of both the whole plant and the seeds of *D. ferox* were extracted by a modification of the Pharmacopœial assay process[6]. An ethereal solution of the total alkaloids was in each case chromatographically fractionated first with ether and then with chloroform on a column of 20 g. of kieselguhr on which was distributed 10 ml. of M/2 phosphate buffer of *p*H 7·3. Details of this technique and the method of collecting fractions of the eluate are described in Part I. The alkaloids in the fractions of eluting solvent were identified as their picrates.

For comparison of the alkaloids of Indian henbane with those of *H. niger*, 500 g. of the Indian drug and 100 g. of *H. niger* were extracted and chromatographically fractionated by the same procedure, the M/2 phosphate buffer having *p*H 6·8 in this instance. Because of the low proportion of alkaloids in the Indian henbane, a total of 10 kg. was extracted in batches of 2 kg. for the identification of the alkaloids after

chromatographic fractionation. The titration liquors corresponding to a given peak were combined; the aqueous layer, containing the alkaloidal sulphate was separated from the organic solvent and indicator was removed from it by shaking with chloroform; after concentrating under reduced pressure, the solution was available for the preparation of solid derivatives by double decomposition with the appropriate reagent.

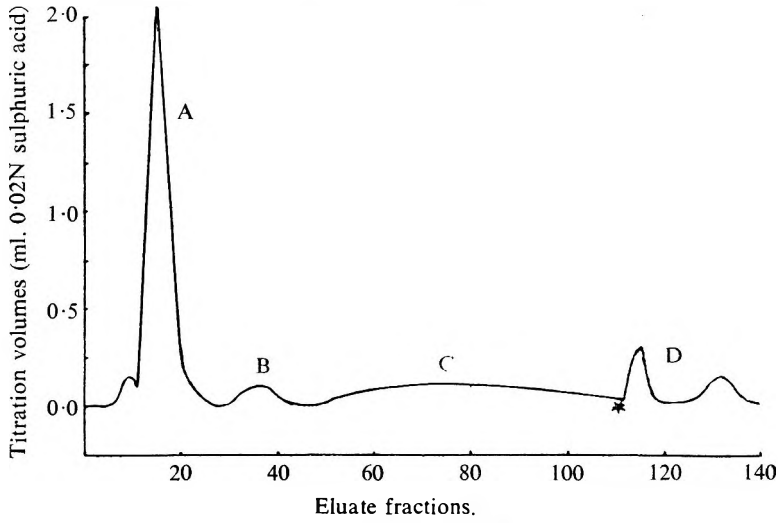

FIG. 1.—Separation of alkaloids from 100g. of Australian *Datura ferox* (whole plant)

Column, 20g. kieselguhr with 10 ml. 0·5 M phosphate buffer, *p*H 7·3.

* Chloroform B.P. used as eluant.

Figure 1 shows the separation of the alkaloids of the whole plant of *D. ferox*. The eluate fractions represented by the large peak A were shown to contain hyoscine by the preparation of the picrate, m.pt. 187°C., not depressed on admixture with authentic hyoscine picrate. The proportion of hyoscine found by titration was 0·06 per cent. and by isolation of the picrate, 0·05 per cent. From the eluate fractions corresponding to peak B, 5 mg. of a picrate, m.pt. 230°C., was isolated. Fractions 52 to 112 of the eluate (peak C) afforded meteloidine picrate, m.pt. 174° to 175°C., undepressed on admixture with authentic material. The melting-point of this picrate was considerably depressed when mixed with hyos-

TABLE I

Alkaloid	*H. niger*	Indian henbane
	per cent.	per cent.
Total alkaloids, calculated as hyoscyamine ...	0·073	0·031
Hyoscine	0·028	0·016
Hyoscyamine	0·041	0·010
Tropine	0·0025	0·0015

cyamine picrate or with atropine picrate. The weight of meteloidine picrate isolated was 3 mg.; no reasonably accurate estimation of the proportion of this alkaloid was possible by a summation of the very small,

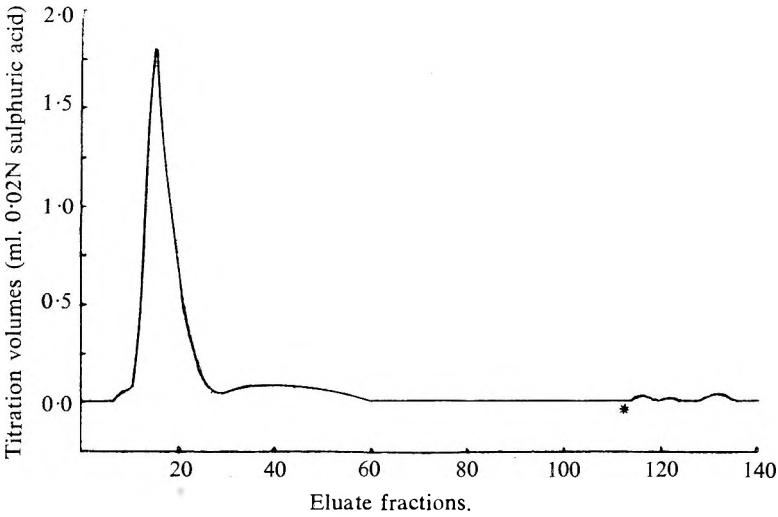

FIG. 2.—Separation of alkaloids from 100g. of Australian *Datura ferox* seeds. Column, 20g. kieselguhr with 10 ml. 0·5 M phosphate buffer, *p*H 7·3. * Chloroform B.P. used as eluant.

individual eluate-fraction titres. The partition chromatogram of *D. ferox* seeds is represented in Figure 2. The only significant peak was shown to refer to hyoscine, 0·093 per cent., which afforded hyoscine

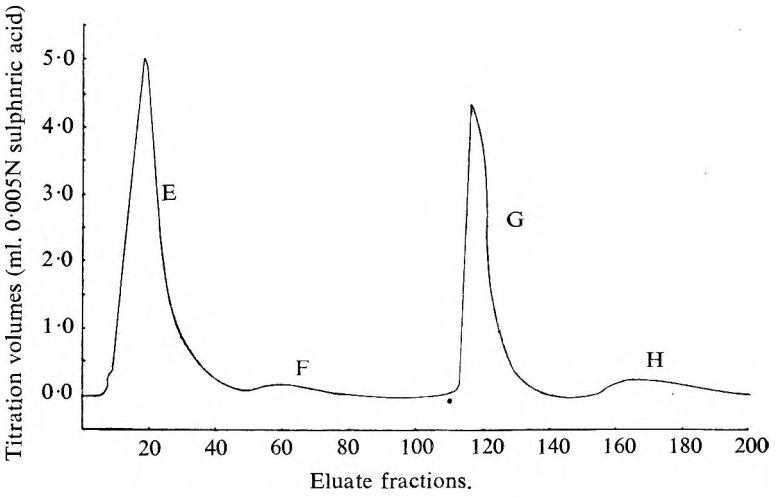

FIG. 3.—Separation of alkaloids from 100g. of Indian henbane. Column, 20g. kieselguhr with 10 ml. 0·5 M phosphate buffer, *p*H 6·8. * Chloroform B.P. used as eluant.

picrate, m.pt. 187°C., undepressed by authentic hyoscine picrate. The proportion of total alkaloids, calculated as hyoscine, was 0·095 per cent.

For Indian henbane and *H. niger*, the partition chromatograms were as shown in Figures 3 and 4. Quantitative data obtained from the respective 500 g. and 100 g. quantities of these two drugs are summarised in Table I. The shape of the chromatogram curve from the larger-scale extraction of Indian henbane was similar to Figure 3.

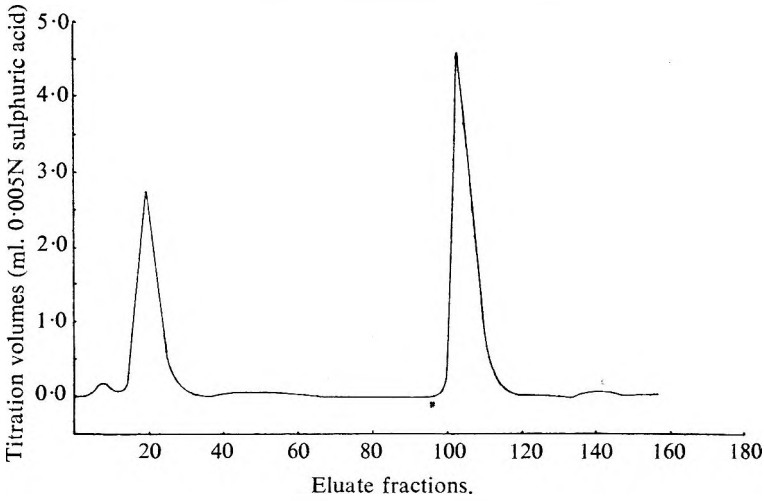

FIG. 4.—Separation of alkaloids from 100g. of *Hyoscyamus niger*.
Column, 20g. kieselguhr with 10 ml. 0·5 M phosphate buffer, *p*H 6·8.
* Chloroform B.P. used as eluant.

Peak E was demonstrated to correspond to hyoscine by the preparation of the picrate, m.pt. and mixed m.pt. 186°C. and the aurichloride, m.pt. and mixed m.pt. 204°C., with decomposition. From the fractions represented by peak F, a picrate, m.pt. 230°C., was isolated; this material was identical with the picrate of the same melting-point isolated from *D. ferox*. The alkaloid corresponding to peak G was found to be hyoscyamine, since it afforded an aurichloride, m.pt. 163°C., not depressed on admixture with authentic hyoscyamine aurichloride, and also a picrate, m.pt 164° to 165°C., not depressed by mixing with authentic material. For the identification of the alkaloid corresponding to peak H, the auribromide and Reineckate were found to be satisfactory. The auribromide crystallised from water or dilute hydrobromic acid as dark brown needles, m.pt. 196°C., with decomposition. Found: C, 14·9; H, 2·4; N. 2·4 per cent. $C_8H_{15}ON,HAuBr_4$ requires C, 14·6; H, 2·4; N, 2·1 per cent. The melting-point of this substance was undepressed on admixture with the auribromide prepared from tropine made by the hydrolysis of hyoscyamine according to Ladenburg[7]. The Reineckate, m.pt. 239° to 240°C., with decomposition, did not depress the melting-point of authentic tropine Reineckate[1]. Found: N, 20·3 per cent. $C_8H_{15}ON,H[Cr(SCN)_4(NH_3)_2]$, H_2O requires N,20·5 per cent.

Discussion of Results

Contrary to previous findings, the experiments described in this communication and in Part I[1] show that samples of *D. ferox* grown in Australia and in this country contain hyoscine as the principal alkaloidal constituent. Its proportion is very variable since material grown in Nottingham contained 0·4 per cent, whereas the Australian material contained only 0·06 per cent. This plant is common in Australia and in view of the low proportion of other alkaloidal constituents, may, if the constancy of alkaloidal type is confirmed for other Australian samples, provide a convenient commercial source of hyoscine. Since the partition chromatogram indicated the complete absence of meteloidine from the seeds, it would appear that this alkaloid is not a product of metabolism of the reproductive organs of the plant. It is hoped that further information on the nature of the alkaloid which afforded a picrate, m.pt. 230° C., and on the alkaloids of *D. ferox* which are eluted with chloroform (peak D) will be presented in a further communication.

The major alkaloids of Indian henbane are hyoscine and hyoscyamine with tropine as a minor alkaloid, but the total alkaloidal content is very low. Notwithstanding anatomical differences, the shape of the partition chromatogram curve, which is a function of the R_F values characteristic of the alkaloids, demonstrates that the only significant difference between Indian henbane and *H. niger* is in the relative proportions of the alkaloids. The small final peak in the curve for *H. niger* is likely to correspond to tropine since a comparison of the curves for *H. niger* and Indian henbane shows that the R_F value must be very close to that of the tropine independently identified in the Indian henbane. This sample of Indian henbane bore no resemblance in its constituents to *H. reticulatus*, which according to Konovalova and Magidson[8], contains hyoscyamine and 1 : 4-*bis*dimethylaminobutane. The characterisation of the small quantity of tropine presented some difficulty. The picrate and aurichloride were too soluble to afford convenient derivatives and the auribromide decomposed slightly on repeated recrystallisation. We now find that the melting-point of the Reineckate varies appreciably with the rate of heating; in Part I the value recorded was 251° to 252°C., with decomposition, after sintering at 246° to 248°C., whereas the best reproducible value is probably 245° to 246°C., with decomposition.

We are greatly indebted to Dr. C. Barnard of the Council for Scientific and Industrial Research, Australia for providing the samples of *D. ferox* and to Mr. J. L. Forsdike for procuring the sample of Indian henbane.

Summary

1. Australian-grown *Datura ferox* has been found to contain as principal alkaloids, hyoscine and meteloidine.

2. The chief alkaloidal constituents of Indian henbane are hyoscine, hyoscyamine and tropine; in this respect. Indian henbane differs from *Hyoscyamus niger* only in the absolute and relative proportions of the alkaloids.

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THE ASSAY OF MERCURIC SALICYLATE

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THE iodometric method of assaying mercuric salicylate of the British Pharmaceutical Codex, 1934, was replaced in the Fourth Supplement by a thiocyanate titration of the mercury. The organic matter is destroyed by oxidation with potassium permanganate, first in alkaline then in acid solution and excess of oxidising agent is removed by adding ferrous sulphate solution.

Several other standard procedures for the determination of mercury in organic compounds or in the presence of organic matter follow the same principle, but oxidation is usually effected by heating with a mixture of nitric and sulphuric acids.[1, 2, 3] While it is accepted that salicylates and other phenols can be oxidised by permanganate, there is no specific information in the literature on the oxidation of mercuric salicylate. The composition of this salt has not yet been established and though it is described as a salt of salicylic acid in which the mercury replaces both the phenolic and carboxylic hydrogen atoms[4], the composition varies with the method of preparation and the B.P.C. allows material containing between 54 and 59·6 per cent. of mercury.

It was found in a preliminary experiment that a carefully homogenised sample assayed differently by the B.P.C. and United States Pharmacopœia XI methods. The latter employs a nitric-sulphuric acid oxidation followed by a thiocyanate titration of the mercury. The results differed by 1·27, representing a divergence of over 2 per cent. of the mercury. The mercury content of the sample was established gravimetrically by decomposing the sample with boiling hydrochloric acid and precipitating as sulphide. The precipitate was filtered, washed and dried to constant weight. A carbon disulphide extraction showed that no sulphur had been precipitated. The results were in close agreement with the U.S.P. figures: B.P.C. method, 56·30 per cent.; U.S.P. XI method 57·57 per cent.; sulphide method 57·55 per cent. (mean of 3 determinations).

BRITISH PHARMACEUTICAL CODEX METHOD

Dissolve 0·3 g. of sample in 10 ml. of 0·1N sodium carbonate. Add 1·5 g. of finely powdered potassium permanganate in small portions and mix well. After 5 minutes add carefully 5 ml. of sulphuric acid and after a further 5 minutes, 40 ml. of water and then acid solution of ferrous sulphate in small quantities, shaking after each addition until the precipitate is dissolved. Add 5 ml. of nitric acid and titrate with 0·1N ammonium thiocyanate, using ferric ammonium sulphate as indicator.

Acid Solution of Ferrous Sulphate. Freshly prepared by dissolving 7 g. of ferrous sulphate in 90 ml. of water, freshly boiled and cooled, and adding sulphuric acid to 100 ml.

Thiocyanate Titration Conditions

The acidity and amount of indicator added in the titration conform to established usage[5,6,7,8], but about 70 ml. of ferrous sulphate solution is required to dissolve the manganese dioxide precipitate, introducing ferric ion equivalent to 8·5 g. of ferric ammonium sulphate or 85 ml. of indicator solution. Variation of the indicator concentration has been found of little significance over a small range[8], but the total ferric concentration here is far above any hitherto tested. Furthermore, the sensitivity of the end-point is reduced in the strongly-coloured solution.

The quantitative effect was determined by titrating a standard solution of mercuric nitrate with thiocyanate at the same final mercury concentration and acidity as in the B.P.C. method using (a) 90 ml. of indicator, (b) 5 ml. of indicator and 85 ml. of water; results: (a) 17·50 ml.; (b) 17·65 ml.

The end-point, taken as the first definite brownish colour, was premature and represented a loss of 1 per cent. on the low burette reading. This accounts for part, at least, of the deficiency shown.

In all subsequent work, the ferrous sulphate solution was replaced by 3 per cent. hydrogen peroxide, added in small portions until the solution was clear; excess of peroxide was removed with 10 per cent. permanganate solution, added to the first permanent pink and decolorised with a small crystal of ferrous sulphate. The suitability of this modification was verified by titrating standard mercuric nitrate with thiocyanate (a) alone (b) in the presence of a solution prepared by carrying out a blank determination on salicylic acid, using the modified method. Identical results were obtained.

Quantity of Permanganate

The bulk of the oxidation occurs in alkaline solution, a heavy brown precipitate of manganese dioxide being thrown down immediately on adding permanganate. The solution becomes purple when all the permanganate has been introduced and the colour and precipitate remain until the reducing agent is added. The excess of permanganate is small since the purple colour fails to develop on increasing the weight of sample by less than 10 per cent.

The relation between sample weight/permanganate ratio and completeness of oxidation was studied in a series of determinations in which the weight of permanganate was fixed at 1·5 g. and the weight of sample varied from 0·2 to 0·45 g. The results were calculated in terms of burette reading per g. of sample.

Reduction of the sample weight below 0·3 g. had no effect on the recovery, whereas from 0·32 g. upwards, low and erratic results were obtained. An adequate safety margin was established by using 2 g. of permanganate, with which recoveries were fully maintained up to 0·4 g. of sample.

Time and Temperature of Oxidation

Increasing the alkaline oxidation time to 25 minutes had no significant effect (Table I). The importance of the acid oxidation stage is shown by the added recovery on increasing the severity of the conditions. Whilst a longer period than the 5 minutes oxidation of the B.P.C. method gave by itself no advantage, due probably to the rapid fall of temperature in the initial few minutes, a higher recovery was obtained on heating the solution for 10 minutes (Table I).

TABLE I

Conditions of oxidation			Mercury per cent.
Alkaline soln.	Acid soln.	Temp. effect	
25 minutes	5 minutes	Not heated	57·32
5 ,, ...	1 ,,	,,	56·94
5 ,, ...	5 ,,	,,	57·44
5 ,, ...	10 ,,	,,	57·43
5 ,, ...	5 ,,	Heated	57·45
5 ,, ...	10 ,,	,,	57·59
5 ,, ...	20 ,,	,,	57·59

Losses During Acidification

Acidification with sulphuric acid produces violent effervescence; the liquid boils and purple fumes are given off. Cooling is rapid in 5 minutes, owing to the relatively large surface area of the small volume of liquid. It was found that 10 ml. of 50 per cent. sulphuric acid gave a strong and more prolonged heat effect and enabled the reaction to be easily controlled, with little spray and no fuming. Recoveries were comparative both with the unheated solutions and on heating for 10 minutes, as suggested in the previous section. Results: sulphuric acid B.P. unheated 57·44 per cent., heated 10 minutes 57·59 per cent.; sulphuric acid (50 per cent.) unheated, 57·43 per cent., heated 10 minutes, 57·57 per cent.

Modified Method

Dissolve 0·3 g. of sample, accurately weighed, in 10 ml. of N (approx.) sodium carbonate. Add in small portions 2 g. of finely powdered potassium permanganate and mix well. After 5 minutes, add carefully 10 ml. of sulphuric acid (50 per cent.) and boil the solution gently for 10 minutes. Cool, add 40 ml. of water and then hydrogen peroxide solution (3 per cent.) in small portions until the precipitate is dissolved. Add potassium permanganate solution (10 per cent.) until the solution becomes a faint permanent pink, decolorise with a small crystal of ferrous sulphate, add 5 ml. of nitric acid and titrate with 0·1N ammonium thiocyanate solution using ferric ammonium sulphate solution as indicator.

The modified method was examined on a range of samples of varying mercury content (Table II), and found to agree closely with the U.S.P. XI and sulphide methods.

Table II also shows that the precision of the modified method is higher than that of the U.S.P. XI and B.P.C. methods.

TABLE II

Sample	Mercury per cent.			
	B.P.C. method	Modified method	U.S.P. XI method	Sulphide method
1	54·53 54·62	55·65 55·67	55·72 55·65	55·55
2	55·29 55·19	56·49 56·44	56·49 56·43	56·45
3	55·98 55·93	57·08 57·11	57·15 57·08	56·99
4	56·29 56·25 56·36	57·57 57·59 57·56	57·68 57·59 57·44	57·57 57·49 57·59
5	57·08 57·04	58·38 58·41	58·44 58·34	58·32
6	58·18 58·29	59·48 59·53	59·51 59·51	59·48

Summary

1. The B.P.C. assay of mercuric salicylate has been shown to give low results, compared with the U.S.P. XI method and a gravimetric sulphide method.

2. A study of the conditions of the B.P.C. method has been made and sources of error revealed.

3. A modification of the B.P.C. method has been proposed which gives results in agreement with the sulphide and U.S.P. XI methods.

I am indebted to Mr. C. Morton, B.Sc., Ph.C., Head of the Chelsea School of Pharmacy, for his helpful criticisms.

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THE PROPERTIES AND REACTIONS OF DECAMETHONIUM IODIDE AND HEXAMETHONIUM BROMIDE

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The work of Paton and Zaimis[1] on decamethonium iodide (α:ω-hexamethyldiaminodecane diiodide, known as C.10) showed that it was a very potent substance, and that it was capable of replacing *d*-tubocurarine chloride in medicine. An antidote exists for decamethonium iodide in hexamethonium bromide (α:ω-hexamethyldiaminohexane dibromide, known as C.6), and owing to its pharmacological properties this substance has been suggested for use in hypertension and vascular diseases, thereby replacing tetraethylammonium iodide. Hexamethonium bromide is 10 to 20 times as active as the tetraethyl compound. In view, therefore, of the possible importance of the new drugs, it was considered desirable that an examination should be made of their physical and chemical properties.

Decamethonium Iodide

Decamethonium iodide is a colourless, odourless, crystalline powder, which when dried for 4 hours at 70°C./20 mm. pressure and then placed in a melting-point apparatus at room temperature, heated rapidly to 235°C. and then at 2°/minute, had m.pt. 245° to 246°C. (corr.). If, however, it was put in the bath at 230°C., it melted at once with decomposition. For analysis it was dried at 70°C. *in vacuo*. Found: C, 38·25; H, 7·45; N, 5·14; I, 47·8 per cent.; $C_{16}H_{38}N_2I_2$ requires C, 37·5; H. 7·4; N, 5·47; I, 49·6 per cent.

It is soluble in water (21°C.) 1 g. in 10 ml. and at 100°C., 5 g. in 1 ml. A 1 per cent. solution is clear and colourless and has *p*H 6·6; a 10 per cent. solution has *p*H 6·4. The sterilised ampouled 0·1 per cent. solution in physiological saline solution has *p*H 6·06. In ethyl alcohol at 20°C., its solubility is less than 1 g. in 100 ml., and at 78·5°C. 1 g. in 3 ml., whereas in methyl alcohol (20°C.) it is 1 g. in 40 ml. and at 64·1°C. 1 g. in 1 ml. In boiling acetone, benzene, chloroform and ether the solubility is less than 1 g. in 500 ml. At 100°C. the material lost 0·26 per cent. and turned a yellow colour. On ignition no ash remained.

Reactions.

Effect of heat.	The solid melts, appears to boil and finally chars. No iodine evolved.
Concentrated sulphuric acid. Cold or hot.	Effervescence. Red brown colour changing to dull violet. Violet colour extracted by carbon tetrachloride to give violet extract.

Concentrated nitric acid.	
Cold.	Effervescence. Brown colour. Insoluble brown particles. On shaking with carbon tetrachloride violet solution obtained.
Hot.	As cold, but solution became colourless and iodine sublimed.
Sodium hydroxide solution 20 per cent.	Immediate white precipitate, which redissolved on heating and reappeared on cooling again.
Aqueous sodium nitrite solution.	Cold. Immediate brown colour. Violet extract in carbon tetrachloride.
0·1 N silver nitrate.	Pale yellow precipitate not soluble in strong solution of ammonia.
Folin-Ciocalteu phenol reagent.	Little or no visible change.
Acid potassium iodate solution.	Brown precipitate. Completely soluble in carbon tetrachloride to violet solution. Probably iodine.
Reinecke salt solution, 4 per cent.	Pink precipitate produced at once.
Saturated aqueous picric acid solution.	Bright yellow precipitate, m.pt. 146° to 148°C. Not changed by recrystallisation from methyl alcohol.
Saturated aqueous picrolonic acid solution.	No immediate precipitate. Slow crystallisation on standing. Dull yellow crystals m.pt. 238 to 239°C.
Aqueous gold chloride solution, 10 per cent.	Immediate brown, precipitate, m.pt. 170°C. Recrystallised from aqueous alcohol, m.pt. 174°C.
Aqueous platinic chloride solution, 10 per cent.	Chocolate-brown precipitate m.pt.. 300°C.
Mayer's reagent.	Pale yellow precipitate.
Halogen determination (Volhard method).	98·74 per cent. purity.

Stability of solution. Solutions at concentrations of 1 in 250, 1 in 500 and 1 in 1000 in physiological saline solution were heated at 95° to 100°C. for 30 minutes and kept in sealed tubes for 8 weeks, some in a cool dark place and others exposed to daylight at room temperature. All the solutions remained clear and colourless.

Assay for non-quaternary material. 0·2 g., accurately weighed, was added to a separating funnel containing water (200 ml.), followed by saturated sodium bicarbonate solution (5 ml.) and extracted with chloroform (3 × 20 ml.). The combined chloroform extracts were

washed with water (10 ml.), filtered through a plug of cotton wool into a tared beaker, and evaporated and dried at 100°C. for 1 hour. Residue on original material, 0·15 per cent.

Hexamethonium Bromide

Hexamethonium bromide is a colourless, odourless, crystalline powder which, when dried for 2 hours at 95°C./20 mm. pressure and placed in a melting-point apparatus at 150°C. or below, heated rapidly to 260°C. and then at 2°/minute, had m.p.t. 272°C. (decomp., corr.). If it was put in the bath above 230°C. it sintered slightly and then had m.pt. 273°C. (decomp.) This salt has a tendency to take up moisture, as shown by the sintering which is observed when its m.pt. is taken some weeks later. For analysis it was dried at 100°C. *in vacuo*. Found: C, 39·9; H, 8·18; N, 7·95; Br, 43·8 per cent.; $C_{12}H_{30}N_2Br_2$ requires C, 39·77; H, 8·28; N, 7·73; Br, 44·21 per cent.

It is soluble in water (21°C.) 1 g. in 1 ml. and at 100°C. 5 g. in 1 ml. A 1 per cent. solution is clear and colourless and has *p*H 6·56; a 10 per cent. solution has *p*H 6·0. The sterilised ampouled 1 per cent. solution in physiological saline solution has *p*H 6·0. In ethyl alcohol at 20°C. its solubility is 1 g. in 30 ml. and at 78·5°C. 1 g. in 3 ml., whereas in methyl alcohol (20°C.) it is 1 g. in 5 ml. and at 64·1°C. 1 g. in 1 ml. It is insoluble in acetone, benzene, chloroform and ether, both hot and cold. At 100°C. the material lost 0·64 per cent. On ignition the ash content was 0·02 per cent.

Reactions.

Effect of heat.	Solid melts and blackens.
Concentrated sulphuric acid.	
Cold.	Light yellow colouration.
Hot.	Very slight colour.
Concentrated nitric acid.	
Cold or Hot.	Deep yellow. Bromine liberated.
Sodium hydroxide solution.	White precipitate which dissolved on heating.
Aqueous sodium nitrite solution, 20 per cent.	Cold. No apparent reaction, very slight yellow colour.
0·1 N. silver nitrate.	Yellow precipitate soluble in concentrated ammonium hydroxide.
Folin-Ciocalteu phenol reagent.	Colour changes to pale yellow after boiling, but on standing in the cold becomes turquoise.
Acid potassium iodate solution.	No reaction—cold or hot.
Reinecke salt solution, 4 per cent.	Pink precipitate formed immediately. The solution on standing did not change.

Saturated aqueous picric acid solution.	Bright yellow precipitate, m.pt. 232° to 234°C. Recrystallisation from methyl alcohol did not change m.pt.
Saturated aqueous picrolonic acid solution.	No immediate precipitate. Crystallised slowly on standing to give greenish-yellow crystals m.p.t 243°C. (decomp.).
Aqueous gold chloride solution, 10 per cent.	Immediate orange - brown precipitate m.pt. 259°C. (decomp). Recrystallised from 50 per cent. aqueous ethyl alcohol, m.pt. 260°C. (decomp).
Aqueous platinic chloride solution, 10 per cent.	Immediate buff-coloured precipitate m.pt. 300°C.
Mayer's reagent.	Yellow precipitate.
Halogen determination (Volhard method).	99·15 per cent. purity.

Stability of solution. Solutions at concentrations of 1 in 250, 1 in 500 and 1 in 1000 in physiological saline were heated at 95° to 100°C. for 30 minutes, and kept in sealed tubes for 8 weeks, some in a cool dark place and others exposed to daylight at room temperature. All the solutions remained clear and colourless.

Assay for non-quaternary material. 0·2 g., accurately weighed, was added to a sperating funnel containing water (200 ml.), followed by saturated sodium bicarbonate solution (5 ml.) and extracted with chloroform (3 × 20 ml.). The combined chloroform extracts were washed with water (10 ml.), filtered through a plug of cotton wool into a tared beaker, and evaporated and dried at 100°C. for 1 hour. Residue on original material, 0·15 per cent.

We are indebted to Mr. F. Ridgway for carrying out some of the determinations.

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

PART VII

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IN the previous paper[1], disinfection data from the reaction between *Bact. coli* and the monoalkyl ethers were analysed statistically. Mean probit-log. time regressions were calculated for each substance and confidence limits for the estimation determined. This present communication is devoted to the analysis of the disinfectant data of experiments conducted at 30°C.; the statistical methods used follow exactly those in Part VI of this series of papers[1].

DISINFECTION STUDIES OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS AT 30°C.

Probit-log. time regressions at 30°C.

EXPERIMENTAL

Concentrations of ethylene glycol and the ethers were prepared and their disinfectant activity tested against *Bact. coli* at 30°C. by means of the standardised technique[2]. In most instances four tests were carried out on each concentration.

RESULTS AND CALCULATIONS

Probit-log. time regressions were calculated for each test. Summaries of the terms necessary to calculate the mean slopes and the error mean square at each concentration are presented in Tables IA to VID.

TABLE IA

SUMMARY OF TERMS FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL AT 30°C.

Observation	Concentrations of ethylene glycol			
	62·5 per cent.	65·0 per cent.	67·5 per cent.	70·0 per cent.
$S[(x-\bar{x})(y-\bar{y})]$	1·591879	5·869394	2·657648	4·573893
$S(x-\bar{x})^2$	1·003779	4·035156	2·876413	3·440898
$S(y-\bar{y})^2$	3·229122	9·206112	3·206060	6·590027
N	5	13	13	12
SS for individual regressions... ...	3·199891	8·641415	3·008845	6·298689
b...	1·588894	1·454565	0·923945	1·329273
SS pool...	2·524539	8·537411	2·488896	6·079953

TABLE Ib

CALCULATION OF THE ERROR MEAN SQUARE OF REGRESSIONS FROM CONCENTRATIONS OF ETHYLENE GLYCOL AT 30°C.

Concentration	Item	Sum of squares	*N*	Mean square
62·5 per cent.	Common regression	2·524539	1	2·524539
	Variation in regression ...	0·675352	2	0·337676
	Total	3·199891	3	
	Residual in *y*	0·029231	5	0·005846
65·0 per cent.	Common regression	8·537411	1	8·537411
	Variation in regression ...	0·104004	3	0·034668
	Total	8·641415	4	
	Residual in *y*	0·564697	13	0·043438
67·5 per cent.	Common regression	2·488896	1	2·488896
	Variation in regression ...	0·519949	5	0·103889
	Total	3·008845	6	
	Residual in *y*	0·197215	12	0·016434
70·0 per cent.	Common regression	6·079953	1	6·079953
	Variation in regression ...	0·218736	3	0·072912
	Total	6·298689	4	
	Residual in *y*	0·291338	12	0·024278

TABLE Ic

SUMMARY OF STATISTICAL DATA FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF CONCENTRATIONS OF ETHYLENE GLYCOL AT 30°C.

Concentration	Residual in *y*		Variation in *b*		*SS* pooled *b*	$S[(x-\bar{x})(y-\bar{y})]$	$S(x-\bar{x})^2$
	SS	*N*	*SS*	*N*			
62·5 per cent.	0·029231	5	0·675352	2	2·524539	1·591879	1·003879
65·0 per cent.	0·564697	13	0·104004	3	8·537411	5·869394	4·035156
67·5 per cent.	0·197215	12	0·519949	5	2·488896	2·657648	2·876413
70·0 per cent.	0·291338	12	0·218736	3	6·079953	4·573893	3·440898
Totals	1·082481	42	1·518041	13	19·630799	14·692814	11·356346

$$\bar{b} = \frac{14 \cdot 692814}{11 \cdot 356346} = 1 \cdot 293798$$

$$SS \text{ for joint regression} = \frac{(14 \cdot 692814)^2}{11 \cdot 356346} = 19 \cdot 009529$$

TABLE Id

MEAN SQUARES OF THE VARIATIONS IN THE PROBIT-LOG. TIME REGRESSIONS FROM DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL AT 30°C.

Item	*N*	Sum of squares	Mean square
Grand regression	1	19·009529	19·009529
Variation in regression between concentrations ...	3	0·621270	0·207090
Variation in regression within concentrations	13	1·518041	0·116772
Residual in *y*	42	1·082481	0·025773

Combined data from the calculations of the probit-log. time regressions for ethylene glycol and its monoalkyl ethers at 30°C.

Table VII presents a summary of the massed statistical data from the calculations of probit-log. time regressions for concentrations of ethylene glycol and its monoalkyl ethers at 30°C. From it has been calculated the mean slope ($\bar{b}$=1·5230) and the sum of squares for the joint regression (121·781173).

TABLE IIA

SUMMARY OF TERMS FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOMETHYL ETHER AT 30°C.

Observation	Concentrations of ethylene glycol monomethyl ether			
	35·0 per cent.	37·5 per cent.	40·0 per cent.	42·5 per cent.
$S[(x-\bar{x})(y-\bar{y})]$	4·661846	4·190510	2·212453	3·945919
$S(x-\bar{x})^2$	3·060121	3·194711	1·676468	3·512930
$S(y-\bar{y})^2$	7·476572	5·949571	3·278853	4·811554
N	11	13	7	14
SS for individual regressions... ...	7·255987	5·632065	3·182261	4·686665
b...	1·523419	1·311702	1·319711	1·123256
SS pool...	7·101944	5·496702	2·919798	4·432276

The analysis of variance of the massed regressions is set out in Table VIII. The *z*'s for the various combinations have been calculated by the technique employed in Part VI[1] and have been presented in Table IX. The mean square for the residual in *y* (0·027826) has been used as

TABLE IIB

CALCULATION OF THE ERROR MEAN SQUARE OF REGRESSIONS FROM CONCENTRATIONS OF ETHYLENE GLYCOL MONOMETHYL ETHER AT 30°C.

Concentration	Item	Sum of squares	*N*	Mean square
35·0 per cent.	Common regression	7·101944	1	7·101944
	Variation in regression ...	0·154043	3	0·051348
	Total	7·255987	4	
	Residual in y	0·220585	11	0·020053
37·5 per cent.	Common regression	5·496702	1	5·496702
	Variation in regression ...	0·135363	3	0·044841
	Total	5·632065	4	
	Residual in *y*	0·317506	13	0·024431
40·0 per cent.	Common regression	2·919798	1	2·919798
	Variation in regression ...	0·262463	3	0·087486
	Total	3·182261	4	
	Residual in *y*	0·096592	7	0·013797
42·5 per cent.	Common regression	4·432276	1	4·432276
	Variation in regression ...	0·254389	3	0·084796
	Total	4·686665	4	
	Residual in *y*	0·124889	14	0·008921

denominator to calculate the variance ratios between the different items. The probabilities of the significance between these items have been computed and included in Table IX.

TABLE IIc

SUMMARY OF STATISTICAL DATA FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF CONCENTRATIONS OF ETHYLENE GLYCOL MONOMETHYL ETHER AT 30°C.

Concentration	Residual in y		Variation in b		SS pooled b	$S[(x-\bar{x})(y-\bar{y})]$	$S(x-\bar{x})^2$
	SS	N	SS	N			
35·0 per cent.	0·220585	11	0·154043	3	7·101944	4·661846	3·060121
37·5 per cent.	0·317506	13	0·135363	3	5·496702	4·190510	3·194711
40·0 per cent.	0·096592	9	0·262463	3	2·919798	2·212453	1·676468
42·5 per cent.	0·124889	14	0·254389	3	4·432276	3·945919	3·512930
Totals	0·759572	45	0·806258	12	19·950720	15·010728	11·444230

$$\bar{b} = \frac{15{\cdot}010728}{11{\cdot}444230} = 1{\cdot}311642$$

$$SS \text{ for joint regression} = \frac{(15{\cdot}010728)^2}{11{\cdot}444230} = 19{\cdot}688695$$

TABLE IId

MEAN SQUARES OF THE VARIATIONS IN THE PROBIT-LOG. TIME REGRESSIONS FROM DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOMETHYL ETHER AT 30°C.

Item	N	Sum of squares	Mean square
Grand regression	1	19·688695	19·688695
Variation in regression between concentrations ...	3	0·262025	0·087342
Variation in regression within concentrations	12	0·806258	0·067189
Residual in y	45	0·759572	0·016879

TABLE IIIa

SUMMARY OF TERMS FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOETHYL ETHER AT 30°C.

Observation	Concentrations of ethylene glycol monoethyl ether			
	12·5 per cent.	15·0 per cent.	17·5 per cent.	20·0 per cent.
$S[(x-\bar{x})(y-\bar{y})]$	5·204340	2·643049	2·917334	2·356392
$S(x-\bar{x})^2$	3·686711	1·924663	2·346900	1·824886
$S(y-\bar{y})^2$	8·478306	4·169134	3·977931	3·174931
N	13	9	11	5
SS for individual regressions... ...	7·867899	3·749695	3·755170	3·122334
b...	1·411649	1·373253	1·243059	1·291254
SS pool...	7·346699	3·629575	3·626417	3·042701

TABLE IIIB

CALCULATION OF THE ERROR MEAN SQUARE OF REGRESSIONS FROM CONCENTRATIONS OF ETHYLENE GLYCOL MONOETHYL ETHER AT 30°C.

Concentration	Item	Sum of squares	*N*	Mean square
12·5 per cent.	Common regression	7·346699	1	7·346699
	Variation in regression ...	0·521200	3	0·173733
	Total	7·867899	11	
	Residual in *y*	0·610407	13	0·046950
15·0 per cent.	Common regression	3·629575	1	3·629375
	Variation in regression ...	0·120120	3	0·040040
	Total	3·749695	4	
	Residual in *y*	0·419439	9	0·046604
17·5 per cent.	Common regression	3·626417	1	3·626417
	Variation in regression ...	0·128753	3	0·042918
	Total	3·755170	4	
	Residual in *y*	0·222761	11	0·020251
20·0 per cent.	Common regression	3·042701	1	3·042701
	Variation in regression ...	0·079633	3	0·026544
	Total	3·122334	4	
	Residual in *y*	0·052597	5	0·010519

TABLE IIIC

SUMMARY OF STATISTICAL DATA FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF CONCENTRATIONS OF ETHYLENE GLYCOL MONOETHYL ETHER AT 30°C.

Concentration	Residual in *y*		Variation in *b*		*SS* pooled *b*	$S[(x-\bar{x})(y-\bar{y})]$	$S(x-\bar{x})^2$
	SS	*N*	*SS*	*N*			
12·5 per cent.	0·610407	13	0·521200	3	7·346699	5·204340	3·686711
15·0 per cent.	0·419439	9	0·120120	3	3·629575	2·643049	1·924663
17·5 per cent.	0·222761	11	0·128753	3	3·626417	2·917334	2·346900
20·0 per cent.	0·052597	5	0·079633	3	3·042701	2·356392	1·824886
Totals	1·305204	38	0·849706	12	17·645392	13·121115	9·783160

$$\bar{b} = \frac{13 \cdot 121115}{9 \cdot 783160} = 1 \cdot 341194$$

$$SS \text{ for joint regression} = \frac{(13 \cdot 121115)^2}{9 \cdot 783160} = 17 \cdot 597960$$

TABLE IIID

MEAN SQUARES OF THE VARIATIONS IN THE PROBIT-LOG. TIME REGRESSIONS FROM DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOETHYL ETHER AT 30°C.

Item	*N*	Sum of squares	Mean square
Grand regression	1	17·597960	17·595960
Variation in regression between concentrations ...	3	0·047432	0·015811
Variation in regression within concentrations	12	0·849706	0·070809
Residual in *y*	38	1·305204	0·034348

Test of significance of the difference between the mean squares for variation in regression between concentrations and variations in regression between individual tests.

The same formulas as have been used in Part VI[1] are again used here.

$N_1=19$ and $N_2=73$, therefore $h=30{\cdot}1522$.

$$\text{Hence } z_{(5 \text{ per cent.})}=\frac{1{\cdot}6449}{\sqrt{30{\cdot}1522-1}}-0{\cdot}7843\left(\frac{1}{19}-\frac{1}{73}\right)=0{\cdot}27416$$

The observed value of $z(1{\cdot}99119)$ is less than that calculated at the 5 per cent. level, hence $P>0{\cdot}05$.

TABLE IVA

SUMMARY OF TERMS FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOPROPYL ETHER AT 30°C.

Observation	Concentration of ethylene glycol monopropyl ether			
	3 per cent.	4 per cent.	5 per cent.	6 per cent.
$S[(x-\bar{x})(y-\bar{y})]$	1·890377	1·439740	2·695830	4·639438
$S(x-\bar{x})^2$	0·897739	0·599865	1·445619	2·113064
$S(y-\bar{y})^2$	4·421353	4·197453	5·949648	11·016602
N	5	5	6	11
SS for individual regressions... ...	4·274127	4·093332	5·760662	10·876641
b...	2·105709	2·400107	1·864827	2·195597
SS pool...	3·980584	3·455530	5·027254	10·186338

TABLE IVB

CALCULATION OF THE ERROR MEAN SQUARE OF REGRESSIONS FROM CONCENTRATIONS OF ETHYLENE GLYCOL MONOPROPYL ETHER AT 30°C.

Concentration	Item	Sum of squares	N	Mean square
3·0 per cent.	Common regression	3·980584	1	3·980584
	Variation in regression ...	0·293543	2	0·146772
	Total	4·274127	3	
	Residual in y	0·147226	5	0·029445
4·0 per cent.	Common regression	3·455530	1	3·455530
	Variation in regression ...	0·637802	3	0·212601
	Total	4·093332	4	
	Residual in y	0·104121	5	0·020824
5·0 per cent.	Common regression	5·027254	1	5·027254
	Variation in regression ...	0·733408	3	0·244469
	Total	5·760662	4	
	Residual in y	0·188986	6	0·031498
6·0 per cent.	Common regression	10·186338	1	10·186338
	Variation in regression ...	0·690303	3	0·230101
	Total	10·876641	4	
	Residual in y	0·139961	11	0·012724

Since there was no significant difference between the two items, the sums of squares have been combined and divided by the appropriate number of degrees of freedom to give an error mean square of 0·094755 (Table VIII).

TABLE IVc

SUMMARY OF STATISTICAL DATA FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF CONCENTRATIONS OF ETHYLENE GLYCOL MONOPROPYL ETHER AT 30°C.

Concentration	Residual in y		Variation in b		SS pooled b	$S[(x-\bar{x})(y-\bar{y})]$	$S(x-\bar{x})^2$
	SS	N	SS	N			
3·0 per cent.	0·147226	5	0·293543	2	3·980584	1·890377	0·897739
4·0 per cent.	0·104121	5	0·637802	3	3·455530	1·439740	0·599865
5·0 per cent.	0·188986	6	0·733408	3	5·027254	2·695830	1·445619
6·0 per cent.	0·139961	11	0·690303	3	10·186338	4·639438	2·113064
Totals	0·580294	27	2·355056	11	22·649706	10·665385	5·056287

$$\bar{b} = \frac{10{\cdot}665385}{5{\cdot}056287} = 2{\cdot}109331$$

$$SS \text{ for joint regression} = \frac{(10{\cdot}665385)^2}{5{\cdot}056287} = 22{\cdot}496832$$

TABLE IVd

MEAN SQUARES OF THE VARIATIONS IN THE PROBIT-LOG. TIME REGRESSIONS FROM DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOPROPYL ETHER AT 30°C.

Item	N	Sum of squares	Mean square
Grand regression	1	22·496832	22·496832
Variation in regression between concentrations ...	3	0·152874	0·050958
Variation in regression within concentrations	11	2·355056	0·214369
Residual in y	27	0·580294	0·021492

TABLE Va

SUMMARY OF TERMS FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOBUTYL ETHER AT 30°C.

Observation	Concentration of ethylene glycol monobutyl ether			
	1·5 per cent.	2·0 per cent.	2·5 per cent.	3·0 per cent.
$S[(x-\bar{x})(y-\bar{y})]$	3·109591	3·810624	4·120585	4·262868
$S(x-\bar{x})^2$	1·924012	2·589282	2·602655	2·416612
$S(y-\bar{y})^2$	5·471177	6·077422	6·713748	7·853541
N	11	11	10	9
SS for individual regressions... ...	5·048367	5·806799	6·591779	7·523255
b...	1·616202	1·471691	1·583224	1·763985
SS pool...	5·025726	5·608063	6·523808	7·519636

TABLE Vb

CALCULATION OF THE ERROR MEAN SQUARE OF REGRESSIONS FROM CONCENTRATIONS OF ETHYLENE GLYCOL MONOBUTYL ETHER AT 30°C.

Concentration	Item	Sum of squares	*N*	Mean square
1·5 per cent.	Common regression	5·025726	1	5·025726
	Variation in regression ...	0·022641	3	0·007547
	Total	5·048367	4	
	Residual in *y*	0·422810	11	0·038437
2·0 per cent.	Common regression	5·608063	1	5·608063
	Variation in regression ...	0·198736	3	0·066268
	Total	5·806799	4	
	Residual in *y*	0·270623	11	0·024602
2·5 per cent.	Common regression	6·523808	1	6·523808
	Variation in regression ...	0·067971	3	0·022986
	Total	6·591779	4	
	Residual in *y*	0·121969	10	0·012197
3·0 per cent.	Common regression	7·519636	1	7·519636
	Variation in regression ...	0·003619	3	0·001206
	Total	7·523255	4	
	Residual in *y*	0·330286	9	0·036699

TABLE Vc

SUMMARY OF STATISTICAL DATA FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF CONCENTRATIONS OF ETHYLENE GLYCOL MONOBUTYL ETHER AT 30°C.

Concentration	Residual in *y*		Variation in *b*		*SS* pooled *b*	$S[(x-\bar{x})(y-\bar{y})]$	$S(x-\bar{x})^2$
	SS	*N*	*SS*	*N*			
1·5 per cent.	0·422810	11	0·022641	3	5·025726	3·109591	1·924012
2·0 per cent.	0·270623	11	0·198736	3	5·608063	3·810624	2·589282
2·5 per cent.	0·121969	10	0·067971	3	6·523808	4·120585	2·602655
3·0 per cent.	0·330286	9	0·003619	3	7·519636	4·262868	2·416612
Totals	1·145688	41	0·292967	12	24·677233	15·303668	9·532561

$$\bar{b} = \frac{15 \cdot 303668}{9 \cdot 532561} = 1 \cdot 605410$$

$$SS \text{ for joint regression} = \frac{(15 \cdot 303668)^2}{9 \cdot 532561} = 24 \cdot 568660$$

TABLE Vd

MEAN SQUARES OF THE VARIATIONS IN THE PROBIT-LOG. TIME REGRESSIONS FROM DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOBUTYL ETHER AT 30°C.

Item	*N*	Sum of squares	Mean square
Grand regression	1	24·568660	24·568660
Variation in regression between concentrations ...	3	0·108573	0·036191
Variation in regression within concentrations	12	0·292967	0·024414
Residual in *y*	41	1·145688	0·027944

Test of significance of the difference between the mean squares for the variation in regression between the different compounds and the residual in y.

Here $N_1=5$ and $N_2=232$; from the statistical tables it is seen that the observed value of z (0·894322) is larger than the theoretical value even at the 0·1 per cent. level, (about 0·7), hence $P<0·001$.

TABLE VIA

SUMMARY OF TERMS FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOHEXYL ETHER AT 30°C.

Observation	Concentration of ethylene glycol monohexyl ether				
	0·325 per cent.	0·350 per cent.	0·375 per cent.	0·400 per cent.	0·425 per cent.
$S[(x-\bar{x})(y-\bar{y})]$	2·915882	1·959550	1·374006	2·103304	2·816573
$S(x-\bar{x})^2$	1·219958	0·753519	0·751145	0·987800	1·619699
$S(y-\bar{y})^2$	7·555418	5·700525	3·132374	4·970084	5·323962
N	11	7	8	7	6
SS for individual regressions ...	7·147758	5·402183	2·776206	4·656061	5·117676
b	2·390150	2·600532	1·829215	2·129281	1·738948
SS pool	6·969394	5·095872	2·513353	4·478526	4·897875

TABLE VIB

CALCULATION OF THE ERROR MEAN SQUARE OF REGRESSIONS FROM CONCENTRATIONS OF ETHYLENE GLYCOL MONOHEXYL ETHER AT 30°C.

Concentration	Item	Sum of squares	*N*	Mean square
0·325 per cent.	Common regression	6·969394	1	6·969394
	Variation in regression ...	0·178364	3	0·059455
	Total	7·147758	4	
	Residual in *y*	0·407660	11	0·037060
0·350 per cent.	Common regression	5·095872	1	5·095872
	Variation in regression ...	0·306311	3	0·102104
	Total	5·402183	4	
	Residual in *y*	0·298342	7	0·042622
0·375 per cent.	Common regression	2·513353	1	2·513353
	Variation in regression ...	0·262853	3	0·087618
	Total	2·776206	4	
	Residual in *y*	0·356168	8	0·044521
0·400 per cent.	Common regression	4·478526	1	4·478526
	Variation in regression ...	0·177535	2	0·088767
	Total	4·656061	3	
	Residual in *y*	0·314023	7	0·04486
0·425 per cent.	Common regression	4·897875	1	4·897875
	Variation in regression ...	0·219801	2	0·109901
	Total	5·117676	3	
	Residual in *y*	0·206286	6	0·034361

TABLE VIc

SUMMARY OF STATISTICAL DATA FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS OF CONCENTRATIONS OF ETHYLENE GLYCOL MONOHEXYL ETHER AT 30°C.

Concentration	Residual in y		Variation in b		SS pooled b	$S[(x-\bar{x})(y-\bar{y})]$	$S(x-\bar{x})^2$
	SS	N	SS	N			
0·325 per cent.	0·407660	11	0·178364	3	6·969394	2·915882	1·219958
0·350 per cent.	0·298342	7	0·306311	3	5·095872	1·959550	0·753519
0·375 per cent.	0·356168	8	0·262853	3	2·513353	1·374006	0·751148
0·400 per cent.	0·314023	7	0·177535	2	4·478526	2·103304	0·987800
0·425 per cent.	0·206286	6	0·219801	2	4·897875	2·816573	1·619699

$$\bar{b} = \frac{11{\cdot}169315}{5{\cdot}332121} = 2{\cdot}094723$$

$$SS \text{ for joint regression} = \frac{(11{\cdot}169315)^2}{5{\cdot}332121} = 23{\cdot}396618$$

TABLE VId

MEAN SQUARES OF THE VARIATIONS IN THE PROBIT-LOG. TIME REGRESSIONS FROM DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL MONOHEXYL ETHER AT 30°C.

Item	N	Sum of squares	Mean square
Grand regression	1	23·396618	23·396618
Variation in regression between concentrations ...	4	0·558402	0·139605
Variation in regression within concentrations	13	1·144864	0·088066
Residual in y	39	1·582479	0·040576

Test of significance of the difference between the mean squares for the variation in regression between concentrations and the residual in y.

Here $N_1=19$ and $N_2=232$, therefore $h=35{\cdot}1235$; from this $z_{(5 \text{ per cent.})}=0{\cdot}24366$.

The observed value of $z(0{\cdot}299278)$ is greater than that calculated at the 5 per cent. level, hence $P<0{\cdot}05$.

Test of significance of the difference between the mean squares for the variation in regression between tests and the residual in y.

Here $N_1=73$ and $N_2=232$, therefore $h=111{\cdot}056$; from this $z_{(5 \text{ per cent.})}=0{\cdot}1497$.

The observed value of $z(0{\cdot}308143)$ is greater than that calculated at the 5 per cent. level, hence $P<0{\cdot}05$.

Test of significance of the difference between the mean squares for the variation in the pooled regression (i.e. between concentrations between tests and residual in y.)

Here $N_1=92$ and $N_2=232$, hence $h=131{\cdot}7531$; from this $z_{(5 \text{ per cent.})}=0{\cdot}1387$.

The observed value of $z(0{\cdot}3063)$ is greater than that calculated at the 5 per cent. level, hence $P<0{\cdot}05$.

TABLE VII

SUMMARY OF MASSED STATISTICAL DATA FROM CALCULATIONS OF PROBIT-LOG. TIME REGRESSIONS FOR CONCENTRATIONS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS AT 30°C.

Compound	Range of concentrations investigated	Residual in y		Variation in b				Grand regression pooled SS	$S[(x-\bar{x})(y-\bar{y})]$	$S(x-\bar{x})^2$
				Between tests		Between concentrations				
		SS	N	SS	N	SS	N			
	per cent.									
Ethylene glycol	62·5 to 70·0	1·082481	42	1·518041	13	0·621270	3	19·009529	14·692814	11·356346
Monomethyl ether ...	35·0 to 42·5	0·759572	45	0·806258	12	0·262025	3	19·688695	15·010728	11·444230
Monethyl ether	12·5 to 20·0	1·305204	38	0·849706	12	0·047432	3	17·597960	13·121115	9·783160
Monopropyl ether ...	3·0 to 6·0	0·580294	27	2·355056	11	0·152874	3	22·496832	10·665385	5·056287
Monobutyl ether	1·5 to 3·0	1·145688	41	0·292967	12	0·108573	3	24·568660	15·303668	9·532561
Monohexyl ether	0·325 to 0·425	1·582479	39	1·144864	13	0·558402	4	23·396618	11·169315	5·332121
Totals		6·455718	232	6·966892	73	1·750576	19	126·758294	79·963025	52·504705

$$\bar{b} = \frac{79{\cdot}963025}{52{\cdot}504705} = 1{\cdot}522969$$

$$SS \text{ for joint regression} = \frac{(79{\cdot}963025)^2}{52{\cdot}504705} = 121{\cdot}781173$$

Calculation of the standard errors of the probit-log. time regression coefficients at 30°C.

The variance of b is given by the formula $V_b = \frac{V_y}{S(x\text{-}\bar{x})^2}$ where V_y is the variance of y (the probit), i.e. the error mean square (0·094755, Table VIII) and $S(x\text{-}\bar{x})^2$ is the corrected sum of squares of x (the log.

TABLE VIII

ANALYSIS OF VARIANCE OF MASSED REGRESSIONS FOR DISINFECTION OF *BACT. COLI* BY CONCENTRATIONS OF ETHYLENE GLYCOL AND ITS MONALKYL ETHERS AT 30°C.

Item	*N*	*SS*	Mean square	Variance ratio	Probability
Massed regression	1	121·781173	121·781173		
Variation in regression between compounds	5	4·977121	0·995424		
Variation in regression between concentrations	19	1·750576	0·092136	see Table IX	
Variation in regression between tests	73	6·966892	0·095437		
Residual in *y*	232	6·455718	0·027826		
Pooled error	92	8·717468	0·094755		

TABLE IX

CALCULATION OF *z*'S FOR THE ITEMS IN THE ANALYSIS OF VARIANCE OF THE MASSED REGRESSIONS IN TABLE VIII

N_1/N_2	Variance ratio (V.R.)	$\log_{10}$V.R.	$\log_e$ V.R. = $\log_{10}$ V.R. × 1·15129	$z = \frac{1}{2}\log_e$ V.R.	Probability
19/73	0·965412	$\bar{1}$·9847	$\bar{1}$·98238	1·99119	>0·05
5/232	35·773162	1·5536	1·78864	0·89432	<0·001
19/232	3·311148	0·5199	0·59856	0·29928	<0·05
73/232	3·429774	0·5353	0·61629	0·30814	<0·05
92/232	3·405000	0·5321	0·6126	0·3063	<0·05

TABLE X

THE PROBIT-LOG. TIME REGRESSION COEFFICIENTS WITH THEIR STANDARD ERRORS, OF THE REACTION BETWEEN *BACT. COLI* AND ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS AT 30°C.

Compound	*b*	*N*	$S(x\text{-}\bar{x})^2$	V_y	$s_b = \frac{V_y}{S(x\text{-}\bar{x})^2}$	Ratio of *b* to s_b
Ethylene glycol	1·293798	13	11·356346	0·094755	0·09135	14
Monomethyl ether	1·311642	12	11·444230		0·09099	14
Monoethyl ether	1·341194	12	9·783160		0·09842	14
Monopropyl ether	2·109331	11	5·056287		0·01369	15
Monobutyl ether	1·605410	12	9·532561		0·09978	16
Monohexyl ether	2·094723	13	5·332121		0·13330	16

time) for the regression lines of each compound. The standard error of the regression coefficients (V_b) have been computed and set out in Table X. As in the experiments at 20°C., the ratio of the regression coefficients to their slopes is large, thereby indicating that b has been satisfactorily estimated. Comparison with the results in Part VI, Table IX, indicates that the values of b are higher at 30°C. than at 20°C.

Conclusions

As with the results from the experiments at 20°C. (Part VI[1]), the analysis of variance indicates that there is a significant variation in regressions between the different compounds, i.e. each substance has its characteristic regression coefficient which differs significantly from the average of the series.

The analysis also shows that the variations in the regressions between the concentrations of the substances are of the same order as the variation between the individual tests at a particular concentration. Hence the regressions of different concentrations of the same substance may be taken as parallel.

Summary

1. The course of the disinfection (at 30°C.) between *Bact. coli* and several concentrations of ethylene glycol and the following ethers : monomethyl, monoethyl, monopropyl, monobutyl and monohexyl has been investigated. Several experiments were conducted at every concentration and probit-log. time regressions calculated for all experiments.

2. For every concentration of a substance the sum of squares for the common regression and for the variation in regression were calculated ; the error mean square of the regression was also computed.

3. The data for every concentration of each compound have been pooled and a mean regression has been calculated for each compound.

4. The statistical data from all the calculations for the terms of the regressions for every concentration of the compounds (at 30°C.) have been massed and an analysis of variance carried out.

5. The probabilities for the differences between the mean squares of the items in the analysis of variance have been deduced by means of the z distribution.

6. No significant difference could be shown between the variation in regression between concentrations and between tests ; these two errors have been pooled in order to establish the error mean square for all the estimations performed.

7. The probit-log.time regression coefficient for every compound has been compared with its standard error ; in all cases the ratio was large, thereby indicating that b had been estimated satisfactorily.

References

1. Berry and Michaels, *J. Pharm. Pharmacol.*, 1949, **1**, 470.
2. Berry and Michaels, *Quart. J. Pharm. Pharmacol.*, 1947, **210**, 331.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Cinchonamine, Quinamine and Yohimbol, Relation between. M. Raymond-Hamet. (*C. R. Acad. Sci., Paris,* 1948, **227,** 1182.) Cinchonamine has the same empirical formula as yohimbol, the ultra-violet absorption curves are identical, and both give similar colour reactions. On the other hand the different physiological actions of these two compounds suggest a difference in the nuclear skeleton. Cinchonamine only differs from quinamine by having one oxygen atom less, and it has been suggested that its structure only differs by having CH_2OH in place of CH_3 in the α-position on the indole group. Iodomethylation of yohimbine considerably decreases the sympathicolytic activity, whereas with cinchonamine and quinamine it produces a considerable increase in this action. Further the Sivadjian reaction gives quite different results with yohimbine and cinchonamine. In presence of an oxidising agent, cinchonamine gives with sulphuric acid an intense blue colour which is not obtained with quinamine, and is possibly due to the 2:3:4:5-tetrahydro-β-carbolinic grouping. The absorption spectra of cinchonamine and of quinamine are quite different. G. M.

ANALYTICAL

Acetone, Determination of. R. E. Byrne. (*Anal. Chem.,* 1948, **20,** 1245.) A method is described which is accurate for amounts of acetone up to 25 μg./ml., being based on the estimation of the hydrochloric acid released after combination of the acetone with hydroxylamine hydrochloride. The release of acid causes a drop in *p*H and there is a definite relation between *p*H change and the quantity of acetone present. Standard curves are given connecting *p*H with acetone content under the prescribed conditions. The effect of ethyl alcohol on the *p*H is also studied. The range of sensitivity of the curves may be extended by varying the concentration of hydroxylamine hydrochloride used. Determinations are not perceptibly affected by variations of room temperature between 20° and 30° C. R. E. S.

Acetylacetone and Related β-Diketones, Colorimetric Determination of. R. F. Witter, J. Snyder and E. Stotz. (*J. biol. Chem.,* 1948, **176,** 493.) Methods are given for the colorimetric determination of 2 to 8 micromoles of a number of β-diketones. The colour reaction involves a condensation of the diketone with *o*-phenylenediamine in acid solution to produce a reddish purple colour. Acetylacetone in this reaction yields 2:4-dimethyl-1:5-benzodiazapine hydrochloride; triacetic acid appeared to be decarboxylated to acetylacetone during the period of colour development; ethyl triacetate either reacted directly or was partly hydrolysed since the colour per mole of ester was less than with acetylacetone or the free acid; triacetic lactone did not react and was determined after conversion to acetylacetone by hot acid hydrolysis. Detailed conditions for the reaction are given in which the colour developing during 30 minutes at room tem-

perature was determined at 500 mμ using a photo-electric colorimeter. No colour was formed with diacetyl, acetoin, acetone, acetaldehyde, succinic acid, fumaric acid, acetoacetic acid, oxalacetic acid, lævulinic acid, citric acid, ethyl acetoacetate, ethyl lævulinate, or diethyl acetonedicarbozylate. The following substances interfered with the reaction; tungstic acid, oxidising agents, bisulphite, hydroxylamine, semicarbazide, pyruvic acid, diacetyl, formaldehyde, crotonaldehyde. The crotonaldehyde interference due to the production of a yellow colour with the reagent could be eliminated by the use of sodium bisulphite. A 10 per cent. glucose solution or 0·4 M urea solution did not interfere. The recovery of acetylacetone or triacetic lactone added to various biological systems was of the order of 95 to 100 per cent.

R. E. S.

Adrenaline, Colorimetric Determination of. M. Péronnet. (*Ann. pharm. Franc*, 1948, **6**, 365.) In the colorimetric determination of adrenaline, using iodine as oxidant, it is usual to remove the excess of the latter by means of thiosulphate. Since secondary reactions result from the thiosulphate, the author recommends that the excess of iodine be removed by shaking with benzene. The colour obtained in this way is stable over a considerable period, whereas that obtained by the thiosulphate method steadily decreases. Details are as follows. To 5 ml. of a solution of adrenaline 5 ml. of a 10 per cent. solution of sodium acetate and 2 ml. of 0·02N iodine solution are added; after 5 minutes, 2·5 ml. of water is added and the excess of iodine is removed by shaking with three successive 5 ml. quantities of benzene; the colour is then determined. G. M.

Adrenaline in Pharmaceutical Products, Photometric Determination of. J. R. Doty. (*Anal. Chem.*, 1948, **20**, 1166.) The determination depends on the addition of an alkaline buffer to a slightly acid solution containing adrenaline and a ferrous salt, when a blue colour begins to develop at about *p*H 6·5 and gradually changes to the characteristic red-blue colour which attains a maximum intensity at about *p*H 8 to *p*H 8·5. The method is mostly of value when the adrenaline content is at least 10 p.p.m., optical density measurements being made at 530 mμ. The reaction appears to be specific for compounds possessing at least two phenolic groups attached to adjacent carbon atoms. No colour is produced with phenol, neosynephrine, resorcinol, hydroquinone, orcinol, phloroglucinol, or phthalic acid but an atypical colour response is observed with as little as 5 mg. of salicylic acid. Typical colour reactions are produced by pyrocatechol, pyrogallol, adrenaline and cobefrin. Among inorganic ions bisulphite affects the colour intensity. The method can be used in the presence of procaine hydrochloride without turbidity. Solutions of tetracaine hydrochloride, metycaine hydrochloride and some other products become cloudy or milky when the buffer reagent is added; in this case isopropyl alcohol is added to the anæsthetic solution and the colour is then developed as usual. R. E. S.

Arsenic, Determination of Small Quantities of. P. Paulssen. (*Pharm. Weekbl.*, 1949, **84**, 33.) The method, as applied to arsenic pills, is as follows. 2 pills are rubbed down with a little 6N hydrochloric acid and transferred to a conical flask with 10 to 15 ml. of the acid. 50 mg. of potassium chlorate is added and, after standing for 5 minutes, the mixture is heated on the water-bath for 10 minutes. The mixture is cooled, and the treatment repeated with a further 50 mg. of potassium chlorate. After filtering through a plug of cotton wool, the filtrate is treated with 10 ml. of a 10 per cent.

solution of calcium hypophosphite in 25 per cent. hydrochloric acid, and a little finely divided asbestos is added to the mixture, which is heated on the water-bath for 30 minutes. The precipitated arsenic is filtered off on asbestos supported on a perforated platinum plate, and washed with water, then with alcohol and finally again with water. The plate with the deposit is transferred to a flask, the filter tube being washed with water, and treated with 25 ml. of 0·008N ceric sulphate solution. After the arsenic has completely dissolved, a few drops of a 0·25 per cent. solution of osmic acid in dilute sulphuric acid are added, and one drop of 0·025M *o*-phenanthroline iron solution, and the excess of ceric sulphate is titrated with 0·005N arsenious acid to the first change to red. The method may also be used for atoxyl and neosalvarsan. G. M.

***p.p'*-Dichlorodiphenyltrichloroethane (D.D.T., Dicophane) in commercial samples.** R. L. Wain and A. E. Martin. (*Analyst*, 1948, **73**, 479.) The determination by the dehydrohalogenation method was studied in detail with particular reference to the behaviour of the *ortho* compound which is the most important impurity in commercial samples. The rates of reaction of samples when treated with standard alcoholic potassium hydroxide solution were determined by estimation of the chloride produced, using Volhard's method. At 23°C. the dehydrohalogenation of both the *o,p'*- and the *p,p'*- compounds was found to be a second-order reaction with rate constants of 0·0008971 and 0·03704 l./mol/sec. respectively. Tables and graphs are given for the rates of dehydrohalogenation of known mixtures of pure *o,p*- and *p,p'* compounds and also for commercial samples of known *p,p'*- content (as determined by Balaban and Calvert's crystallisation method). A reaction time of 60 minutes at 23°C. was chosen for the determination since this effected complete dehydrohalogenation of the *p,p'*- compound while only 0·2 equivalent of chloride ion per mol. was lost from the *o,p'*-isomer. A graph of chloride ion liberated from commercial samples treated under these conditions against *p,p'*-isomer content is given and the following equation: percentafie of *p,p-isomer* = (1·56 x mg. of Cl'/g. of sample) − 58·1, is tentatively suggested. R. E. S.

Phenol and Structurally Related Compounds, Determination of, by the Gibbs Method. M. B. Ettinger and C. C. Ruchhoft. (*Anal. Chem.*, 1948, **20**, 1191.) A detailed study is made of the determination of phenols by means of 2:6-dibromoquinone chloroimide. For the determination of phenol in aerobic surface waters, extraction of the colour produced with *n*-butyl alcohol is recommended. The reaction itself should be carried out at room temperature (±1°C.) for both sample and standard at *p*H 9·4 during 6 to 24 hours. The colour produced differs in the peak light absorption wave-length according to the phenolic compound used and a further difference is found between the value in water and in the *n*-butyl alcohol extract. The following phenols were studied: phenol, *o*-cresol, *m*-cresol, *a*-naphthol, *o*-chlorophenol, *p*-chlorophenol. The colour intensification obtained by *n*-butyl alcohol extraction based upon increase in extinction at the wave lengths of maximum adsorption was 7·3 and 7·5 times for phenol and *o*-cresol, respectively. The limits of linear relationship between the reaction products and the colour formed were found to be up to 0·1 p.p.m. for phenol and at least to 0·3 p.p.m. for *o*-cresol. On the basis of these studies a detailed procedure for phenols and cresols in surface waters, using spectrophotometric examination at the wave-lengths of maximum adsorption, is proposed. An accelerated procedure for obtaining early information, with concurrent

loss of some sensitivity and accuracy, is also given. On waters containing 0·1 p.p.m. of the pollutants the recommended procedure was capable of results with a probable error of only ±2·3, ±1·7, and ±2·3 per cent. for one observation for phenol, *o*-cresol, and *m*-cresol, respectively. R. E. S.

ANIMAL SUBSTANCES

Gelatin, Behaviour of Solutions of. G. Rosi and P. M. Strocchi. (*Ann. Chim. appl., Roma,* 1948, **38,** 571.) Experiments were made with a sample of gelatin which contained 2·5 per cent of ash (A) and the same sample after partial (B) and complete (C) electrodialysis, which reduced the ash to 0·26 per cent. and 0·05 per cent. respectively, to see what effect the ash had on its behaviour. The *p*H of the original sample A was 6·76, of B 4·52, and of C 4·65 in a concentration of 0·8065 per cent. Progressive amounts of hydrochloric acid were added, the final amount of 10 ml. of acid to 50 ml. of solution reduced the *p*H of A to 2·17, B to 2·01 and C to 2·06, intermediate quantities showing intermediate results. Ten solutions of each grade containing 0·4032 per cent. of gelatin were adjusted by the addition of hydrochloric acid or sodium hydroxide to a series of *p*H between 2 and 7 and tested for conductivity, viscosity and surface tension. The results of the conductivity tests all showed a minimum in the neighbourhood of *p*H 4·63 and 4·72, the actual figures being highest for A and lowest for C. The curves of viscosity show a maximum between *p*H 2·85 and 3 and a minimum between *p*H 4·74 and 4·78. The minimum is more pronounced with greater purity of the gelatin. Surface tensions show a minimum at *p*H 4·48 for solution A and 4·32 for B and C; solutions B and C show a maximum at *p*H 2·9 and 3·3. H. D.

GUMS AND RESINS

***Aloe vera*, A Mucilage from.** E. Robez and A. J. Haagen-Smit. (*J. Amer. chem. Soc.,* 1948, **70,** 3248.) After drainage of the latex by cutting the leaves near the basal end, the leaves of *Aloe vera* readily yield their mucilaginous layer by scraping after cutting them parallel to the leaf-blade. The mucilaginous parenchyma so obtained, on drying, contains 0·2 per cent. of mucilage and the dried assimilatory tissue 1·73 per cent. Extraction of the ground dried mucilaginous material with alcohol (50 per cent.) and precipitation of the extract by addition of 4 volumes of alcohol (95 per cent.) yielded as precipitate a crude mucilage and a filtrate containing aloin not removed from the leaves by drainage of the latex. The crude mucilage contained 12·9 per cent. of ash which was removed by dialysis from a solution in 0·01N hydrochloric acid against distilled water and the mucilage recovered by precipitation on addition of alcohol. So purified the mucilage was a white amorphous powder decomposing at 271° to 276° C., insoluble in organic solvents, soluble in water to give an optically inactive viscous solution. Elemental analysis indicated the possible composition as a hexosan. With sulphuric acid, the mucilage yielded a hydrolysate free from ketoses, and containing 89 per cent. of aldoses (equal parts of mannose and glucose) and 2·37 per cent. of uronic acid. F. H.

Carob Gum, Constitution of. F. Smith. (*J. Amer. chem. Soc.,* 1948, **70,** 3249.) Carob gum, a polysaccharide obtained from the carob bean (*Ceratonia Siliqua* L.) by extraction of the seeds with water or aqueous

alkaline solution, is shown to be composed of D-galactose (20 per cent.) and D-mannose (80 per cent.). The methylated gum obtainable either directly or through the acetate by treatment with methyl sulphate and sodium acetate, yielded on methanolysis the glycosides of 2 : 3 : 4 : 6-tetramethyl-D-galactose (1 part), 2 : 3 : 6-trimethyl-D-mannose (2 to 3 parts) and 2 : 3-dimethyl-D-mannose (1 part), identified by formation of the crystalline derivatives. The structure of the galacto-mannan polysaccharide is discussed. F. H.

ORGANIC CHEMISTRY

Pteridine, A Synthesis of. W. G. M. Jones. (*Nature*, 1948, **162**, 524.) 2-Chloro-4-amino-5-nitropyrimidine, m.pt. 232°C. is hydrogenated in methyl alcohol over a nickel catalyst to give 2-chloro-4:5-diaminopyrimidine, m.pt. 232°C. which is dehalogenated catalytically over palladium on charcoal in the presence of barium oxide to give 4:5-diaminopyrimidine, m.pt. 204°C. (nitrate decomposes without melting above 260°C.) Reaction of the diaminopyrimidine in aqueous solution with glyoxal bisulphite gave pteridine, crystallizing from alcohol in pale yellow plates, m.pt. 140°C. Pteridine was soluble in water and alcohol and readily sublimed *in vacuo*. The ultra-violet absorption spectrum in aqueous solution at *p*H 5·8 showed a sharp maximum at $299m\mu.\epsilon = 7{,}890$, with a violet-blue fluorescence in the ultra-violet in neutral or alkaline solution. It formed a picrate, m.pt. 117·5°C. and an oxalate which decomposed without melting above 128°C. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Benzylpenicillinic Acid, a Crystalline Form of. N. R. Trenner and R. P. Buhs. (*J. Amer. chem. Soc.*, 1948, **70**, 2897.) The free acid, benzylpenicillinic acid, associated with one molecular proportion of di-*iso*propyl ether of crystallisation, is obtainable in crystalline form by extraction of an aqueous solution of sodium benzylpenicillinate (sodium penicillin G) at *p*H 2·5 with di-*iso*propyl ether, drying the ether phase over anhydrous sodium sulphate and allowing evaporation at room temperature. This procedure enabled crystalline benzylpencillinic acid di-*iso*propyl etherate to be separated from other penicillins and from a crude penicillin sodium salt. Crystalline benzylpenicillinic acid di-*iso*propyl etherate was found to retain its stability over long periods even when exposed to ordinary laboratory air. It was not hygroscopic and is considered to be superior to any other form of benzylpenicillin (penicillin G) as a primary standard of purity. Dissolved in a *p*H 7 phosphate buffer it contained 1420 units/mg. when assayed by the cup-plate method against *Staphylococcus aureus*, this activity being equivalent to 1740 units/mg. in the sodium salt. The optical activity, equivalent weight potentiometrically, ultra-violet and infra-red spectra were examined and are discussed as evidence of the purity and nature of the crystalline material. That the di-*iso*propyl ether merely permits the formation of a well-defined crystal lattice and so is present as a simple solvate of crystallisation and does not form an oxonium salt with benzylpenicillin is supported by the inability to induce crystallisation of benzylpenicillinic acid in the presence of a wide range of other ethers. F. H.

N-Methylvaline, Optical Resolution of, A New Phenomenon. A. H. Cook, S. F. Cox and T. H. Farmer. (*Nature*, 1948, **162**, 61.) A new and simple

method of resolution of N-methylvaline has been found in connection with the study of the nature and configuration of hydrolytic products of the antibiotic lateritiin-I derived from *Fusarium lateritium*. It was found that the lactone derived from D-α-hydroxy*iso*valeryl-D-N-methylvaline is more strongly adsorbed on an acid-washed alumina column than that derived from D-α-hydroxy*iso*valeryl-N-methylvaline and undergoes hydrolysis. *Laevo*-α Brom*iso*valeryl chloride and DL-N-methylvaline were condensed together and the product was refluxed with pyridine and water when the hydroxy-acid condensation product was formed. It was extracted and heated for several hours on a steam-bath under reduced pressure to yield a lactone having $[\alpha]_D$ +52° in alcohol. On passing an ethereal solution of the lactone through an acid-washed alumina column about half the material was lost. The remainder had $[\alpha]_D$ + 79° in alcohol and on hydrolysis yielded L(+)-N-methyvaline. F. H.

Penicillins, Synthetic, Preparation and Antibacterial Properties of Crude Sodium Salts. F. H. Carpenter, G. W. Stacey, D. S. Genghof, A. H. Livermore and V. du Vigneaud. (*J. biol. Chem.*, 1948, **176,** 915.) Minute yields of crude sodium salts of several penicillins were obtained by synthesis. In the reaction used, appropriate oxazolones were condensed with α-amino-β-mercapto acids to yield penicillenic acids which were isolated as amorphous solids characterised by their ultra-violet absorption spectra. The penicillenic acids were converted in small yield to the corresponding penicillins, the reaction conditions depending on the type of acid involved. In this manner benzylpenicillin analogues in which the D-penicillamine fraction was replaced by two isomeric DL-β-methyl cysteines, by DL-β,β-diethylcysteine, or by DL-β-ethyl-β-methyl cysteine were prepared. In addition, crude sodium D-phenylpenicillin and D-styrylpenicillin were synthesised. Antibiotic activities against *Staphylococcus aureus* H, *Bacillus subtilis*, and *Vibrio metchnikovii* were determined. Qualitative tests showed that the crude synthetic penicillins did not possess marked antibacterial properties against *Aerobacter aerogenes*, *Klebsiella pneumoniæ*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Proteus vulgaris OX*-19, *or Pseudomonas æruginosa*, organisms which also showed a high degree of resistance to the action of crystalline sodium benzylpenicillin. R. E. S.

Pituitary, Anterior, Crystalline Growth Hormone, A New Preparation of. A. E. Wilhelmi, J. B. Fishman and J. A. Russell. (*J. biol. Chem.*, 1948, **176,** 735.) A new method is described for the preparation of crystalline growth hormone from fresh bovine anterior pituitary glands. Fractionation of calcium hydroxide extracts of the ground glands, using ethyl alcohol at low temperatures following the method of Cohn *et al.* (*J. Amer. chem. Soc.*, 1946, **68,** 459) for the separation of plasma proteins, yielded an abundance of crude fractions with high growth-promoting activity. The active fractions were redissolved in dilute potassium chloride solution followed by removal of the bulk of impurities in a precipitate formed at *p*H 5·0 and by a fractional precipitation with ethyl alcohol from *p*H 8·5 to 8·7, when a crystalline protein was obtained, electrophoretically homogeneous, which from its biological activity and other properties was identified as the anterior pituitary growth hormone. The yield of the crude primary fractions averaged about 33 g./kg. of fresh glands, with a corresponding yield of the order of 3 g. of pure crystalline growth hormone. The pure crystalline

product had the expected effects upon body growth and upon the width of the proximal epiphyseal cartilage of the tibia in hypophysectomised rats. It was also the most active glycostatic hormone preparation yet isolated.

R. E. S.

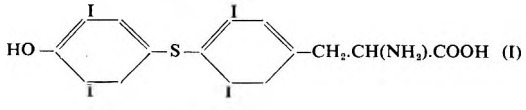
Riboflavine and Allied Substances, Production during the Growth of *Corynebacterium diphtheriæ*. A. J. Woiwod and F. V. Linggood. (*Nature*, 1948, **162,** 219.) The report of Wadsworth and Crowe (*J. Infect. Dis.*, 1943, **73,** 106) that a special strain of *C. diphtheriæ*, grown on a synthetic medium, elaborates a flavine, has been confirmed. Park Williams No. 8 strain of *C. diphtheriæ*, grown on casein hydrolysate produced a flavine which gave two fluorescent spots on a paper partition chromatogram, using *n*-butyl alcohol-acetic acid as a solvent. Evidence has been produced that the faster-running spot was riboflavine, and it has been suggested that the slower running spot was either riboflavine phosphate or flavine adenine dinucleotide.

F. H.

Sodium Ascorbate, Crystalline Form of. S. L. Ruskin and A. T. Merrill. (*Science*, 1948, **108,** 713.) A stable sodium ascorbate is obtained by the reaction of ascorbic acid with sodium methylate in methyl alcohol. The product was more stable than ascorbic acid itself and showed no decline in potency after 500 hours at 45°C. in closed glass containers. For the preparation 88 g. of ascorbic acid was dissolved in 600 ml. of hot absolute methyl alcohol. While still hot, it was treated under stirring with 250 ml. of a warm solution of sodium methylate containing 12·5 g. of sodium. The combined solutions were stirred until the resulting precipitate of sodium ascorbate crystallised (about 15 minutes); the sodium ascorbate was then filtered with suction and washed with a little methyl alcohol. It could be dried *in vacuo* at a temperature as high as 100°C. producing a 95 per cent. yield of pure product. Analysis indicated the presence of about 1 per cent. of water; the rotation was + 102·99° and the assay by iodine titration gave a result of 87·55 per cent. (theory 88·9). Further procedures were developed using sodium hydroxide, sodium carbonate, or sodium hydride with similar results. Petrographic studies showed the variety of crystallisations occurring and, together with the nature of the hydroxyl group reaction during neutralisation, provided evidence suggesting a possible explanation of the stability of the sodium ascorbate crystals prepared.

R. E. S.

Thyroxine, Synthesis of a Sulphur-containing Analogue of. C. R. Harington. (*Biochem. J.* 1948, **43,** 434.) The possibility of controlling thyroid function by means of compounds inhibiting the output of thyrotrophic hormone from the anterior pituitary gland is discussed. The compound (I) which is identical with thyroxine save that sulphur replaces oxygen, was selected for trial.



An attempt was made to synthesise the compound on the lines of thyroxine itself and by starting with 4-methoxythiophenol and 3:4:5-triiodonitrobenzene, 3:5-diiodo-4(4′-methoxy)phenylsulphidobenzaldehyde was obtained by a series of reactions similar to those employed in the thyroxine synthesis. At this stage the modified Erlenmeyer method did not yield

3 : 5-diiodo-4(4′-hydroxy)phenylsulphidophenylalanine and a different route had to be used. The aldehyde was reduced to the corresponding alcohol by means of aluminium *iso*propoxide; the alcohol was converted into the chloride and the latter condensed with ethyl acetamidomalonate; appropriate treatment of the resulting ester with a mixture of hydrobromic and acetic acids gave the hydroxydiiodoamino-acid in satisfactory yield and final iodination in ammoniacal solution afforded the desired thyroxine analogue. In acute experiments the thyroxine analogue was non-toxic to mice in doses up to 0·5 g./kg. (subcutaneous) and 0.25 g./kg. (intravenous). Tested on tadpoles (*Xenopus lævis*) it accelerated metamorphosis, its activity in this respect being approximately one-fifth of that of thyroxine. R. E. S.

Vitamin B_{12} Group of Factors, Chromatography of. W. F. J. Cuthbertson and E. L. Smith. (*Biochem. J.* 1949, **44,** v.) A combination of partition chromatography on paper with microbiological assay on a solid medium enables the two red clinically active substances, thymidine, and a fourth microbiologically active component present in liver extract to be demonstrated. Water-saturated *n*-butyl alcohol is used, with upward or downward development, two techniques being available for observing the developed spots. A drop of adequately purified material, containing at least 10μg. of the factors, gives directly visible red spots. The crystalline factor gives a single spot, but the mother liquors usually give a second fainter and slower-moving spot and occasionally a third, which travels fastest and appears to be a microbiologically inactive degradation product. Fairly crude extracts can be used for the other technique since only 0·005 and 0·1 μg. of the factors are required: the developed strip is applied to the surface of nutrient agar seeded with *Lactobacillus lactis* Dorner, being removed after 10 min. and the plate incubated overnight. The usual pattern is an ellipse of growth not far from the origin and another a few cm. along, while much farther along, and beyond the position occupied by riboflavine (if present), are one or two zones of attenuated growth; the first two zones are due to the red factors, the third to a substance not yet characterised, and the fourth to thymidine. Samples of the 'animal protein factor' of bacterial origin were examined by this technique and two zones of strong growth and one or two of diffuse growth were always observed, these being always in the same relative positions as the zones from liver extract. It is concluded that the bacteria elaborate the anti-pernicious anæmia factor and probably also the second active red factor, as well as thymidine. R. E. S.

BIOCHEMICAL ANALYSIS

Aerial Bactericides, Evaluation of. (*Chem. Ind.*, 1949, **68,** 115.) This report of the Aerosols Panel of the British Disinfectant Manufacturers' Association proposes that aerosols and other gaseous products for which bactericidal claims are made should be recommended for use at such concentrations as are capable of reducing the bacterial content of the test chamber by not less than 85 per cent. when tested by the technique described. The test chamber is preferably cubical, of a capacity of 500 to 1,000 cu. ft., and suitably equipped with a filter for incoming air, fans for mixing the air and for ventilation, and an arrangement for sampling the air. The procedure is to count the normal bacterial population of the chamber, to spray it with a culture of the test organism and count again, and finally to disperse the

bactericidal agent and determine the percentage survival in the period 4 to 6 minutes from the commencement of the dispersal of the bactericidal agent. The whole procedure is carried out at a temperature of 20 ±3°C., and a relative humidity of 60 per cent. ±5 per cent. The test organism is a special non-pathogenic strain of *Staphylococcus albus* deposited at the National Collection of Type Cultures under the name *Staphylococcus albus*, Aerosols strain. It is grown on a special liquid culture medium and the culture is diluted with broth for spraying; for the production of the spray an M.A.3 Aerolyser Spray Assembly is used. The bactericidal agent is dispersed from an electrically heated boat of nickel-chrome alloy. The air in the chamber is sampled by means of a slit sampler and petri dishes containing a special solid culture medium and the plates are incubated for 24 hours at 37°C. before counting. To enable different workers to ensure that the strain of bacteria they are using is exhibiting normal resistivity and also to indicate the degree of reproducibility being attained by different laboratories, *cyclo*-pentanol-1-carboxylic acid is recommended as a standard reference bactericide. The technique described is also suitable as a preliminary sorting test for potential bactericides and for comparing the efficiencies of apparatus designed to disperse aerial bactericides.

G. R. K.

Calciferol, Colorimetric Determination of. P. B. Nielsen (*Dansk Tidsskr. farm.*, 1949, **23**, 21.) Calciferol may be determined colorimetrically within less than 3 per cent. by means of the antimony chloride reaction, provided alcohol is absent. Ordinary chloroform is purified by washing 3 times with water, drying over sodium sulphate, and fractionating, rejecting the first and last tenth. The middle fraction is collected over sodium sulphate. It should be tested for freedom from chlorides, oxidising agents (potassium iodide-starch) and alcohol—the latter by shaking 5 ml. with 20 ml. of water, and adding to 10 ml. of the aqueous solution 2 drops of 1·7 per cent. potassium dichromate solution and 5 ml. of concentrated sulphuric acid. The yellow colour of the solution should not change within 5 minutes. The reagent is prepared by dissolving 100 g. of antimony chloride in chloroform to 500 ml., filtering, and adding 10 g. of redistilled acetyl chloride. A quantity, up to 1 ml., of the solution to be tested, is treated with 3 or 4 ml. of the reagent, and the extinction (using filter S50) is measured within 1 to 2 minutes. For crystalline calciferol the extinction $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 500 mμ is about 1700. Solutions in arachis oil can be assayed directly, without saponification, down to 75,000 I.U./ml., but at lower concentrations allowance must be made for the absorption of the oil itself, determined in a similar manner. Solutions in alcohol may be assayed as follows: a suitable quantity of the solution is weighed into a 50-ml. measuring flask, 50 cg. of arachis oil is added, and carbon dioxide, previously washed with concentrated sulphuric acid, is passed through the flask. The oil should be clear within 45 minutes. The glass tube and neck of the flask is washed down with a little purified chloroform, and the gas is again passed for 30 minutes. The washing is repeated and the flask is filled up with purified chloroform, the test being then continued as before. A solution of calciferol in alcohol (96 per cent.), of 300,000 I.U./g., was found to be unchanged in strength after 14 months. G. M.

Caronamide, Colorimetric Determination of. C. Ziegler and J. M. Sprague. (*J. Lab. clin. Med.*, 1948, **35**, 96.) Caronamide (4′-carboxy-

phenylmethanesulphonanilide, $C_6H_5.CH_2.SO_2NH.C_6H_4.COOH$) was found to be resistant to hydrolytic cleavage except under conditions that resulted in the destruction of the *p*-aminobenzoic acid produced. The action of Raney catalyst (powdered nickel aluminium alloy) in alkaline solution, however, gave complete cleavage and the *p*-aminobenzoic acid could be determined by established procedures. Details are given for the determination of caronamide in water, urine, plasma and blood, using this process. In the procedures for plasma and blood, alcohol (90 per cent.) was used as a protein precipitant; in an alternative method, plasma in alkaline solution was treated directly with the alloy. The liberated *p*-aminobenzoic acid was determined colorimetrically by diazotisation using N-(1-naphthyl)-ethylenediamine dihydrochloride. Results obtained from aqueous solutions of *p*-aminobenzoic acid of known concentration showed that the alloy treatment caused a loss of from 10 to 20 per cent. of this compound although the recoveries at this level were fairly constant and reproducible. Since recovery experiments on caronamide itself gave losses of a similar order it appeared that the caronamide was split quantitatively but that an average of 15 per cent. of the liberated *p*-aminobenzoic acid was either destroyed or lost by adsorption on the finely divided nickel. The use of standard reference curves, prepared from readings of the depth of colour obtained when known amounts of caronamide in urine, plasma, or blood were subjected to the method, is therefore necessary.

R. E. S.

CHEMOTHERAPY

β-Aminoethyl Heterocyclic Nitrogen Compounds, Histamine Activity of. H. M. Lee and R. J. Jones (*J. Pharmacol.* 1949, **95,** 71.) A series of 24 β-aminoethyl heterocyclic nitrogen compounds was tested for histamine activity in the hope that some correlations between chemical constitution and histamine activity might be drawn. Eleven of the compounds were found to be active. The one structural feature common to all of the active compounds tested is the system in which the portion $-N{=}\overset{|}{C}-$ or $=N-\overset{\|}{C}-$ is part of an aromatic nucleus; $-N{=}\overset{\|}{C}-CH_2CH_2NH_2$ or $=N-C-CH_2CH_2NH_2$. The presence of this structural fragment does not, however, guarantee that a compound will have histamine activity; most of the inactive compounds also have the system in their structures. The size and shape of the aromatic nucleus appears to have a definite bearing on the activity of the compounds. Substitution by a methyl group in the 2- position of histamine lowered the activity several-fold, but similar substitution in the 1- position lowered the activity several hundred fold. The spatial disposition of the various atoms in the aromatic ring, and the nature of the atoms themselves, have a profound influence on the histamine activity. The authors concluded that from this series of compounds no correlations could be drawn between histamine activity and the intrinsic chemical properties of the aromatic nuclei.

S. L. W.

Diamines, 2-Thenyl Substituted, with Antihistamine Activity. F. Leonard and U. V. Solmssen. (*J. Amer. chem. Soc.,* 1948, **70,** 2064.) A series of 6 new and 2 previously reported 2-thenyl substituted alkylene-diamines which conform to the general type $C_4H_3SCH_2$

$$\begin{matrix} C_4H_3SCH_2 \\ \rangle N-R_2B, \\ R_1 \end{matrix}$$

where R_1 is phenyl or 2-pyridyl, R_2 is a straight or branched alkylene chain containing two or three carbon atoms and B is a dimethylamino or piperidine group, were prepared. Intermediate secondary amines R_1NHR_2B (I) and $R_1NHCH_2C_4H_3S$ (II) were prepared by condensing aniline with a dialkylaminoalkyl chloride hydrochloride or 2-thenyl chloride, and 2-aminopyridine with dialkylaminoalkyl chlorides or 2-thiophenealdehyde followed by reduction. The tertiary amines were obtained by the alkylation of the intermediates (I) and (II) in benzene or toluene solution in the presence of sodamide. These were converted to water-soluble salts in which form they were evaluated pharmacologically. Preliminary results showed that several of the compounds possessed antihistamine activity similar to that of currently available substances. N,N-dimethyl-N′-phenyl-N′-(2-thenyl)-ethylenediamine was characterised by a very low toxicity and a high order of activity. Attempts to form either hydrochlorides or hydrobromides of N_2,N_2-dimethyl N_1-phenyl-N_1-(2-thenyl)-1 : 2-propanediamine and N_2N_2-dimethyl-N_1-(2-pyridyl)-N_1-(2-thenyl)-1:2-propanediamine resulted in their decomposition. The decomposition of N_2,N_2-dimethyl-N_1-phenyl-N_1-(2-thenyl)-1,2-propanediamime under conditions of hydrochloride formation, observed after the removal of solvents from the neutralisation mixture was investigated in some detail; breakdown products were identified and a mechanism of the procedure suggested. R. E. S.

Sulphadiazine and Paludrine, Resistance of the Malaria Parasite of the Fowl *(Plasmodium gallinaceum)* to. A. Bishop and E. W. McConnachie. (*Nature*, 1948, **162**, 541.) Treatment with gradually increasing doses of sulphadiazine yielded a strain of *Plasmodium gallinaceum* in young chicks in which the resistance to the drug is 32 times greater than that of the untreated strain. Whereas the minimum dose of drug which produces an adverse effect upon the growth-rate of the normal strain, when given twice daily on successive days, is 0·625 mg./20 g. of body-weight, in the resistant strain growth of the parasites occurs in birds receiving 20 mg. twice daily. The production of this 32-fold resistance to sulphadiazine took approximately 12 months. The degree of resistance acquired has been assessed at intervals, by comparing the intensities of infections produced by standard inocula (that is 50,000,000 parasites per chick intravenously) of (a), the resistant strain, into groups of chicks receiving doses ranging from 2·5 to 20 mg. of sulphadiazine twice daily for 3½ days, and (b), the normal parent strain, into groups of chicks receiving similar drug treatment. The sulphadiazine-resistant strain produced infections in chicks receiving doses of drug which inhibited the development of the normal strain (that is, 2·5 to 20 mg.); but the resulting infections were not as heavy as those produced by the parent strain in untreated birds. The sulphadiazine-resistant strain produced, on the whole, less intense infections in untreated birds than did the parent strain. In the sulphadiazine-resistant strain of *P. gallinaceum*, exoerythrocytic schizonts were found in chicks during treatment with this drug. This sulphadiazine-resistant strain of *P. gallinaceum* was also resistant to sulphathiazole, sulphanilamide and sulphapyridine. The cross resistance to sulphadiazine and paludrine was also studied; the sulphadiazine resistant strain of *P. gallinaceum*, when tested by the method described was found to be resistant to 0·1 mg. of paludrine although it had not been subjected to treatment with that drug. Since acquired resistance to sulphadiazine was found to confer resistance to paludrine, the effect of sulphadiazine upon a paludrine-resistant strain of *P. gallinaceum* was studied; the strain was found

to be resistant to doses of 1·25 mg. of sulphadiazine. In one strain tested the development of resistance to paludrine was not accompanied by resistance to sulphadiazine. Various aspects of the drug-resistance are discussed.

R. E. S.

PHARMACY

GALENICAL PHARMACY

Isotonic Solutions, Preparation of. C. G. Lund, K. Pedersen-Bjergaard and E. B. Rasmussen. (*Dansk. Tidsskr. Farm.*, 1949, **23**, 119.) The apparatus used for the micro determination of osmotic pressure consists essentially of a short length of constantin wire, bent into an inverted U, and soldered at each end to a manganin wire. The junctions are formed into small loops, each capable of holding about 0·01 ml. of liquid. This apparatus is used as a delicate thermocouple to determine difference of temperature between two drops of liquid. A drop of liquid under examination is placed on one loop, and a drop of sodium chloride solution, of suitable concentration, on the other one. The apparatus, enclosed in a suitable vessel, is kept in a constant temperature bath, and, after 10 minutes, the potential difference is noted with the aid of a sensitive galvanometer. By adjustment of the concentrations, it is possible to find the concentration of a sodium chloride solution which has the same osmotic pressure or depression of freezing point as the solution under examination. The table summarises the results.

Substance	Concentration per cent.	Depression of freezing-point
Sodium chloride	0·9	0·52
Bensulphamide hydrochloride	3·72	0·52
Calcium lævulinate ($2H_2O$)	3·57	0·52
Histidine hydrochloride (H_2O)	3·44	0·52
Neostigmine bromide	4·95	0·52
Pethidine hydrochloride	4·79	0·52
Pilocarpine nitrate	4·62	0·52
Sodium acetate ($3H_2O$)	2·03	0·52
Sodium metabisulphite	1·38	0·52
Sulphadiazine sodium	4·24	0·52
Sulphamerazine sodium	4·53	0·52
Sulphathiazole sodium ($1\frac{1}{2}H_2O$)	4·82	0·52
Cetyltrimethylammonium bromide	5·0	0·233
Morphine sulphate ($5H_2O$)	6·0	0·298
Oxyquinoline sulphate	8·0	0·441
Strychnine hydrochloride ($2H_2O$)	2·5	0·191
Tubocurarine hydrochloride	5·0	0·271

G. M.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and norAdrenaline Injections, and Further Studies on Liver Sympathin. G. B. West. (*Brit. J. Pharmacol.*, 1948, **3**, 189.) Examination of the pressor effects of *dl*-noradrenaline and *l*-adrenaline, when injected into the jugular, femoral and splenic veins, and the splenic and external iliac arteries, of cats and rabbits, showed both substances to be less active by portal

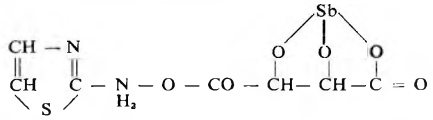
than by jugular vein, though whereas with adrenaline the ratio value for equipressor doses by these routes decreased as the pressure rise increased, with noradrenaline the ratio value remained constant. Unlike adrenaline, noradrenaline when injected into the portal circulation is not potentiated by the simultaneous administration of guanidine or cocaine, and is therefore not rapidly absorbed from the blood stream during its passage through the liver. Both adrenaline and noradrenaline by intra-arterial and intrajugular injection failed to show potentiation by the simultaneous administration of guanidine by the same routes, though both were enhanced by cocaine. Experiments to obtain further evidence of the similarity between intraportal injections of noradrenaline and hepatic nerve stimulation, showed that hepatic nerve stimulation and small intraportal doses of noradrenaline both produced depressor responses, while corresponding doses of adrenaline were without effect; guanidine and cocaine by intraportal injection were also found not to potentiate the action of liver sympathin. When injected into the artery supplying the caudal end of the spleen, adrenaline in small doses caused a small rise followed by a large fall in blood pressure, the depressor effect being possibly due to the liberation of histamine; noradrenaline, on the other hand, produced a pure rise of blood pressure at all dose levels. Noradrenaline is therefore a much more potent pressor agent by splenic artery than adrenaline, and, in addition, relatively more adrenaline than noradrenaline is inactivated in the spleen. S. L. W.

Amidone, Pharmacology of the Optical Isomers of. R. H. Thorp. (*Brit. J. Pharmacol.*, 1949, **4**, 98.) The relative analgesic properties of *d*-, *l*-, and *dl*-amidone were determined on rats in comparison with that of morphine; *dl*-amidone hydrochloride was shown to be a rather more powerful analgesic than morphine sulphate. The activity of the *lævo* isomer was very much greater than that of the racemic form, which failed to produce a graded increase in pain threshold when given in large doses. The acute toxicity for the three isomers is approximately equal, and is due to direct action on the cardiac muscle; it is increased with the *l*- or *dl*-isomers by the central nervous depression which these two drugs produce. The depressant effect of *l*-amidone on rabbit respiration is twice as great as that of the racemic compound, the effect of the *dextro*-isomer being negligible in comparable doses. Local anæsthetic action is greatly influenced by the optical isomerism; it is shown by all three isomers, but is greatest in the *lævo* isomer which is 3·5 times as potent as procaine. Amidone and its optical isomers are rather more active than pethidine as spasmolytic drugs; the effect is not associated with the optically active carbon atom but is a function of the molecule as a whole. The reported property of analgesic drugs of producing a state of " acute vascular tolerance " to the depressor action resulting from intravenous injection was confirmed with *l*-amidone. S. L. W.

Antihistamine Drugs, Pharmacology of. A. M. Lands, J. O. Hoppe, O. H. Siegmund and F. P. Luduena. (*J. Pharmacol.*, 1949, **95**, 45.) This is a report of an investigation on the antihistamine drugs N′-(2-pyridyl)-N′-(3-thenyl)-N,N-dimethylethylene-diamine (WIN 2848), N′-(2-pyridyl)-N′-(2-chloro-3-thenyl)-N,N-dimethylethylene-diamine (WIN 2875) and N-(2-pyridyl)-N′-(2-bromo-3-thenyl)-N,N-dimethylethylene-diamine (WIN 2876). WIN 2848 was shown to be a highly active histamine antagonist, and was potent in this respect than WIN 2875 and WIN 2876. In a dose of 0·013 mg./kg. it protected guinea-pigs against 2·8 intravenous lethal doses

of histamine: a dose of 0·028 mg./kg. of pyribenzamine was required to give the same protection. The three WIN compounds appear to be of the same order of toxicity as pyribenzamine when administered intravenously in mice. WIN 2848 was shown to diminish markedly the severity of the effects of histamine given percutaneously in man. It is suggested that WIN 2848 may be found useful in the treatment of allergic conditions. S. L. W.

Antimonyl 2-Aminothiazole Tartrate, Preparation and Toxicity of. D. B. Meyers and J. W. Jones. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 41.) An organic compound of antimony which might prove highly toxic to protoza in quantities safe for administration to the host was required. Properties desired were: solubility in water so that parenteral administration was possible; a low index of irritation at the site of injection; a high parasiticidal action; and a sufficient margin of safety. The compound prepared was a water-soluble trivalent antimony salt, which was found to be less toxic and less irritating than tartar emetic when injected intravenously into the femoral vein of white rats. Analyses for antimony and sulphur indicated the following structural formula:



The LD50 of the compound for rats was shown to be 75 to 95 mg./kg.

G. R. K.

Decamethonium Iodide (C10), Effects of on Respiration and on Induced Convulsions in Man. D. L. Davies and A. Lewis. (*Lancet*, 1949, **256,** 775.) From a study of the effects of C10 and *d*-tubocurarine chloride in 18 patients with depressive illness subjected to electrically induced convulsions, no distinguishable difference was observed, either in respect of the pareses and paralyses induced or the characteristics of the modified convulsion. The effect of the two drugs on respiratory movement showed no significant difference. Small doses of either produced thoracic movements of lesser amplitude, with slight increase in the rate but no change in the amplitude of abdominal movements. Large doses abolished thoracic movement and diminished the amplitude of abdominal movements, while greatly increasing their rate. Although the efficiency of respiration is impaired by both drugs in large doses C10 spares the diaphragm more than does *d*-tubocurarine chloride, and would thus appear to offer a wider margin of safety. The clinical use of C10, in combination with thiopentone sodium, in a series of 40 patients who were being treated by electrically induced convulsions, showed it to be safe and effective for this purpose and without disagreeable side-effects.

S. L. W.

Digitalis Products, The Use of Pigeons for the Assay of. A. Lavallee and M. G. Allmark. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 45.) Comparative data are given on the intravenous pigeon and U.S.P. cat methods for powdered digitalis, digitalis tinctures, tablets and capsules, purified glycosidal products and amorphous digitalin. The pigeon method used has been described by Cook (*Bull. U.S.P.*, 1948, **35,** 89.), and by Braun and Lusky (*J. Pharmacol.*, 1948, **93,** 81.) The two methods showed fairly

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BOOK REVIEWS

THE CHEMISTRY OF PENICILLIN, edited by Hans T. Clarke, John R. Johnson and Sir Robert Robinson. Pp. 1042 and Appendix. Princeton University Press, New Jersey (London: Geoffrey Cumberlege) 1949, £9 9s. 0d.

Many of the successful results achieved by scientific workers during the war are now known to the general public, and many are aware that chemists in this country and the U.S.A. working together under the auspices of the Medical Research Council and the Office of Scientific Research and Development respectively, determined the probable constitution of penicillin but failed to discover a commercial method of synthesising it. Because the results were not of immediate practical value, there may have been a tendency to dismiss them as of little importance and to regard the effort and money spent on this project—and there was a considerable expenditure of both—as having been wasted.

It was, of course, disappointing that no practical synthesis of penicillin was forthcoming and that none of the simple synthetic compounds related to penicillin had therapeutic properties of any value, but it was important to know this in order that due weight might be given to improving the fermentation process for making penicillin and, in fact, a knowledge of the structure of penicillin helped to improve very materially the amount produced by the mould.

The complete results obtained in this collaborative investigation have now been published. During the 2½ years in which nearly 40 teams on both sides of the Atlantic collaborated, about 700 reports, officially classified as secret, were prepared and circulated. Although these served their immediate purpose of informing other workers of the progress made by any particular group, they were not suitable as a permanent record, and, when the time came for the results to be made public, it was decided to correlate the observations of all the groups and present them systematically and in detail in a separate monograph instead of in the scientific journals as originally proposed. The result is this well-produced volume, similar in page size and format to the *Journal of the American Chemical Society*. In each chapter, a description of the results obtained and the conclusions reached is given in ordinary type and is followed by the relevant experimental details in smaller type. The book is equipped with a good index.

The first chapter, containing a brief outline of the chemistry of penicillin, has already been published *verbatim* in *Nature* and *Science*. Of the other 28 chapters, the first three describe the results obtained prior to 1943 when collaboration commenced. The others deal with the chemistry of particular penicillin degradation products or of groups of substances related in one way or another to the penicillin molecule; with the infra-red absorption spectra of penicillin and related substances; with the application of X-ray analysis and other physical methods to the elucidation of chemical structure; with the biosynthesis of the penicillins; with chemical modifications of the penicillin molecule; with methods of assay; and with the various methods used to synthesise penicillin, most of which were so strikingly unsuccessful!

It is important that all concerned with chemical work in the pharmaceutical field should appreciate the uniqueness of this book. It is not a text-book in which existing knowledge is summarised and critically assessed, but a genuine source book containing information not available elsewhere. It has therefore the same status as the journal of a learned society and will

presumably be abstracted in the same way so that its contents may become known to those who have not ready access to the book itself.

" The Chemistry of Penicillin " covers a far wider field than its title implies. Chapters 21, 25 and 26, for example, contain probably most of what is known of the chemistry of oxazoles and oxazolones, thiazolidines and β-lactams, and subsequent work in these fields will doubtless contain many references to what may familiarly become known as *Chem. Pen.* Again, chapters 11 and 12 give an account of a new and extremely important method of determining the disposition of atoms within a complex molecule, that will doubtless be used increasingly in future work, whilst chapter 13 contains much fundamental data on the infra-red absorption spectra of penicillin, its degradation products, related compounds and simpler substances examined for purposes of comparison.

It is impossible in the space available to give more than this brief outline of the volume under review. It is a book that should be added to every scientific library of importance, especially as its price must put it beyond the reach of the individual chemist, and a book that every organic chemist interested in chemotherapy should browse through in order to familiarise himself with its contents; for it contains much unexpected information that may be of value in other fields.

F. A. ROBINSON.

ABSTRACTS (*Continued from Page* 633)

close agreement, with the possible and inexplicable exception of digoxin tablets. Results differed from those of the cat method by less than 10 per cent. in the majority of the samples. For the whole leaf products the maximum difference was found to be 19·9 per cent. Pigeons were less variable than cats for the assays reported and as a result fewer pigeons are required to meet the present U.S.P. requirement. G. R. K.

Insulin, Potentiation of, by Sulphones. A. B. Macallum. (*Canad. J. Res.*, 1948, **26E**, 232.) Sulphones in trace quantities combined with a diet rich in fresh vegetables were shown to produce in rabbits an increased sensitivity to insulin, both in the rate of fall of blood sugar levels and maintenance of hypoglycæmia. In order to relate the molar concentration of sulphone to the unit value of insulin, 1 ml. of a 0·01 M sulphone solution was used in conjunction with 1 unit of insulin. In the case of less soluble solutions more dilute solutions were used but the volume of the dose increased to keep the amount of sulphone in relation to the amount of insulin constant. The sulphone solutions were injected hypodermically into the side of the animal opposite the site of insulin administration in order to avoid formation of possible insulin-sulphone complexes. In the case of simple sulphones the potentiation did not appear until sulphamide was used, and the maximum effect in this group was attained with phthalyl tauramide. In the case of the sulphonamides the potency was least with sulphanilamide, but increased in the succeeding members of this series (sulphathiazole, sulphaguanidine, sulphadiazine), the last, No. 307, a disulphone under experimental trial, being the most effective. The benzenesulphonic derivatives (ethyl benzenesulphonate, saccharin, benzenesulphonamide) were the most active of all the sulphones investigated. The sensitivity is not contingent on the presence of sulphone compounds, since it may persist for several

[*Continued on page* 637

SCIENTIFIC MEETINGS

CHROMATOGRAPHY

Summary of a Lecture delivered by Professor A. H. Cook, D.Sc., Ph.D., at the Royal Institution

DR. COOK related how chromatography has helped in the isolation and purification of natural substances occurring in high dilution. This was especially so in recent years in the fields of hormones, vitamins, penicillin and vitamin B_{12}. Carotene, one of the first substances to be studied by the method, occurs at about 1 part in 1,000, lactoflavine is present at 1 part in 30,000 to 40,000 of culture medium and the anti-pernicious anæmia factor at about 1 in 80×10^6. Another difficulty in the study of natural products lies in the accompanying impurities which are very often similar in physical and chemical properties and indistinguishable by ordinary chemical or physical methods. Chromatography frequently provides a solution to such problems. When there are great differences in the extent to which certain substances are adsorbed some of them may pass straight through the column, but they are not lost as they appear in the liquid at the end of the column in an orderly fashion. This is the principle of the liquid chromatogram. Sometimes a zone may contain more than one substance when it will be necessary to fractionate them by repeating the process in a number of columns. An American apparatus provides for up to 200 repetitions. Colourless compounds are detectable by viewing them in ultra-violet light, when many fluoresce, by the addition of a suitable dyestuff whose relative behaviour on the column is known or by the conversion of the substance to a coloured or fluorescent derivative. Chromatography has been the key to the chemistry of the carotenoids. It has also played an important part in micro-chemistry (detecting 0·01 g.) and in the examination of wines, foodstuffs and drugs.

The degree to which a substance is adsorbed is related to molecular structure. The carotenoids which are characterised by a varying number of OH groups and conjugated or isolated double bonds, are a good example of this.

Oxygen atoms, double bonds (conjugated more than isolated bonds) are associated with increased adsorption. Thus fucoxanthin containing 10 double bonds and from 4 to 6 OH groups is most strongly adsorbed whilst α-carotene is about the least. The stereoisomeric methyl bixins give chromatograms which vary with the different molecular shapes of these compounds. This method of identification is much more sure than that of melting-points.

Recently the method of mechanically separating the zones on a chromatogram has been replaced by one where the zones are eluted into a special container where the liquid is observed either polaragraphically or by continuous measurements of the refractive index. In this way Tisellius and Claesson have separated mixtures of lauric, palmitic and myristic acids. Ion exchange is a development of chromatography in which the ionic charge of the column and eluent plays an important part. It has been used to study the nucleotides and amino-acids. Substances which form salts in a medium of acid *p*H may be separated on a cationic column and those forming salts in one of alkaline *p*H on an anionic column.

Another recent development is the partition method in which the column is packed with silica gel containing water. The passage of the zones

down the column depends on their relative partition coefficients between water and the organic solvents. Adsorption by the silica is sometimes a problem which may be avoided by the use of strips or sheets of filter paper. This is a very convenient way of testing urine and has led to the detection of cysteine which was not formerly suspected in abnormal urine. The strip of filter paper is spotted with a drop of solution on a line drawn near one end. It is then suspended vertically in a glass cylinder so that the spotted end is immersed in a trough or organic solvent saturated with water at the top of the cylinder. The atmosphere is kept saturated with organic solvent and water vapour by placing the cylinder in a shallow dish of the mixture. The movement of the zone of substance in solution relative to the distance moved by the advancing front of liquid is measured. Various reagents are used to detect the zones of substances present. By using a sheet of filter paper a number of solutions may be examined at the same time and, under standardised conditions, it is possible to use maps to identify the different substances by their relative positions. Amino-acids both in the free and bound forms, gramicidin, penicillin, purines, sugars and anthocyanins have all been studied by this method. Vitamin B_{12} was investigated by a modified method in which the chromatographic strip was laid across a seeded agar plate and the effect of the various zones on the bacterial growth was noted. Observations have been made on the products of photosynthesis in algae with the aid of radio-active particles. The cells were extracted with solvent and chromatograms prepared which were photographed on X-ray film. By comparison with chromatograms of known substances no less than 15 substances, hitherto unknown in photosynthesis, were detected. Partition chromatography has also shown the toxic factor in flour caused by treatment with nitrogen trichloride to be neither an amino-acid nor a protein. Because little change ensues in the nature of substances isolated by chromatography, it is indispensable in the investigation of natural products especially in such complex problems as the nature of bacterial toxins and the specificity of proteins. It has enabled us to speculate on the course of photosynthesis and the biogenesis of amino-acids.

ABSTRACTS (*Continued from Page* 635)

days after these compounds have been detoxicated or eliminated. The potentiating effect is due to the combined effect of the sulphone sensitisation and some element in fresh vegetables in the standard diet (fresh cabbage, lettuce, carrots, hay and oats). S. L. W.

Testosterone, Long-Acting Preparation of. E. Carlinfanti, F. D'Aló and L. Cutolo. (*Lancet*, 1949, **256**, 479.) To a solution of 1 g. of crystalline testosterone in 10 ml. of alcohol (96 per cent.) is slowly added, with constant stirring, 20 ml. of an equeous suspension of aluminium phosphate 7 mg./ml. The mixture is allowed to sediment and the supernatant fluid decanted off. The residue is made up to 40 ml. with saline solution. The preparation is stable and can be administered with a fine needle. Experiments were carried out on castrated guinea-pigs, one group receiving one injection of testosterone propionate in oil in a dose of 25 mg./100 g., and another group the same amount of pure testosterone adsorbed on to aluminium phosphate. It was found that one injection of the ester-in-oil preparation leads to a rapid rise and fall in the weight of the seminal vesicles, whereas the new preparation produces a greater and more continuous action, reaching a peak not earlier than 30 days after administration. S. L. W.

NEW REMEDIES

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

Eulissin* is a synthetic curarising agent for use in anæsthesia and convulsive therapy. It is a sterile solution of decamethonium iodide (bistrimethylammonium decane iodide (C10) for intravenous injection. It is supplied in ampoules containing 5 mg. in 2·5 ml., in boxes of 6, 12 and 100 ampoules.

S. L. W.

Fel-evac* is a standardised fatty meal for use in cholecystography, replacing the unstandardised meal hitherto employed, usually consisting of eggs, with bacon or milk. The use of Fel-evac does not necessitate any departure from the usual procedure. A fat-free meal at 6 p.m. is followed an hour later by the opaque substance; only fluids are allowed until after the first radiograph at 9 or 10 a.m. the following morning. If a clear shadow is obtained, Fel-evac is then given and further radiographs taken at intervals of 15, 30 and 60 minutes. A dose of 1½ fl. oz. in a small glass of warm milk is usually satisfactory; in a normal gall-bladder this induces a good contraction within 30 minutes. It is supplied in 10 oz. and 20 oz. jars.

S. L. W.

Pyribenzamine* is N-benzyl-N-(α-pyridyl)-N′-dimethylethylenediamine monohydrochloride. It is a white, crystalline substance, which is stable, non-hygroscopic, and soluble in water. It is an anti-histamine, as little as 5 mg. protecting guinea-pigs against 50 mg. of histamine, or 100 times the lethal dose. It is indicated in the prevention and treatment of allergic conditions, such as allergic rhinitis, acute and chronic urticaria, allergic eczema, and drug reactions; it has also been employed in a number of pruritic conditions. Less encouraging results have been obtained in the treatment of bronchial asthma and migraine. It is administered in the form of tablets containing 0·05 g., the average adult dose being from 1 to 4 tablets daily. In young children dosage is determined on a body-weight basis, and in older children approximately one-half the adult dose is employed. Side-effects are usually mild, the commonest being drowsiness and gastro-intestinal disturbances; dryness of the mouth, vomiting and diarrhœa occur occasionally. Rare manifestations include insomnia, tachycardia, diplopia and urinary disturbances. It is issued in packages of 20, 100 and 500 tablets of 0·05 g., and as an elixir in bottles of 100 ml. containing 0·005 g. per ml.

S. L. W.

Visco-Pyelosil* is a 35 per cent. aqueous solution of diodone rendered viscous with a neutralised polymer of methacrylic acid and esters. Injected subcutaneously or intramuscularly, and introduced into the uterus and peritoneal cavity it causes no irritation. It must not be injected intravenously or intrathecally. It is employed as a radiological contrast medium in hysterosalpingography especially in the investigation of sterility. The miscibility with body fluids avoids globulation which can confuse definition or produce misleading appearances. If any enters the circulation it disperses rapidly and no compilation from oil embolism can arise. Rapid dispersal and excretion also avoids risk of subsequent irritation due to unabsorbed contrast medium remaining in the cavities. The amount of contrast medium required usually does not exceed 10 ml. and all exposures should be completed within 10 minutes. It is issued in boxes containing 1 and 5 ampoules of 10 ml.

S. L. W.

NEW APPARATUS

A SIMPLE METHOD FOR PHASE-CONTRAST MICROSCOPY

PHASE contrast microscopy is particularly valuable in the examination of living cells with high powers and an ordinary microscope using an oil immersion lens may be easily adapted. This method was demonstrated by Dr. J. R. Baker[1] and his associate workers of the Department of Zoology of Oxford University at the Royal Society's Conversazione on May 26, 1949. A phase-plate is prepared from a circle of glass, 1 mm. thick, of the same diameter as the back lens of the objective, and with the sides optically plane and exactly parallel to one another. This phase-plate is held in place behind the objective lens by means of a hollow brass cylinder which fits into the objective. The phase-plate is glued to the lower end of this cylinder (Fig. 1). The phase-plate is uniformly bloomed one side with magnesium fluoride to such a thickness as to give a retardation of a quarter-wave of apple green light, in comparison with light passing through the same thickness of air. (Deposition of magnesium fluoride is done by Messrs. R. and J. Beck.)

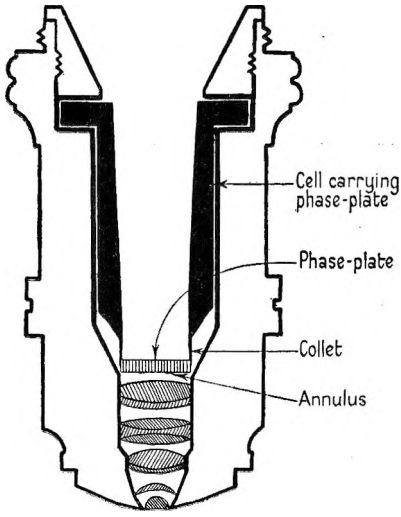

FIG. 1.—Diagram of the microscope objective showing the method of fitting the phase-plate. (Reproduced by courtesy of the Quarterly Journal of Microscopical Science.)

The plate, glued to the end of the cylinder, is accurately mounted in the centre of the revolving disc of a turntable used for mounting microscopical slides, and an annulus is dug out by scraping part of the bloom away with a chisel-pointed needle mounted on a specially constructed arm. The annulus should lie a little less than half-way from the centre of the phase-plate to its circumference.

In order to balance the direct light coming through the annulus with that of the diffracted light which passes through the rest of the phase-plate, carbon must be deposited on the annulus to reduce its transmission. This is done by passing the plate through a small xylene or benzene flame. The smoking should be sufficient to reduce light-transmission by about 30 per cent. The carbon is then removed from the phase-plate, except from the annulus itself. An illuminating annulus is next prepared. An annular space is cut away from a piece of black paper, which is then stuck on to a sheet of glass. The illuminating annulus is placed immediately in front of a 150 watt "Helios" enlarging lamp, which is the source of light. To set up the apparatus a square of fine ground glass is placed between the light source and the microscope mirror (Fig. 2). The microscope may now be used ordinarily. Some living cells are now placed on a slide and focused with a low-power objective. The condenser (preferably a high-power achromatic one) is then adjusted so that a pencil held against the ground glass is seen in focus with the cells. The low power objective is now replaced by the oil-immersion lens

carrying the phase-plate, and one of the cells is carefully focused. Then the slide is moved so that no cell is sen; the draw-tube is removed and a 3 in. objective is screwed into it at the bottom. The draw-tube is replaced and adjusted so that the phase-plate annulus is carefully focused. The sheet of

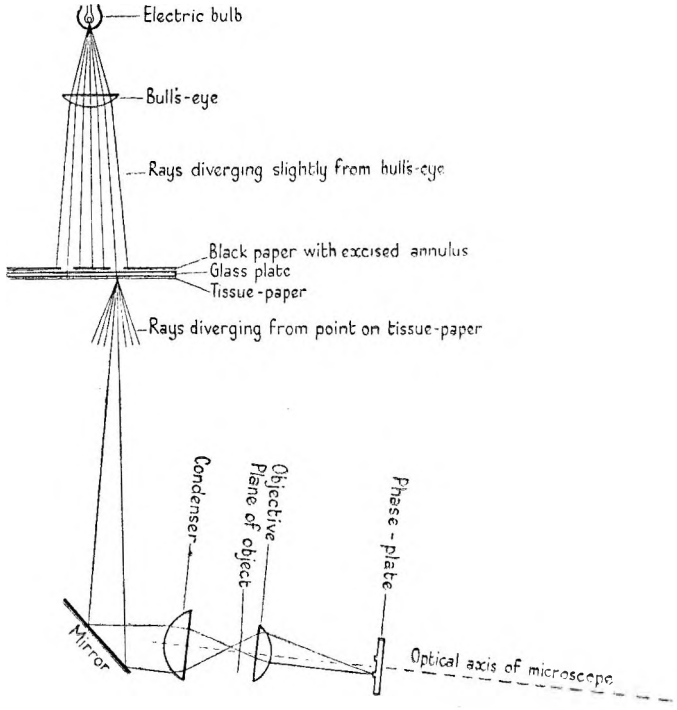

FIG. 2.—Optical diagram of the apparatus for Phase-contrast Microscopy. The system described in the text is an improved and simplified form. (Reproduced by courtesy of the Quarterly Journal of Microscopical Science.)

ground glass is next exchanged for the bright annulus, and the condenser is now lowered until the bright annulus is in focus at the same time as the phase-plate annulus. At this position, the image of the bright annulus thrown by the condenser lies in the plane that is conjugate to the plane of the phase-plate placed on the other side of the objective. To make the two annuli exactly coincide the mirror is adjusted and the bright annulus and lamp moved towards or away from the mirror, whilst corresponding movements of the condenser are made to keep the bright annulus in focus. The 3 in. objective is then removed and the object once more brought into the field of view. The microscope will show the cells in " positive " phase-contrast: the field will be bright, transparent objects of high refractive index will appear black or grey.

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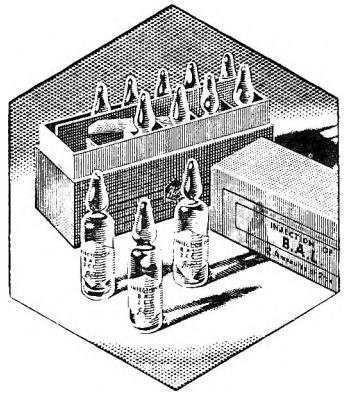

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