The Journal of
PHARMACY
and
PHARMACOLOGY

VOLUME VII No. 8 AUGUST, 1955

Published by Direction of the Council of

THE PHARMACEUTICAL SOCIETY OF GT. BRITAIN

33 BEDFORD PLACE, LONDON, W.C.1

'REVECTOR' Microscopical Stains

Preparation of sections in the Morbid Histology Laboratory of the Royal Free Hospital, London.

'REVECTOR' Microscopical Stains have established a high reputation for the techniques used in bacteriology, haematology and histology. 'Revector' Microscopical Stains are of the same uniform high quality and reliability as all the fine chemicals and reagents listed by Hopkin and Williams Ltd. The following is a selection from the full range of 'Revector' Microscopical Stains and a comprehensive list will be provided on request.

Basic Fuchsin
Carmine
Crystal Violet
Eosin, yellowish W.S.
Giemsa's Stain
Haematoxylin
Leishman's Stain
Methylene Blue.

HOPKIN & WILLIAMS Limited

Manufacturers of fine chemicals for Research and Analysis

CHADWELL HEATH, ESSEX, ENGLAND

The Journal of

PHARMACY *and* PHARMACOLOGY

Successor to The Quarterly Journal of Pharmacy and Pharmacology

33 BEDFORD PLACE, LONDON, W.C.1

Telephone: CHAncery 6387

Telegrams: Pharmakon, Westcent, London

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Annual Subscription **50s.** Single Copies **5s.**

Vol. VII. No. 8 **August, 1955**

CONTENTS PAGE

[*Continued on page ii*

CONTENTS

PAGE

REVIEW ARTICLE

THE INTERRUPTION OF GANGLIONIC TRANSMISSION AND SOME OF ITS PROBLEMS

BY ELEANOR ZAIMIS, M.D., B.Sc.

Reader in Pharmacology, Royal Free Hospital School of Medicine, University of London

DURING the last few years considerable attention has been paid to ganglionic blocking substances; first because they proved to be clinically useful and second because they are of great interest to physiologists and pharmacologists. Substances such as penta- and hexamethonium, tetraethylammonium, azamethonium, pentolinium and Arfonad have been frequently discussed[1–15] and detailed descriptions may be found even in textbooks[16,17]. For this reason I will restrict myself to the discussion of new information and of a few problems which are still unsolved and which are interesting both from an academic and a clinical point of view.

Structure-action Relationships

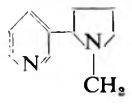
A glance at the formulæ of Tables I and II will at once suggest the difficulty of reaching conclusions about the structural features necessary for ganglionic activity. Table I gives compounds known to mimic acetylcholine at the ganglionic synapse. Such molecules might be expected to be modelled on acetylcholine or at least on parts of acetylcholine. This, however, is not always so, and the compound most difficult to account for is nicotine. In his recently published book Barlow[18] reviews in detail the literature relevant to structure-action relationships, and in discussing the requirements for acetylcholine-like activity at the ganglionic synapse concludes that "the idea that it is the electron density at various points which is important rather than the presence of some particular group or groups, would seem to be a much more realistic approach to the problem." "But," he continues, "electronic activation is not the only factor; steric effects and Van der Waal's forces may also play a part." Table II shows compounds able to compete with acetylcholine at the ganglionic synapse. Again no convincing relationships can be found among these substances. It appears that with an increasing number of active compounds the problem has become not less but more complicated, and will remain so, until more is known about the intimate properties of the different effector cells upon which drugs act.

Looking at the problem from a more general point of view, the primary difficulty is to comprehend the relation between acetylcholine itself and the effector cells. Acetylcholine is known to be active at several sites, the ganglionic synapse, the neuromuscular junction, the effector cells innervated by post-ganglionic cholinergic nerve fibres, and possibly also the central nervous system, producing what we call "different actions." It depolarises motor end-plates and ganglion cells, depresses some smooth muscles and stimulates others, stimulates glandular secretions, and so on.

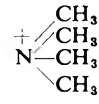
It seems almost incredible that one single molecule should produce so many different actions. One possible explanation is that its structure is such that it can fit and activate receptors with different properties. On the other hand possibly the initial stage of the trigger mechanism of all these actions is the same but our methods are still too crude to detect it. Whatever be the explanation the molecule of acetylcholine is a masterpiece and possesses to perfection the properties requisite for producing these actions. It is therefore not surprising that none of the innumerable synthetic compounds can really imitate acetylcholine in all its actions.

TABLE I

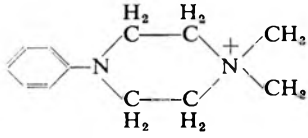
COMPOUNDS MIMICKING ACETYLCHOLINE



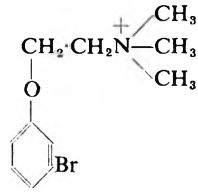
Nicotine



Tetramethylammonium (TMA)



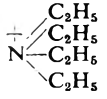
1 : 1-Dimethyl-4-phenylpiperazinium (DMPP)



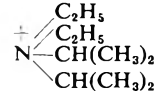
2-*m*-Bromophenoxy-trimethyl-ethylammonium (MBF)

TABLE II

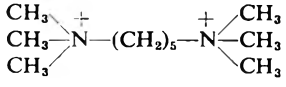
COMPOUNDS COMPETING WITH ACETYLCHOLINE



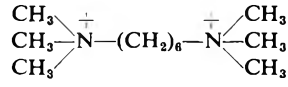
Tetraethylammonium (TEA)



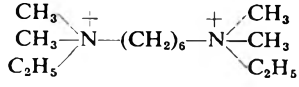
Diethyldi-*iso*propylammonium



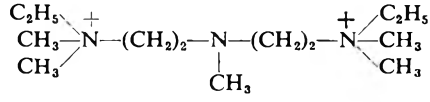
Pentamethylene-α : ω-bistrimethyl-ammonium (Pentamethonium)



Hexamethylene-α : ω-bistrimethy-ammonium (Hexamethonium)l

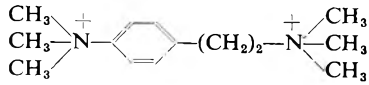


Hexamethylene-α : ω-bis-ethyl-dimethylammonium

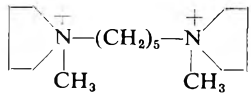


3-aza-pentane-3-methyl-α : ω-bis-ethyldimethyl-ammonium (Azamethonium)

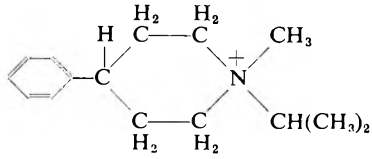
TABLE II—*continued*



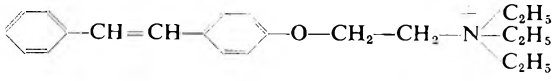
1-ω-trimethylethylammonium-4-trimethylammonium benzene



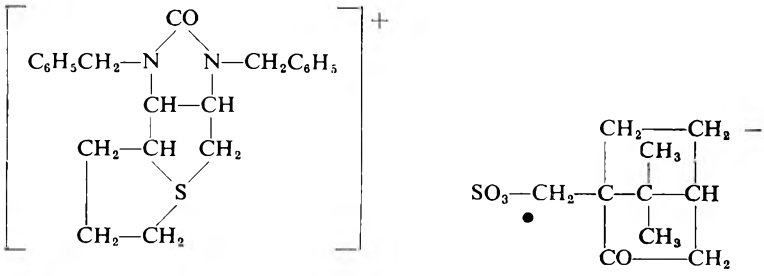
Pentamethylene-α: ω-bis (1-methylpyrrolidinium)
(Pentolinium)



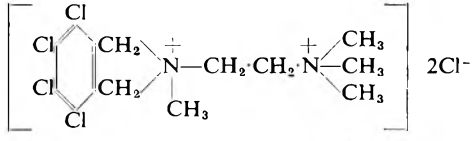
1-Methyl-1-*iso*propyl-4-phenylpiperidinium



2-Stilbenoxy-ethyl-triethylammonium
(Elvetil)



(+)-3:4(1′:3′-dibenzyl-2′-keto-imidazolido)-1:2-trimethylene thiophanium
(+)-camphor sulfonate
(Arfonad)



4:5:6:7-Tetrachloro-2-(2-dimethyl-aminoethyl)-*iso*indoline dimethochloride

The Modes of Action

The autonomic ganglia are distributing centres from which impulses from a single pre-ganglionic fibre may be relayed to one or several post-ganglionic fibres. The nerve fibres of the autonomic nervous system act

by the release of either acetylcholine or of adrenaline and noradrenaline. Dale[19] called *cholinergic* those nerve fibres which act by the release of acetylcholine and *adrenergic* those nerve fibres which act by the release of adrenaline or noradrenaline. All pre-ganglionic, sympathetic and parasympathetic nerve fibres are cholinergic—in other words the synaptic transmission across an autonomic ganlion is effected by acetylcholine. In 1953 Paton and Perry[20] obtained direct evidence that injected acetylcholine can cause depolarisation of the cells of the superior cervical ganglion, and Perry and Talesnik[21] obtained the same effect in the ciliary ganglion. These electro-physiological analyses on the two types of ganglion cells form a valuable addition to our knowledge of the mechanism by which acetylcholine mediates the transmission of the nervous impulse from the pre-ganglionic to the post-ganglionic fibre.

According to the chemical theory of ganglionic transmission the arrival of a nerve impulse at the nerve endings of the pre-ganglionic fibre is followed by the release of acetylcholine. Acetylcholine has a powerful and rapid action producing a depolarisation of the ganglion cell, in other words a stimulation of the ganglion cell, thus initiating an impulse in the post-ganglionic fibre. The release of acetylcholine is very rapid, and its destruction, by a specific enzyme, cholinesterase, is also rapid, being complete in a few milliseconds. After this the synapse is ready to transmit another impulse. However, when very large amounts of acetylcholine accumulate at the ganglionic synapse depolarisation will persist[20]. During this process electrical inexcitability develops and ganglionic blockade results. Consequently acetylcholine has two actions at the ganglionic synapse: (*a*) it may stimulate and (*b*) it may interrupt ganglionic transmission.

There are a great number of substances which interfere with the transmission at the ganglionic synapse, but their effects are produced by different modes of action. In the first instance they may be broadly divided into two categories:

1. Substances whose chemical structures bear some resemblance to acetylcholine and act by mimicking acetylcholine, competing with or allowing it to accumulate at the ganglionic synapse.

2. Substances whose chemical structures bear little or no resemblance at all to acetylcholine and which probably act on the pre-ganglionic nerve endings or on the effector cells, changing some of the properties essential for normal transmission.

The first group includes:

(*a*) Substances whose properties must be very similar to those of acetylcholine and which imitate its action at the ganglionic synapse. The depolarisation of the ganglion cells, which drugs of this kind produce, is initially associated with increased excitability, but as the depolarisation persists the phase of increased excitability passes, and transmission of excitation across the synapse no longer occurs.

(*b*) Substances competing with acetylcholine for the ganglion cell receptors, thus reducing the effectiveness of acetylcholine. In order to combine with the same receptors such molecules must obviously share

many of the properties of acetylcholine, but must also be deficient in some important quality needed to initiate depolarisation.

(*c*) The anticholinesterases, which cause an accumulation of acetylcholine by preventing its normal destruction. Thus their effects are indirect and all the phenomena following their administration are produced by acetylcholine itself.

The second group includes miscellaneous molecules which apparently act on the pre-ganglionic nerve endings or on the effector cells, and whose ganglionic blocking activity is small in comparison with their main pharmacological actions. For instance, local anæsthetics can affect ganglionic transmission by interfering with the release of the chemical transmitter and by reducing the sensitivity to acetylcholine of the ganglion cells[22–24].

Larrabee and Posternak[25] studied the ganglionic activity of some general anæsthetics. According to their results, chloroform and ether depress synaptic transmission through a sympathetic ganglion in concentrations similar to those known or assumed to exist in the blood during surgical anæsthesia. At the same time these substances depress synaptic transmission more readily than conduction along any type of axon.

Exley[26] has made a comprehensive study of the action of barbiturates at the ganglionic synapse. His results show, first, that not all barbiturates are equally active. The most active are butobarbitone and amylobarbitone; the least active are the thiobarbiturates; second, that the ganglionic and the central depressant activities show little correlation, and thirdly that the active barbiturates neither interfere with the release of acetylcholine nor depolarise the ganglion cells, but reduce the sensitivity to acetylcholine. In discussing his results Exley reaches the conclusion that the mechanism by which barbiturates diminish the excitability of the ganglion cells to acetylcholine must be different from that of the more specific quaternary ammonium compounds, and proposes to substitute the term "ganglion depressant" for "ganglion blocking."

A ganglionic blocking activity can be demonstrated also among tertiary amines known mainly as antihistamine drugs (diphenhydramine, promethazine), or as substances antagonising acetylcholine at the post-ganglionic cholinergic nerve endings (atropine, scopolamine, Trasentin)[26a]. Longo, von Berger and Bovet[27], applied the term "central ganglionic blocking substances" to a group of drugs (caramiphen, ethopropazine, benzhexol) which possess both ganglionic blocking properties and a relatively specific type of central depressant activity.

It is obvious that molecules such as those just mentioned in the second group, in whose properties there are basic differences, cannot produce a ganglionic blocking effect through the same mechanism. But to analyse such mechanisms is very difficult and little more can be achieved until our knowledge of the intimate properties of the effector cells is more complete.

Papers and reviews by various hands have recently appeared in which problems arising round different modes of action have been discussed in detail. To these I should like to add one comment. All the above

substances can produce a block at the ganglionic synapse at some stage of their action, and if we consider the problem from the point of view of the final result, all can be described as ganglionic blocking substances. On the other hand, if we apply the classical definition which requires that the substance should leave the nerve endings and the effector cells unaffected, the field is greatly narrowed. Possibly the term ganglionic blocking substances should be applied only to substances competing with acetylcholine. A conservative attitude must be preserved even towards substances blocking by depolarisation until more is known about the condition of a ganglion cell which has been submitted to a long-lasting depolarisation.

Substances Competing with Acetylcholine

Substances blocking ganglionic transmission by depolarisation start their activity with a powerful stimulation of the whole of the autonomic system, and obviously cannot be used clinically. On the other hand, the ganglionic blocking activity of the substances discussed in the second group is rather small in comparison with their main effects. Because of their high activity and specificity only substances blocking by competition with acetylcholine will be discussed.

Tetraethylammonium

Tetraethylammonium appears to be the simplest quaternary ammonium derivative which blocks ganglionic transmission by competing with acetylcholine, and it was the first substance to be used clinically[1]. However it has now been superseded and is largely used as a tool in pharmacological analysis. It has always been thought that the main pharmacological actions of tetraethylammonium are due to the paralysis of the autonomic ganglia. Zamboni[28] has, however, produced some evidence that the fall in blood pressure after tetraethylammonium administration is not due only to the ganglionic blockade, but also to a depressant action on the vasomotor centre.

The blocking action of tetraethylammonium is increased by the introduction of methyl side-chains on the alkyl carbon(s) *alpha* to the quaternary nitrogen, and it appears that diethyl-di*iso*propylammonium has a molar potency 12·6 times that of tetraethylammonium.[29]

Penta- and hexamethonium, pentolinium, azamethonium

These substances at present hold the centre of the picture as they have proved to possess great activity coupled with high specificity and their pharmacological actions both in animals and man have been studied in great detail[2,3,5,6,8,9,11–14,30–32].

The absorption of the methonium compounds and allied substances from the intestine is poor, rendering oral therapy difficult to control. This is not surprising as they are quaternary ammonium molecules. Harington[33], studying the absorption and excretion of hexamethonium, found that the rate of absorption of various hexamethonium salts differs considerably. For instance a comparison of the bromide with the chloride

salt showed that more than twice as much hexamethonium appeared in the urine after the administration of the bromide. In addition the excretion of the bitartrate and methosulphate is significantly smaller than for either the bromide or the chloride. This is a very interesting and still unexplained fact.

All studies made in connection with the fate of these drugs in the body show that their elimination depends almost entirely on the kidney. Excretion is rapid and this is one of the difficulties which occur in treating hypertension with methonium compounds. Attempts have been made to lengthen the action of the drugs. Smirk[34] has found that the effects of both hexamethonium and pentolinium are more prolonged when dissolved in 25 per cent. polyvinylpyrrolidine, and Goldsmith *et al.*[35] confirm this finding. However, excretion studies made by Harington and reported by Rosenheim[36] have shown that hexamethonium is as rapidly excreted in the urine when injected in this form as when given in aqueous solution.

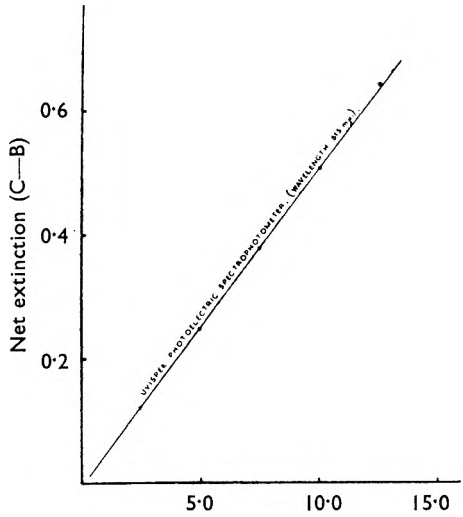


FIG. 1. Standard curve for the determination of hexamethonium using a uvispek photoelectric spectrophotometer.

For the estimation of rather high concentrations of methonium compounds (not less than 2 mg. of substance present in the fraction of biological fluid to be analysed) the method described by Zaimis[37] and that modified by Harington[33] is satisfactory. In this method the methonium ion is precipitated as the reineckate salt and measured photometrically in acetone solution. Up to the present only biological methods have been possible in detecting low concentrations of these substances. As these methods, however, are time-consuming and need skilled hands and complicated equipment, a chemical method sufficiently sensitive to detect low concentrations of the drugs in plasma and suitable for routine use in a clinical laboratory was sought. For the first attempts to estimate chemically low concentrations of hexamethonium, methods already in use for low concentrations of tetraethylammonium were tried. However, neither the method employed by Cochin and Wood[38] (personal experience) nor that employed by Mitchell and Clark[39] (Child, Ph.D. Thesis[40]) could be used for the estimation of hexamethonium. After many attempts with different dyes, Child[40] has finally succeeded in developing

a chemical method for the estimation of hexamethonium in plasma. This method is based on the extraction of the methonium ion from the plasma by the use of Amberlite IRC-50, a weak cation exchange resin, and subsequent elution with dilute acid and complex formation with bromothymol blue. The complex is extracted with chloroform and a part of the chloroform layer is then re-extracted with dilute alkali. Thirty minutes later the intensity of colour developed in the alkaline layer is determined spectrophotometrically. This method may be used for plasma levels of hexamethonium as low as 2 μg. base/ml. (Fig. 1). The method has been tried in order to estimate plasma levels in patients under hexamethonium treatment. From the results obtained it is clear that further refinement is needed before it can conveniently be used for routine purposes. Such work is at present in progress. The formation of a complex between bromothymol blue and hexamethonium has also been reported by Ballard *et al.*[41] and Gottlieb[42].

The main clinical uses of ganglionic blocking substances have been in the treatment of hypertension, in the reduction of bleeding during operations, in peripheral vascular diseases and in the treatment of peptic ulcer[30–32,34,36,43–52]. None of these substances shows any appreciable selective preference for sympathetic or parasympathetic ganglia, and unless the intention is to block the whole of the autonomic system their clinical use is always complicated by undesired effects. For instance a fall in blood pressure is inconvenient during the treatment of a peptic ulcer, while a decreased motility of the gastrointestinal tract is equally undesirable during the treatment of hypertension. These difficulties are, however, circumvented by clinicians who use appropriate counter-agents to reduce the unwanted activity. Pilocarpine, carbacol, eserine and neostigmine are successfully used to overcome paralysis of accommodation, dry mouth, paralytic ileus, urinary retention, i.e., the unwanted effects of parasympathetic blockade. Methedrine or noradrenaline infusion have been found quite satisfactory in diminishing hypotension. Considering the mode of action of these substances it was expected that anticholinesterase drugs would antagonise their effects at the ganglionic synapse. However, the evidence for such an antagonism is as yet inconclusive.

Dr. Goetzee and myself[53] are studying the influence of lowered body temperature on the effects produced by different drugs. Hexamethonium has been one of the drugs studied. Experiments have been carried out on cats, under chloralose anæsthesia. Body temperature is lowered at the rate of about 4° C. per hour by the circulation of cold water through a thin rubber bag inserted into the abdominal cavity. Figure 2 shows the result of an experiment in which contractions of the nictitating membrane were elicited by stimulation of the cervical sympathetic. The first two doses of hexamethonium were administered at an interval of one hour while the body temperature was constant at 39° C. The third dose was administered after the body temperature had fallen to 30° C. It is clear that the intensity of the block is increased at this temperature and the duration of activity prolonged. Thus body temperature must be included among the other factors that influence the intensity and duration of a

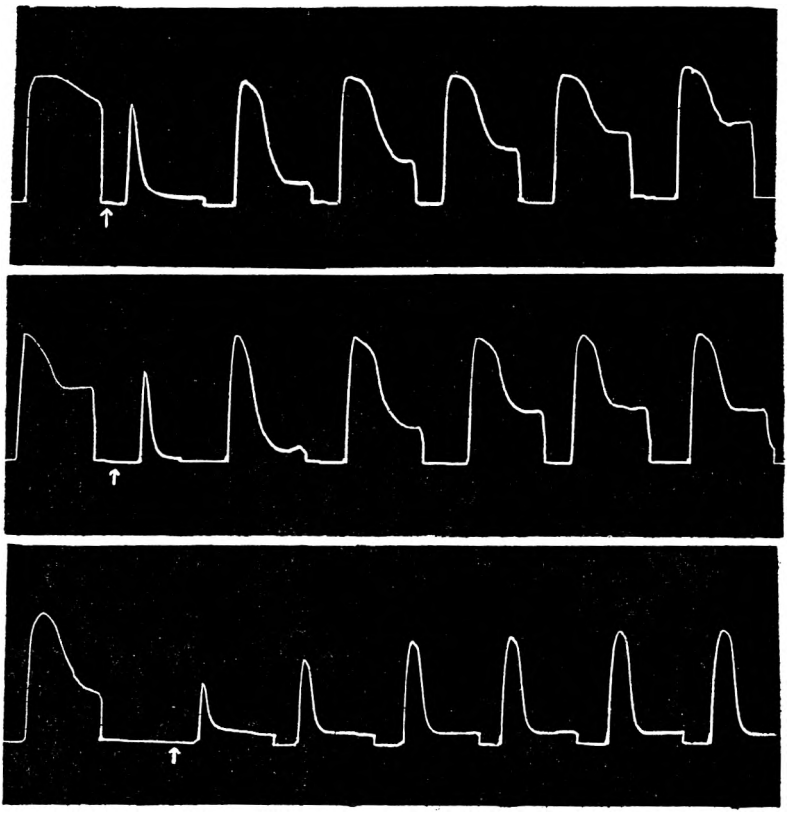


FIG. 2. Cat, 2·3 kg. The effect of three successive doses of 1·5 mg. hexamethonium dibromide. Contractions of nictitating membrane elicited every 10 minutes by pre-ganglionic stimulation at a frequency of 20 shocks per second. Period of stimulation 2 minutes. First and second doses at 39° C. body temperature, third dose at 30° C.

ganglionic blocking substance. This effect should be of particular interest to anæsthetists making use of ganglionic blocking substances during operations carried out on patients whose body temperature has been lowered.

The clinical results achieved in the treatment of hypertension by the methonium compounds vary very greatly and there is no doubt that they cannot be so used without a considerable understanding of their mode of action. Rosenheim[36], using hexamethonium for almost all his cases, feels "that hexamethonium and its homologues should be used in those patients with severe benign hypertension in whom the progress of the disease, the presence of fluffy exudates in the fundi, or the occurrence of hæmaturia suggests the approach of the malignant phase." Smirk, on the other hand[34] uses the methonium compounds and allied substances widely in the benign phase of the disease. According to him "a true basal blood pressure over 175 systolic, 100 diastolic, is probably a

sufficient indication for treatment even in a symptomless patient." Commenting on their usefulness Pickering[44] remarks that "methonium compounds offer by far the most promising therapy that has been produced for hypertension." "But," he continues, "their use makes the most exacting demands on the physician and patient. To use them successfully the physician must have faith in them, and know how to help his patient over the difficulties their use involves. The patient must be sufficiently aware of the gravity of his condition to tolerate the discomforts, have sufficient morale to persevere, and sufficient intelligence to adjust his way of life to the changes in behaviour which the use of these drugs entails."

In 1948 Brown and Gray[54] showed that a close-arterial injection of acetylcholine or nicotine into the skin or mesentery causes a discharge of impulses in the sensory nerves. Their experiments, although providing conclusive evidence that acetylcholine is not involved in the normal function of sensory endings, clearly demonstrate that the latter have properties very similar to the receptive parts of ganglion cells or motor end-plates. Douglas and Gray[55] using a similar method found that an intravenous injection of hexamethonium completely abolished the sensory discharge initiated by the close-arterial injection of acetylcholine although there was no change in the responses to potassium or to touch. The concentrations of acetylcholine used by them were of the same order of magnitude as those which Brown, Dale and Feldberg[56] used to excite a muscle twitch. This fact shows that the sensitivity of the sensory pathways in the skin is similar to that of the motor end-plate, where the sensitivity to acetylcholine is high and associated with normal function. Moreover Armstrong and Keele[57] showed that acetylcholine applied to the exposed base of a cantharidine blister, produces pain in human subjects. This cantharidine induced blister is formed by a separation of the epidermis from the underlying dermis and when the blister is opened the pain nerve terminals are directly accessible to applied solutions. The blister technique is more sensitive and also less unpleasant than other procedures. In their experiments Armstrong and Keele use a specially developed apparatus for producing a continuous and almost simultaneous graphic record of the subjects' assessment of the intensity of pain. Pain thus produced is readily antagonised by the previous application of hexamethonium.

Hexamethonium abolishes in the carotid body the respiratory stimulant actions produced by acetylcholine, nicotine and lobeline but does not interfere with those produced by oxygen lack, potassium or cyanide. Douglas[58] in discussing his results, arrives at the conclusion that: (*a*) the selective abolition by hexamethonium of the response to the nicotine-like drugs indicates that these do not act at a synapse on the chemo-sensory afferent pathway, (*b*) that the pathway from the oxygen-sensitive elements of the cat's carotid body runs to the respiratory centre uninterrupted by any ganglion-like synapse, as hexamethonium in doses much greater than those causing profound block of sympathetic and parasympathetic ganglia failed to exert any apparent action on the carotid body responses to anoxia and (*c*) that the behaviour of the carotid body is strictly analogous

to the behaviour of the skin. Since then other workers have obtained similar results[59–61]. Thus we have now experimental evidence that ganglionic blocking substances are capable of antagonising sensory responses, elicited in the skin and carotid body by a mechanism which is specific against the stimulating properties of acetylcholine, nicotine and similar substances. The use of ganglionic blocking substances in the human subject often results in striking relief of certain types of pain. The types of pain most readily relieved are hypertensive headaches, peptic ulcer pain, anginal pain, causalgia after peripheral nerve injury or pain in herpes zoster and pulmonary infarction. (For references see Moe and Freyburger[1], and Paton and Zaimis[2].) This antagonism to pain is probably associated with the release of smooth muscle spasm and improvement of blood supply which these drugs effect. But we cannot overlook the possibility that a ganglionic blocking substance might act in some instances directly on certain sensory receptors.

Tolerance to all the methonium compounds develops, possibly at slightly varying rates. Mohanty[62], trying to elucidate the mechanism by which tolerance develops, found that the ganglia of an eviscerated or dehepatised cat did not develop tolerance after an intravenous injection of hexamethonium; but when by cross-circulation experiment, the isolated liver of another animal was incorporated into the circulation, tolerance developed in the whole animal. In addition, a solution of hexamethonium treated with liver homogenate, when applied to isolated ganglia, produced resistance to the effects of further hexamethonium doses. Mohanty concludes that the action of the liver on hexamethonium gave rise to a substance which competed with hexamethonium at the ganglia without itself producing any important degree of ganglionic block. These results are interesting but it is difficult to see how they are to be correlated with certain clinical findings. First there is definite evidence[30] that as far as parasympathetic ganglia are concerned, either tolerance does not develop, or it develops much more slowly. It seems improbable that a substance produced by the action of the liver on hexamethonium would compete with hexamethonium at the sympathetic and not at the parasympathetic ganglia. Second, it is known[30] that there is a remarkable difference in the speed at which tolerance develops. Again, if the appearance of tolerance is linked with the formation of a substance in the liver the lack of uniformity in the development of tolerance can hardly be accounted for. The possibility has occurred to me that these facts about tolerance may be linked with two other phenomena. First, with the much discussed potentiation of adrenaline and noradrenaline which always occurs in the presence of ganglionic blocking substances. Experimental results clearly show that this potentiation cannot be attributed only to the abolition of the normal compensatory nervous mechanisms, as it is found both after vagotomy and after section of the spinal cord at a high level. Bartorelli *et al.*[63] discussing their own results together with those obtained by previous workers conclude that there is as yet no satisfactory explanation of this potentiation. Second, during the administration of ganglionic blocking substances for the reduction of blood pressure in operations a

very interesting phenomenon has been observed. When the first dose of a ganglionic blocking substance is administered a lowering of the blood pressure is produced, but the blood pressure returns apparently quite rapidly to its initial level, and any subsequent doses are practically without effect[64]. This very acutely developing tachyphylaxis is not in accordance with the results obtained in animal experiments, where no matter what ganglionic pathway is tested no such phenomenon occurs. The possibility of ganglionic blocking substances acting at other sites has been previously discussed. I should now like to put forward the suggestion that hexamethonium or the other ganglionic blocking substances may sensitise the receptors to adrenaline and noradrenaline, an effect more apparent under such conditions as surgical anæsthesia. If an action of this kind occurs the effect of ganglionic blocking substances will be of

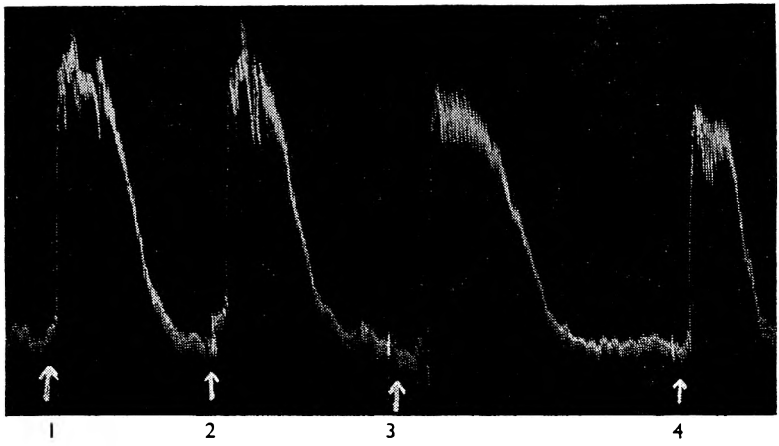


FIG. 3. Cat, 2·7 kg. Chloralose anæsthesia. Blood pressure record. At arrows 1, 2 and 4, 20 μg. adrenaline. At arrow 3, 0·5 mg. tetramethyl ammonium iodide. All doses were given intravenously.

short duration and subsequent doses will be ineffective, not because they fail to block ganglionic transmission again, but because this is masked by the peripheral sensitisation of the receptors to adrenaline and noradrenaline. This too could be a possible explanation of the potentiation of adrenaline and noradrenaline in the presence of ganglionic blocking substances and a reason for the decreasing sensitivity of the sympathetic ganglia to the action of these substances, a phenomenon usually described as tolerance development. In a series of experiments with tetramethylammonium it was observed that this substance reduces the rise of blood pressure produced by adrenaline. Figure 3 shows the result of such an experiment. Is it then possible that a ganglionic stimulant substance reduces the sensitisation of the receptors to adrenaline while a ganglionic blocking substance increases it? The answer to this question can only be found by experiment, but such a possibility is to some extent supported by the findings of Bülbring[64a] that hexamethonium has a stimulant action

and sensitizes the taenia coli of the guinea-pig to different forms of stimulation, and also those of Zauder[65] that hexamethonium potentiates the response of the isolated intestine to both acetylcholine and histamine. According to Zauder this potentiation is independent of innervation and is due to "an increase of the excitability of the muscle in a manner as yet unexplained."

Elvetil

The pharmacology of Elvetil has been described by Cavallini and his colleagues[66]. According to them the substance blocks the transmission in the autonomic ganglia and its duration of action is unusually prolonged. At the same time the substance shows a preference for the parasympathetic system. For instance, in dogs and cats, an intravenous dose of 0·5 mg./kg. reduces or abolishes for about 30 minutes the response to vagus stimulation, but to block the superior cervical ganglion a dose of 3 to 4 mg./kg. is required.

Arfonad

Arfonad[15] is a substance much used by anæsthetists for lowering blood pressure during operations[67–69]. It is popular because its effect is short-lasting so the anæsthetist feels more confident of controlling the blood pressure. Substances which possess high specificity are appreciated by pharmacologists who are inclined to regard them as ideal for clinical applications. For the pharmacologist Arfonad cannot be so classified for while it blocks the transmission of the autonomic ganglia it also liberates histamine[15,70], has a direct action[71], and it is uncertain which of these actions is the main cause of the fall in blood pressure in human beings. If I, as a pharmacologist, had to analyse the substance and give an opinion I should certainly feel very worried and doubtful about its clinical usefulness. Its successful application however, proves that the final value of a substance can only be assessed after both pharmacological and clinical tests.

SU-3088

A few months ago there appeared a short report[72] of a new drug, 4:5:6:7-tetrachloro-2-(2 dimethylaminoethyl)-*iso*indoline dimethochloride, with promising properties. The substance was described as being very potent, of long-lasting activity, easily absorbed when administered orally, and free from tolerance development. However, from the recently published first clinical report[73] it is apparent that expectations have not been fulfilled. The article ends as follows: "Results at present seem to indicate that control of blood pressure has been a little more consistent than with pentolinium; certainly effects continue longer and fewer mg. are required each day. Side actions and variations of effect are similar. It is not yet certain whether a schedule of therapy can be worked out for Su-3088 which will achieve results comparable to those we have obtained with hexamethonium, given orally, for four years. At least this new drug should be a useful addition to the agents now available for control of

hypertension and the reduced mg. requirement and decrease in number of tablets needed each day should predict a financial saving for patients." This is regrettable as an orally active ganglionic blocking substance would have great clinical advantages and is much needed.

Reading through the clinical literature it is interesting to see that every one of the ganglionic blocking substances has its supporters, possibly because the difficulty of dealing with the autonomic nervous system makes clinicians prefer to use a drug to which they are well accustomed. But for the pharmacologist and the physiologist all of them are little gold-mines of information, for through their differences physiological events can be clarified and the modes of action of different drugs studied.

References

1. Moe and Freyburger, *Pharmacol. Rev.*, 1950, **2**, 61.
2. Paton and Zaimis, *ibid.*, 1952, **4**, 219.
3. Bein and Meier, *Schweiz. med. Wschr.*, 1951, **81**, 446.
4. Wien, Mason, Edge and Langston, *Brit. J. Pharmacol.*, 1952, **7**, 534.
5. Bein, Gross, Schuler and Tripod, *Schweiz. med. Wschr.*, 1952, **82**, 1143.
6. Zaimis, *Acta Neurovegetativa*, 1953, **7**, 115.
7. Wien and Mason, *Brit. J. Pharmacol.*, 1953, **8**, 306.
8. Paton, *Arch. int. Pharmacodyn.*, 1954, **97**, 267.
9. Wien, *ibid.*, 1954, **97**, 395.
10. Ambache, *ibid.*, 1954, **97**, 427.
11. Paton, Lectures on the Scientific Basis of Medicine, London University Press, 1954, **2**.
12. Paton, *Pharmacol. Rev.*, 1954, **6**, 59.
13. Bein and Meier, *Der Anaesthesist*, 1954, **3**, 25.
14. Mason and Wien, *Brit. J. Pharmacol.*, 1955, **10**, 124.
15. Randall, Peterson and Lehmann, *J. Pharmacol.*, 1949, **97**, 48.
16. Drill, *Pharmacology in Medicine*, 1st Ed., McGraw-Hill Book Co., 1954.
17. Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, 2nd Ed., The MacMillan Co., 1955.
18. Barlow, *Chemical Pharmacology*, 1st Ed., Methuen and Co., Ltd., 1955.
19. Dale, *J. Physiol.*, 1934, **80**, 10P.
20. Paton and Perry, *ibid.*, 1953, **119**, 43.
21. Perry and Talesnik, *ibid.*, 1953, **119**, 455.
22. Harvey, *Bull. Johns Hopk. Hosp.*, 1939, **65**, 223.
23. Mason and Pelmore, *Brit. med. J.*, 1953, **1**, 250.
24. Paton and Thompson, *ibid.*, 1953, **1**, 991.
25. Larrabee and Posternak, *J. Neurophysiol.*, 1952, **15**, 91.
26. Exley, *Brit. J. Pharmacol.*, 1954, **9**, 170.

26*a*. Bovet, *Rendiconti dell' Istituto Superiore di Sanita*, 1953, **16**, 608.

27. Longo, von Berger and Bovet, *J. Pharmacol.*, 1954, **111**, 349.
28. Zamboni, *Arch. Sci. Farmacol.*, 1954, **4**.
29. Winbury, Cook and Hambourger, *Arch. int. Pharmacodyn.*, 1954, **97**, 125.
30. Morrison, *Brit. med. J.*, 1953, **1**, 1291.
31. Morrison and Paton, *ibid.*, 1953, **1**, 1299.
32. Harington and Rosenheim, *Lancet*, 1954, **266**, 7.
33. Harington, *Clin. Sci.*, 1953, **12**, 185.
34. Smirk, *N.Z. med. J.*, 1953, **52**, 325.
35. Goldsmith, Beaven and Lambert, *Lancet*, 1955, **268**, 371.
36. Rosenheim, *Brit. med. J.*, 1954, **2**, 1181.
37. Zaimis, *Brit. J. Pharmacol.*, 1950, **5**, 424.
38. Cochin and Woods, *J. Pharmacol.*, 1951, **101**, 7.
39. Mitchell and Clark, *Proc. Soc. exp. Biol. N.Y.*, 1952, **81**, 105.
40. Child, Ph.D. Thesis Univ. London
41. Ballard, Isaacs and Scott, *J. Pharm. Pharmacol.*, 1954, **6**, 971.
42. Gottlieb, *Dansk. Tidskr. Farm.*, 1953, **27**, 199.
43. McMichael, *Brit. med. J.*, 1952, **1**, 933.
44. Pickering, *High Blood Pressure*, 1st Ed., J. and A. Churchill, Ltd., 1955.
45. Bartorelli, *Journées Thérapeutiques de Paris*, 1953, p. 177.

46. Hampton and Little, *Lancet*, 1953, **264**, 1299.
47. Enderby, *Lancet*, 1954, **267**, 1097.
48. Armijo, *Therapie*, 1953, **8**, 323.
49. Willcox and Trèmouroux, *ibid.*, 1953, **8**, 293.
50. Donati, *Atti. Soc. lombarda Sci. med. biol.*, 1954, **9**, 475.
51. Bozza, *ibid.*, 1954, **9**, 466.
52. Oselladore and Damia, *ibid.*, 1954, **9**, 466.
53. Goetzee and Zaimis (work in hand).
54. Brown and Gray, *J. Physiol.*, 1948, **107**, 306.
55. Douglas and Gray, *ibid.*, 1953, **119**, 118.
56. Brown, Dale and Feldberg, *ibid.*, 1936, **87**, 394.
57. Armstrong and Keele (unpublished work).
58. Douglas, *J. Physiol.*, 1953, **119**, 118.
59. Liljestrand, *Pharmacol. Rev.*, 1954, **6**, 73.
60. Dontas and Nickerson, *Fed. Proc.*, 1954, **13**, 420.
61. Douglas, *Pharmacol. Rev.*, 1954, 6, 81.
62. Mohanty, *Nature, Lond.*, 1954, **174**, 184.
63. Bartorelli, Capri and Cavalca, *Brit. J. Pharmacol.*, 1954, **9**, 476.
64. Enderby (personal communication).
64a. Bülbring, *J. Physiol.*, 1955, **128**, 200.
65. Zauder, *Fed. Proc.*, 1954, **13**, 420.
66. Cavallini, Mantegazza, Massarini and Tommasini, *Il Farmaco*—Ed. Sc. **8**, fasc. 6.
67. Anderson and McKissock, *Lancet*, 1953, **265**, 754.
68. Kilduff, *ibid.*, 1954, **266**, 337.
69. Scurr and Wyman, *ibid.*, 1954, **267**, 338.
70. Mitchell, Neuman and Gillivray, *Fed. Proc.*, 1951, **10**, 325.
71. McCubbin and Page, *J. Pharmacol.*, 1952, **105**, 437.
72. Plummer, Trapold and Earl, *ibid.*, 1955, **113**, 44.
73. Grimson, Tarazi and Frazer, *Circulation*, 1955, **11**, 733.

RESEARCH PAPERS

SPECTROPHOTOMETRIC STUDIES OF THE IODATE AND PERSULPHATE OXIDATIONS OF ADRENALINE AND NORADRENALINE

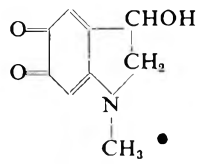
By F. G. Stock and L. W. Hinson

City Analyst's Laboratory, 152, *Great Charles Street*, *Birmingham*, 3

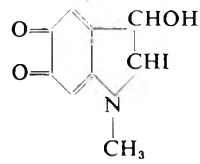
Received March 21, 1955

Introduction

The colorimetric determination of adrenaline has been the subject of many papers, a useful review of which has been made by Jackerott[1]. Most of the suggested methods depend on the oxidation of adrenaline to a coloured substance, but one of the major difficulties in the use of the oxidation process is the necessity for the careful control and standardisation of the working conditions. It was therefore decided to subject the method to a spectrophotometric study, with a view to following the course of the reaction under different series of conditions. For this purpose the two oxidants chosen were potassium persulphate and potassium iodate which are probably the most popular. If potassium persulphate is used, the red primary oxidation product formed is adrenochrome, while in the case of oxidation by potassium iodate an iodine-substituted adrenochrome is obtained; this latter compound has been identified by Richter and Blaschko[2], as 2-iodo-3-hydroxy-1-methyl-2:3-dihydroindole-5:6-quinone.



Adrenochrome



Iodo-adrenochrome

Adrenochrome and its iodo-derivative have very similar, but not identical, absorption spectra, the difference being easily detectable by visual inspection of their solutions. The possibility of the occurrence of noradrenaline in a sample of pharmaceutical adrenaline made it necessary to study the behaviour on oxidation of this substance also. It was found that the oxidation of noradrenaline proceeds at a much slower rate than that of adrenaline, and this fact made it possible to obtain an approximation of the percentages of the two substances present when in admixture.

Von Euler and Hamburg[3], using iodine as oxidant (which produces adrenochrome and not iodo-adrenochrome) found that at *p*H 4·0 adrenaline is quantitatively converted to adrenochrome in 1½ minutes, but that only 10 per cent. of noradrenaline is so converted. They also

found that 3 minutes treatment with iodine at *p*H 6·0 resulted in maximal formation of adrenochrome and noradrenochrome. These facts were used by them to estimate noradrenaline in the presence of adrenaline. For the accurate determination of noradrenaline in adrenaline the method of Auerbach and Angell[4] is probably the best available at the moment, but it is a somewhat lengthy process, and the short simple method outlined in this paper, while making no pretence of superseding that of the two authors mentioned does, it is claimed, give an estimate of the amounts of noradrenaline and adrenaline in a mixture sufficiently accurate for any practical purpose.

Methods of Oxidation

The method of persulphate oxidation employed was the accepted procedure originally proposed by Ewins[5] and later modified by Barker, Eastland and Evers[6], in which a buffer solution of *p*H 5·5 is used, containing 0·239 per cent. $Na_2HPO_4 \cdot 12H_2O$, 0·937 per cent. $NaH_2PO_4 \cdot 2H_2O$, 1·0 per cent. sodium chloride and 0·2 per cent. potassium persulphate; the oxidation is effected by mixing equal volumes of adrenaline solution and buffer at 22° C. and allowing to stand for 30 minutes. For the iodate oxidation a modification of the procedure described by Jackerott was used; 5 ml. of a dilute adrenaline solution are mixed with 1 ml. of 5 per cent. phosphoric acid and 4 ml. of 0·0125 molar potassium iodate solution, the mixture is heated in a boiling water bath for 60 seconds and cooled immediately: it is extremely important that the water in the bath should actually be boiling. The standard adrenaline solution was prepared either from adrenaline bitartrate or from adrenaline base using the calculated quantity of 0·1 N sulphuric acid required to effect solution. A solution containing 10 mg. per cent. was found to be convenient because when diluted for oxidation with an equal volume of reagent, the final concentration of 5 mg. per cent. is suitable for use in the spectrophotometer; the expression $E_{1\text{ cm.}}^{5\text{ mg. per cent.}}$ proved to be very useful for the comparison of spectrophotometric data.

Spectroscopic Properties of Adrenochrome and Iodo-adrenochrome

It was decided to prepare both adrenochrome and its iodine-substituted derivative and determine their spectroscopic characteristics. Adrenochrome was prepared by a method described by Harley-Mason[7], and the iodo-compound by one published by Richter and Blaschko[2]; repeated preparation of these compounds by the methods quoted, gave the same products in so far as they quantitatively exhibited the same absorption spectra. Analyses of the compounds obtained by Harley-Mason[7], and Richter and Blaschko[2], are given by these authors in the original papers, and in both, agree quite well with theoretical considerations.

Adrenochrome gave maxima at 220, 302 and 485 mμ, the ratios of the absorption values at these peaks being 5·53 : 2·45 : 1·00; the iodo-compound gave maxima at 233, 305 and 520 mμ, with ratios of 5·83 : 2·94 : 1·00. The $E_{1\text{ cm.}}^{1\text{ per cent.}}$ values obtained for the visible peaks were

231 for adrenochrome and 111 for its iodine derivative. The $E_{1\text{ cm.}}^{5\text{ mg. per cent.}}$ $\lambda_{max.}$ values obtained in the visible region when adrenaline was subjected to persulphate and iodate oxidations respectively were approximately 0·930 and 0·810; as 1 g. of adrenaline produces on oxidation 0·978 g. of adrenochrome or 1·666 g. of iodo-adrenochrome, it therefore follows that the average conversions obtained at the times of measurement for the persulphate and iodate oxidations, under the conditions described in these methods, represented approximately 82 and 88 per cent. of the theoretical total conversions respectively. The absorption curves of adrenochrome and its iodo-derivative are illustrated in Figure 1, and are identical in characteristics with those obtained in the persulphate and iodate assays under the conditions at which they are carried out. The colorimetric assay is obviously based upon the absorption band in the visible spectrum, but there is no reason why the other two peaks in the ultra-violet region should not be used for a spectrophotometric assay. The peak in the far ultra-violet region, however, seems to be unsuitable because of the irrelevant absorption frequently encountered at these wavelengths; the peak in the 300 mμ region could be used with the advantage of a threefold increase in sensitivity over the peak in the visible region.

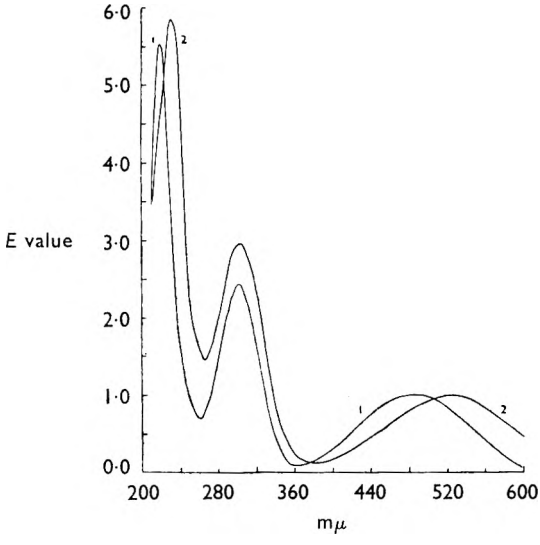


FIG. 1. Absorption spectra of adrenochrome (1) and its iodine-substituted derivate (2).

PECULIARITIES OF IODATE AND PERSULPHATE OXIDATIONS

It will be convenient at this stage to consider in detail some aspects that are peculiar to either the persulphate or iodate oxidations.

(*a*) *Potassium Persulphate Oxidation*

Using the standard method of potassium persulphate oxidation at *p*H 5·5, the peak in the visible spectrum for adrenochrome was slightly displaced from 485 to 490 mμ, consequently all measurements of $E\lambda_{max.}$ were taken at this wavelength. It has long been known that the oxidation process is greatly affected by variation of temperature. At 22° C. the

oxidation reached its maximum after about 20 minutes and then gradually the indicated adrenochrome concentration began to fall, so that the reading taken at 30 minutes was slightly less than the maximum value obtained; but for obvious reasons it represents a point of greater stability for measurement in a comparative assay. The results obtained for the

TABLE I

POTASSIUM PERSULPHATE OXIDATION AT 22° C. FOR DIFFERENT SAMPLES OF ADRENALINE AND NORADRENALINE

Sample	$E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 490 mμ value			
	Time of oxidation in minutes			
	20	25	30	35
(−)-Adrenaline (synthetic)	0·940	0·937	0·925	0·915
	0·940	0·930	0·912	0·902
(−)-Adrenaline (A)	0·940	0·935	0·920	0·912
	0·935	0·935	0·920	0·910
	0·930	0·947	0·935	0·920
	0·915	0·937	0·947	0·925
,, (B)	0·922	0·910	0·890	0·880
	0·915	0·920	0·912	0·900
,, (C)	0·912	0·900	0·885	0·872
	0·910	0·920	0·910	0·887
Adrenaline B.P.	0·763	0·817	0·852	0·870
(suspected to contain noradrenaline)	0·820	0·855	0·870	0·880
(−)-Noradrenaline	0·137	0·153	0·175	0·200
	0·133	0·159	0·191	0·220
	0·130	0·157	0·186	0·219

$E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 490 mμ values of various samples of adrenaline and noradrenaline are given in Table I. The oxidation of noradrenaline proceeded at a much slower rate than that of adrenaline, and this fact is illustrated by Figure 2. In the same diagram the effect of the presence of metals upon the oxidation is shown; copper accelerated and iron retarded the oxidation of both adrenaline and noradrenaline. For mixtures of adrenaline and noradrenaline the $E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 490 mμ value fell as the proportion of noradrenaline in the mixture increased and some results obtained for such mixtures are given in Table II.

TABLE II

POTASSIUM PERSULPHATE OXIDATION AT 22° C. SHOWING $E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 490 mμ VALUES FOR MIXTURES OF ADRENALINE AND NORADRENALINE

Mixture		$E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 490 mμ value			
		Time of oxidation in minutes			
Adrenaline	Noradrenaline	20	25	30	35
95	5	0·922	0·922	0·920	0·912
90	10	0·898	0·902	0·898	0·895
85	15	0·825	0·853	0·864	0·871
80	20	0·768	0·806	0·826	0·838

(b) Potassium Iodate Oxidation

With iodate oxidation the experimental conditions are not so critical, for we found that the concentrations of potassium iodate and phosphoric acid could be varied within wide limits and reproducibility still be achieved.

Furthermore, unlike the persulphate oxidation the effects of copper and iron were found to be negligible. Luhr and Rutschel[8] compared the results of the iodate method with biological examinations and found that there was good agreement between them. The time of immersion in the water bath and the necessity for maintaining a good "rolling boil" were really the only critical factors in the oxidation. Under the conditions of iodate oxidation and the measurement of the absorption in phosphoric acid solution the absorption maximum in the visible spectrum was displaced slightly from 520–5 to 530 mμ, and accordingly $E\lambda_{max.}$ values were measured at this wavelength. The relationship between the $E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 530 mμ value and the time of oxidation is illustrated by Figure 3; in the case of adrenaline the absorption increased rapidly to reach its maximum at about 60 seconds and there was little significant difference between the values obtained over the range 60 to 80 seconds. Even at 120 seconds the value was only 10 per cent. below its peak value, on the other hand, the oxidation of noradrenaline proceeded at a much slower rate. The results obtained with mixtures of these two substances are set out in Table III; a mixture of 20 per cent. noradrenaline and 80 per cent. adrenaline had a higher absorption value at 120 seconds than at 60 seconds; for a 15:85 mixture the absorption was much the same at 60 and 120 seconds, but its maximum value was reached at 100 to 110 seconds; a mixture containing 10 per cent.

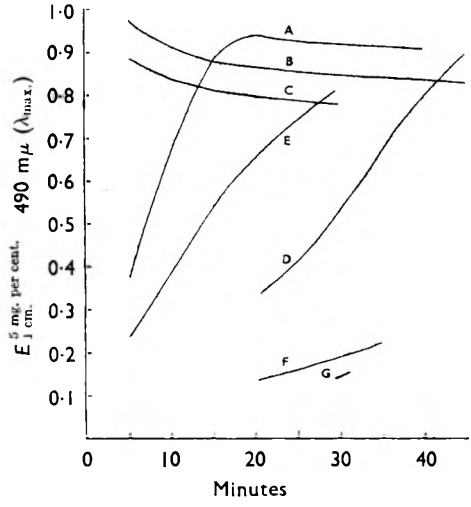


FIG. 2. Potassium persulphate oxidation at 22° C. showing relationship of the absorption at 490 mμ ($\lambda_{max.}$) with time for adrenaline and noradrenaline and the effect of copper and iron.

A. Adrenaline, normal curve.
B. ,, 10 p.p.m. copper.
C. ,, 1 ,, ,,
D. ,, 10 ,, iron.
E. Noradrenaline. 10 p.p.m. copper.
F. ,, normal curve.
G. ,, 10 p.p.m. iron.

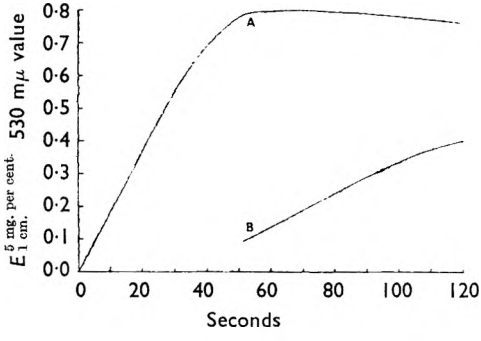


FIG. 3. Potassium iodate oxidation showing relationship between $E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 530 mμ and time of oxidation for A, adrenaline and B, noradrenaline.

of noradrenaline had its period of constant absorption prolonged over the range 60 to 100 seconds. Much useful information on a particular sample of adrenaline B.P. (which may contain noradrenaline) can be obtained from a study of the effects of different periods of oxidation upon both the magnitude of $E\lambda_{max.}$ and the shape of the absorption curve itself. It should be possible from an examination of data of this type to detect the presence of 10 per cent. of noradrenaline in a sample of adrenaline

TABLE III

POTASSIUM IODATE OXIDATION OF SYNTHETIC (−)-ADRENALINE, (−)-NORADRENALINE AND MIXTURES OF THE TWO

		$E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 530 mμ value								
		Time of oxidation in seconds								
Substance		50	60	70	80	90	100	110	120	180
(−)-Adrenaline (synthetic)		0·786	0·800	0·797	0·796	0·790	0·778	0·772	0·766	0·710
(−)-Noradrenaline		0·087	0·137	0·200	0·241	0·275	0·326	0·376	0·398	0·464
Mixtures of the above										
Adrenaline	Noradrenaline									
80	20	0·658	0·668	0·678	0·677	0·678	0·680	0·680	0·683	0·658
85	15	0·660	0·696	0·696	0·700	0·702	0·705	0·706	0·698	0·666
90	10	0·691	0·738	0·734	0·732	0·732	0·730	0·720	0·720	0·703
95	5	0·728	0·766	0·766	0·768	0·763	0·761	0·755	0·750	0·702

B.P. If, however, one starts with a solid which is known to be a mixture of adrenaline and noradrenaline and nothing else, then it is possible from a determination of the E value at $\lambda_{max.}$ alone to assess the proportions of the two substances in the mixture. The complete absorption curves obtained for varying times of oxidation from 1 to 40 minutes for both adrenaline and noradrenaline are given in Figures 4 and 5. In the case of adrenaline there was a rapid rise to a maximum at about 1 minute after which the peaks fell slowly at first, accompanied by a corresponding rise in the absorption at $\lambda_{min.}$ around 390 mμ; the absorption in this region rose rapidly as the peaks in the visible and ultra-violet regions fell. A very good internal check of the stage of oxidation reached may be obtained by examining the relationship between the absorption at $\lambda_{max.}$ and $\lambda_{min.}$ This also illustrates the fact that under controlled conditions one obtains the typical red coloured primary oxidation product; the occurrence of secondary oxidation is shown by the more yellowish solutions obtained when this stage is reached. With noradrenaline the maximum absorption was reached in about 3 minutes, but its magnitude was still only just over 60 per cent. of that reached by adrenaline at its maximum; as time increased there was a general rise in the absorption on the short wave side of the peak as that of the peak itself fell, in much the same manner as for adrenaline.

In Table IV we record data for the $E^{5\ mg.\ per\ cent.}_{1\ cm.}$ 530 mμ values obtained by 1 minute iodate oxidation on various samples of adrenaline supplied as laboratory reagents, including a sample of synthetic (−)-adrenaline and also one of (+)-adrenaline together with samples of both (±)- and

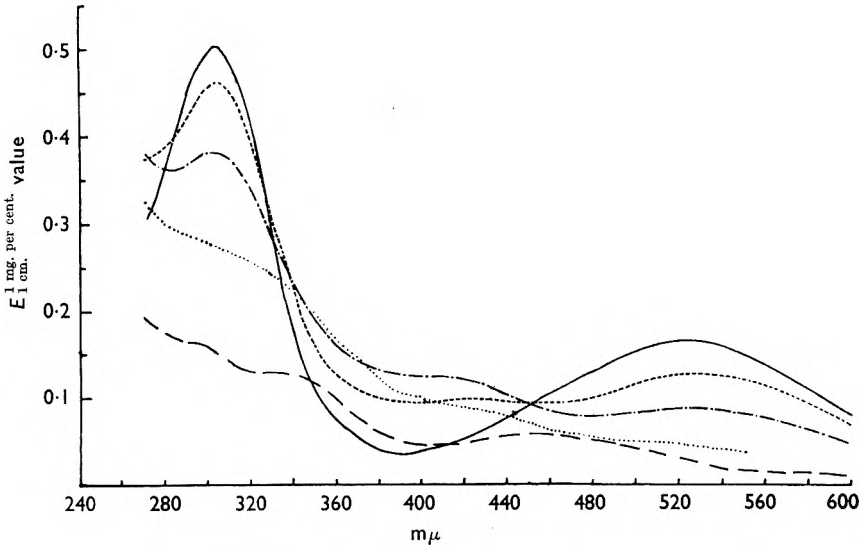


FIG. 4. Potassium iodate oxidation of adrenaline showing the effect of the time of oxidation on the $E^{1\%}_{1\,cm.}$ value.

——— 1 minute. ..... 20 minutes.
– – – – 5 minutes. — — 40 „
– · – · 10 „

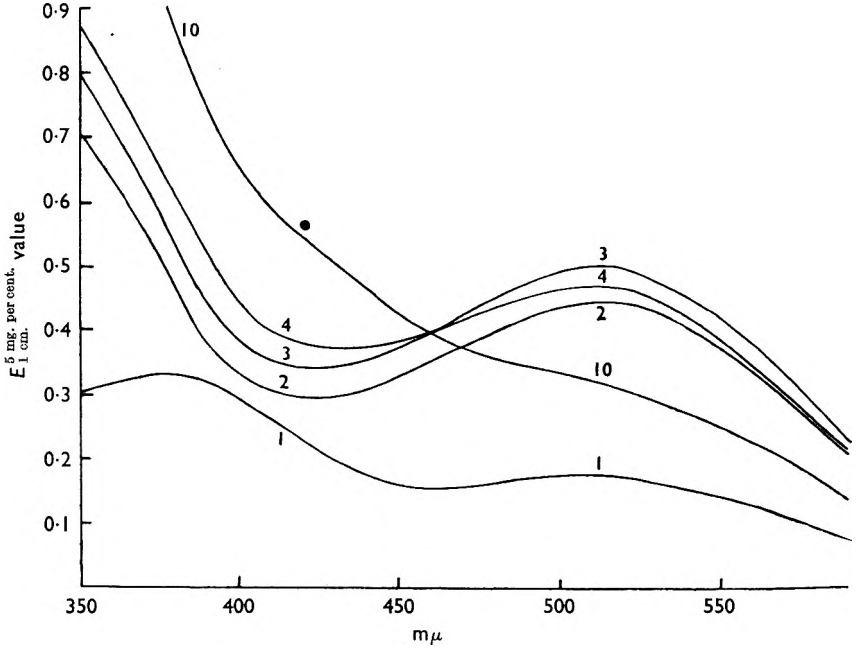


FIG. 5. Potassium iodate oxidation of noradrenaline showing the relationship between absorption and time of oxidation (given in minutes by the figures on the curves).

TABLE IV

POTASSIUM IODATE OXIDATION OF VARIOUS SAMPLES OF ADRENALINE AND NORADRENALINE; $E^{2\ \text{mg. per cent.}}_{1\ \text{cm.}}$ 530 mμ VALUES AT 1 MINUTE

Sample							
(−)-Adrenaline (synthetic)	0·810	0·812	0·815	0·805	0·812		
(±)-Adrenaline	0·812	0·815	0·800	0·800			
(−)-Adrenaline (A)	0·820	0·835	0·830	0·812	0·805	0·805	
,, (B)	0·798	0·792	0·812	0·805			
,, (C) (i)	0·800	0·803	0·822	0·813	0·820		
,, ,, (ii)	0·800	0·805					
,, (D)	0·805	0·810					
(−)-Adrenaline bitartrate	0·812	0·812	0·815	0·815			
Adrenaline B.P. (suspected to contain noradrenaline)	0·736	0·730	0·730	0·738	0·735	0·750	0·750
(−)-Noradrenaline bitartrate ..	0·135	0·138					
(±)-Noradrenaline bitartrate ..	0·137	0·132	0·150	0·148			

(−)-noradrenaline. The agreement although not exact is extremely good, and most of the samples of adrenaline appear to be free from noradrenaline except one supplied as of B.P. quality. This gave a significantly lower E value, which on detailed examination proved to be due to the presence of noradrenaline, estimated very approximately at somewhere in the 10 to 15 per cent. region.

THE APPROXIMATE ASSESSMENT OF ADRENALINE AND NORADRENALINE IN MIXTURES

Since the rates of oxidation of adrenaline and noradrenaline by potassium iodate are significantly different and the changes in the absorptions of the pure substances with time are known, it is possible to calculate the proportions present in a mixture by comparing the changes in its absorption at a suitable wavelength between two times t_1 and t_2.

If an exact determination of the noradrenaline and adrenaline content of a mixture is required the method of Auerbach and Angell already referred to should be used; there are, however, times when an approximation is all that is required, in such cases the following method is suitable because the necessary information can be obtained in minutes rather than in hours required for the application of the former method.

Assume that the following data are available.

Adrenaline: ratio of absorption at t_2 to absorption at t_1 is R_A
Noradrenaline: ratio of absorption at t_2 to absorption at t_1 is R_N
Mixture: ratio of absorption at t_2 to absorption at t_1 is R_S.
Let x be the absorption due to adrenaline at t_1,
then, xR_A is the absorption due to adrenaline at t_2,
and, if y is the absorption due to noradrenaline at t_1,
then, yR_N is the absorption due to noradrenaline at t_2.
If, b is the absorption of the mixture at t_1, then $b = x + y$,
and, if a is the absorption of the mixture at t_2, then $a = xR_A + yR_N$.
Now $R_S = a/b = (xR_A + yR_N)/(x + y)$.

Hence
$$x = \frac{R_S - R_N}{R_A - R_S} \cdot y = \frac{R_S - R_N}{R_A - R_S} \cdot (b - x).$$

Thus $$x = \frac{(R_N b - a)}{(R_N - R_A)}.$$

The $E^{5\ \text{mg. per cent.}}_{1\ \text{cm.}}$ 530 mμ values obtained for the potassium iodate oxidations of adrenaline and noradrenaline at different values of t from 1 to 7 minutes are given in Table V; each value represents the mean of 24 determinations. If the data are examined in order to choose

TABLE V

$E^{5\ \text{mg. per cent.}}_{1\ \text{cm.}}$ 530 mμ VALUES FOR THE POTASSIUM IODATE OXIDATION OF ADRENALINE AND NORADRENALINE AT DIFFERENT VALUES OF t

t in minutes	1	2	3	4	5	6	7
(−)-Noradrenaline	0·141	0·384	0·458	0·450	0·414	0·379	0·351
(−)-Adrenaline	0·811	0·767	0·719	0·676	0·619	0·580	0·529

TABLE VI

APPLICATION OF THE METHOD TO MIXTURES OF ADRENALINE AND NORADRENALINE OF KNOWN COMPOSITION

Composition of mixture, per cent.		$E^{5\ \text{mg. per cent.}}_{1\ \text{cm.}}$ 530 mμ value		Determined composition, per cent.	
Adrenaline	Noradrenaline	1 minute	5 minutes	Adrenaline	Noradrenaline
90	10	0·728	0·562	89	6
		0·732	0·575		
		0·730	0·577		
		0·730	0·586		
		0·732	0·572		
		0·730	0·570		
		Mean 0·730	0·574		
75	25	0·637	0·562	75	23
		0·645	0·565		
		0·640	0·557		
		0·642	0·548		
		0·644	0·555		
		0·636	0·568		
		Mean 0·641	0·559		
50	50	0·460	0·497	49	46
		0·460	0·490		
		0·462	0·493		
		0·458	0·488		
		0·460	0·485		
		0·453	0·490		
		Mean 0·459	0·491		
25	75	0·312	0·468	25	77
		0·300	0·468		
		0·308	0·473		
		0·308	0·473		
		0·316	0·472		
		0·302	0·473		
		Mean 0·308	0·471		
10	90	0·203	0·442	11	91
		0·203	0·442		
		0·215	0·442		
		0·215	0·431		
		0·230	0·440		
		0·230	0·442		
		Mean 0·216	0·440		
Unknown sample labelled B.P.		0·725	0·602	87	14
		0·727	0·606		
		0·727	0·592		
		0·728	0·590		
		0·720	0·594		
		0·728	0·590		
		Mean 0·726	0·596		

suitable values for t_1 and t_2 we find that the ratios of the absorptions at 7 minutes to those at 4 minutes are the same for the two substances, hence t_1 must be less than 4 minutes; the times 1 and 5 minutes seem to be as good as any and these were the values for t_1 and t_2 selected. Using the standard data,

$E_{1\text{ cm.}}^{5\text{ mg. per cent.}}$ 530 mμ; adrenaline 1 minute 0·811, 5 minutes 0·619:
noradrenaline 1 minute 0·141, 5 minutes 0·414:

so that, R_A is 0·763 and R_N is 2·936, the general equation becomes:

$$x = (2{\cdot}936b - a)/2{\cdot}173.$$

The above method was then applied to mixtures of adrenaline and noradrenaline and the results obtained are shown in Table VI, and the agreements with theory were good. It must be stressed, however, that the solutions used were of the pure substances and consequently deductions made by application of the method to impure extracts would not of necessity be valid.

Summary

1. A spectrophotometric study of the persulphate and iodate oxidations of adrenaline and noradrenaline has been made; adrenochrome and its iodine-substituted derivative have been prepared and studied.

2. The spectrophotometric examination of the coloured solutions given by adrenaline on oxidation seems in certain cases to offer a more logical approach to the use of this reaction for the assay of the hormone since the shape of the absorption curve gives an internal check on whether or not the primary oxidation stage has been passed and secondary oxidation commenced.

References

1. Jackerott, *Dansk. Tidsskr. Farm.*, 1941, **15**, 217.
2. Richter and Blaschko, *J. chem. Soc.*, 1937, 601.
3. Von Euler and Hamburg, *Science*, 1949, **110**, 561.
4. Auerbach and Angell, *ibid.*, 1949, **109**, 537.
5. Ewins, *J. Physiol.*, 1910, **40**, 317.
6. Barker, Eastland and Evers, *Biochem. J.*, 1932, **26**, 2129.
7. Harley-Mason, *J. chem. Soc.*, 1950, 1276.
8. Luhr and Rietschel, *Pharm. Zentralh H.*, 1938, **79**, 193.

THE QUANTITATIVE ESTIMATION OF DIGITALIS GLYCOSIDES BY MEANS OF KELLER-KILIANI AND PESEZ-DEQUEKER REAGENTS

By R. Dequeker and M. Loobuyck

From the Department of Pharmacognosy, University of Leuven, Belgium

Received March 21, 1955

Previous work in this department[1] has established that the Pesez reaction[2,3,4] for the detection and estimation of gitoxin in commercial digitoxin may be employed for the quantitative estimation of digitoxose and glycosides containing digitoxose. The yellow colour produced has a maximum light-absorption at 4740 Å (Fig. 1) and we have shown that the reaction obeyed the Lambert–Beer law over the concentration range 2 to 4 μg./ml. of digitoxose (Fig. 2). The reaction is given by 2 only of the 3 molecules of digitoxose present in each of the primary glycosides

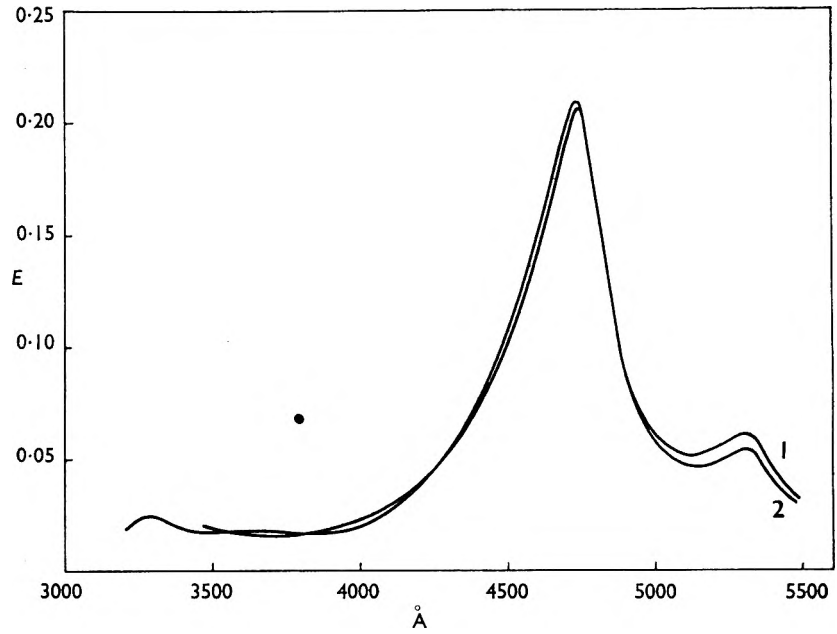


Fig. 1. Absorption spectra of digitoxose in the Pesez-Dequeker reaction. 1. 10·4 μg./ml. cell length 10 mm. 2. 20·8 μg./ml. cell length 5 mm.

desacetyldigilanids A and B and digilanids A, B and C. The sugars cymarose and sarmentose give the same absorption curve as digitoxose with the reagent; thus the glycoside cymarin may be estimated by this reaction.

The method of carrying out the Pesez–Dequeker reaction is as follows. Dissolve 10 to 20μg. of digitoxose or an equivalent quantity of glycoside in 1 ml. of dry acetone in a 12-mm. diameter hard glass test tube, add

phosphoric acid (density 1·70) to produce 5 ml., mix with a glass rod and immerse in a water bath at 35° C. for 15 minutes. Gitoxin or the dry residue derived from decolourised tincture of digitalis are incompletely soluble in 1 ml. of acetone and it is sufficient to suspend these materials in the acetone, a clear solution resulting upon the addition of phosphoric acid. Cool the mixture to 20° C. and determine the light absorption at 4740 Å in a 1-cm. cell by means of a spectrophotometer, using as a blank a mixture of acetone and phosphoric acid treated in the same manner as the assay process. This present work has been carried out on a Unicam S500 photoelectric absorptiometer.

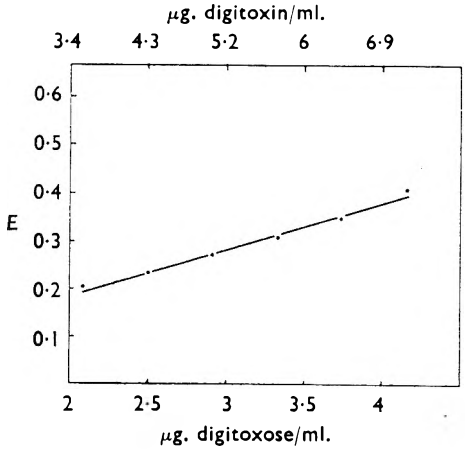


FIG. 2. Density concentration ratio of digitoxose/digitoxin in the Pesez-Dequeker reaction at 4740 Å.

Our previous publication[1] has shown comparative results for the estimation of digitoxose-containing glycosides present in tincture of digitalis by means of the Pesez–Dequeker method and by the Keller–Kiliani method as described by Lindewald and Soos[5,6]. The purpose of this present paper is to compare the sensitivity and the accuracy of the Pesez–Dequeker method and the Keller–Kiliani method as described by Rowson[7] when applied to pure digitalis glycosides and to mixtures of such glycosides with known digitoxose contents.

EXPERIMENTAL

The Pesez–Dequeker reagent and the Keller–Kiliani reagent (Rowson[7]) were initially standardised against a sample of pure digitoxin (Roche), shown to be free from gitoxin by the methods of Pesez[2,3] and Tattje[8]. Aliquot portions of a standard solution of the glycoside containing 0·140 g./l. were employed and the density readings for both reagents are recorded in Table I. The straight-line graph of colour density plotted against digitoxin concentration is shown in Figure 3.

TABLE I

COLOUR DENSITY OF DIGITOXIN WITH KELLER–KILIANI AND PESEZ–DEQUEKER REAGENTS

Method	Digitoxin used (μg.)	Digitoxose equivalent (μg.) $f = 0{\cdot}58108$	Density readings
Keller–Kiliani	350	203	0·325
	420	244	0·392
	490	285	0·465
Pesez–Dequeker	28	16·3	0·222
	35	20·3	0·284
	42	24·4	0·348

TABLE II

THE STANDARD SOLUTIONS USED IN THE COMPARATIVE ASSAYS

Test Substances	Concentration (g./l.)	Solvent
Digitoxin	0·140	Ethanol (94 per cent.)
Digitoxigenin (Roche)	0·030	Ethanol (94 per cent.)
Desacetyldigilanid A (Sandoz)	0·070	Ethanol ,,
Gitoxin (Roche)	0·070	Ethanol ,, + chloroform in equal volume
Gitoxigenin (Roche)	0·015	Ethanol (94 per cent.)
Desacetyldigilanid B (Sandoz)	0·035	Ethanol ,,
Digitalinum verum (Roche)	0·070	Ethanol ,,

Standard solutions of 6 other digitalis glycosides or genins were used for the further comparison of both methods of estimation and they are indicated in Table II. The genins and a digitoxose-free glycoside (digitalinum verum) were selected since they are probably normal constituents of digitalis leaf and, although they give no reaction with either reagents under investigation, it was thought desirable to determine if their presence influences the behaviour of digitoxose-containing glycosides with the reagents. By taking appropriate volumes of one or more of the standard solutions, the individual glycosides or their mixtures were subjected to estimation by the Keller–Kiliani or the Pesez–Dequeker reagents; the solution being evaporated and the residue dissolved in 10 ml. of Keller–Kiliani reagent or in 1 ml. of acetone for the Pesez–Dequeker reaction. Only the pure gitoxin was suspended in the acetone and phosphoric acid added to the suspension.

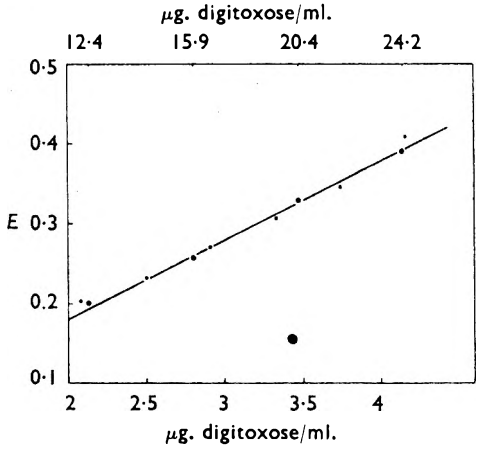


FIG. 3. Curve of density-digitoxin concentration, expressed as digitoxose in the Keller-Kiliani (Rowson) (•) and Pesez-Dequeker reaction (•).

Table III gives the results of the comparative assays using both reagents for the estimation of the individual glycosides containing digitoxose and of their mixtures. It will be seen that the quantitative estimations are closely comparable when using the 2 different reagents; the Pesez–Dequeker method is more sensitive and it is easier to carry out. The presence of genins or of digitalinum verum does not exert any influence upon the results.

CONCLUSIONS AND SUMMARY

1. The Pesez–Dequeker reaction and the Keller–Kiliani reagent of Rowson have been used to estimate desacetyldigilanid A, digitoxin,

TABLE III

COMPARATIVE RESULTS OF THE KELLER–KILIANI AND PESEZ–DEQUEKER TEST ON DIGITOXOSE GLYCOSIDES AND MIXTURES

Concentrations and volumes of the test substances (see Table II)		Digitoxose				
			Keller–Kiliani		Pesez–Dequeker	
Test substances	Standard solution (ml.) see Table II	Content (μg.)	found (μg.)	deviation (per cent.)	found (μg.)	deviation (per cent.)
Gitoxin	2·00	80·0	81	+1·25		
	2·50	100·0	100	0·0		
	3·00	120·0	120	0·0		
	0·40	15·9			16·2	+1·9
	0·50	19·9			20·2	+1·5
	0·60	23·9			24·0	+0·4
Desacetyldigilanid A	7·00	157·0	172	+8·7		
	8·00	179·0	196	+8·7		
	9·00	201·0	223	+11·0		
	0·60	13·4			14·0	+4·0
	0·70	15·7			16·5	+4·8
	0·80	17·9			19·0	+5·7
Desacetyldigilanid B	10·00	110·0	113	+2·7		
	13·00	143·0	147	+2·8		
	16·00	176·0	181	+2·8		
	1·20	13·2			13·2	0·0
	1·40	15·4			15·1	−2·0
	1·60	17·6			17·3	−1·7
Mixture A:—digitoxin + digitoxigenin	2·50*	203·0	201	−1·0		
	3·00*	244·0	241	−1·2		
	3·50*	285·0	280	−1·7		
	0·20*	16·3			15·8	−3·0
	0·25*	20·3			20·3	0·0
	0·30*	24·4			23·8	−2·5
Mixture B:—as mixture A + desacetyldigilanid A	1·70*	176·0	181	+2·8		
	2·00*	207·0	209	+1·0		
	2·30*	238·0	238	0·0		
	0·15*	16·0			17·1	+6·8
	0·20*	21·0			22·1	+5·2
	0·25*	25·6			27·5	+7·4
Mixture C:—as mixture B + gitoxin	1·30*	186·0	187	+0·5		
	1·50*	215·0	218	+1·5		
	1·70*	244·0	246	+0·8		
	0·10*	14·4			14·5	+0·7
	0·15*	21·5			21·9	+1·9
	0·20*	28·7			26·2	−8·7
Mixture D:—as mixture C + gitoxigenin	1·30*	186·0	187	+0·5		
	1·50*	215·0	218	+1·5		
	1·70*	244·0	246	+0·8		
	0·10*	14·4			14·5	+0·7
	0·15*	21·5			21·1	−1·9
	0·20*	28·7			28·8	+0·3
Mixture E:—as mixture D + desacetyldigilanid B	1·10*	170·0	174	+2·4		
	1·30*	200·0	207	+3·5		
	1·50*	231·0	240	+3·5		
	0·10*	15·4			15·7	+2·0
	0·15*	23·2			23·3	+0·5
	0·20*	30·9			30·8	−0·3
Mixture F:—as mixture E + digitalinum verum	1·10*	170·0	177	−4·0		
	1·30*	200·0	208	−4·0		
	1·50*	231·0	242	−4·7		
	0·10*	15·4			16·3	+7·0
	0·15*	23·2			23·6	+1·7
	0·20*	30·9			31·1	+0·6
Number of assays = 27						
Average deviation, per cent.				2·72		2·71
Standard deviation				3·93		3·76

* ml. standard solution of each of the test substances.

desacetyldigilanid B and gitoxin as individual glycosides or in mixtures, also when in association with digitoxigenin, gitoxigenin and digitalinum verum.

2. Both reactions give the same accuracy of results in each estimation.

3. The presence of the genins and of digitalinum verum does not influence the results.

4. The Pesez–Dequeker reaction is the more sensitive and is more convenient to employ; it is thus recommended as the method of choice for the estimation of digitoxose-containing glycosides.

The authors are indebted to Messrs. Sandoz Products Ltd., to Roche Products Ltd., of Basle, and to Laboratoires Nativelle, of Paris, for gifts of pure glycosides and genins.

References

1. Dequeker, *Verhandelingen van de Koninklijke Vlaamse Academie voor Geneeskunde van België*, 1953, **15**, 335.
2. Petit, Pesez, Bellet and Amiard. *Bull. Soc. Chim. Fr.*, 1950, **17**, 288.
3. Bellet, *Ann. Pharm. franç.*, 1950, **8**, 471.
4. Pesez, *ibid.*, 1950, **8**, 746.
5. Lindewald, *Pharm. Zentralh.*, 1944, **85**, 80.
6. Soos, *Sci. Pharm.*, 1948, **16**, 1, 29.
7. Rowson, *J. Pharm. Pharmacol.*, 1952, **4**, 814.
8. Tattje, *ibid.*, 1954, **6**, 476.

THE MICROCHEMICAL DIFFERENTIATION OF MORPHINE AND NALORPHINE

By E. Pedley

North-Western Forensic Science Laboratory, Preston

Received March 29, 1955

Nalorphine (*N*-allylnormorphine) is a drug recently introduced as an antagonist of morphine and other analgesic drugs such as methadone and pethidine. It is therefore liable to be encountered in toxicological and other cases of medico-legal interest in the presence of such analgesic drugs or it may be mistaken for morphine or its compounds under certain circumstances. Thus it is of importance to have a method of distinguishing the two drugs on the micro-scale.

Colour reagents are of little value for this purpose as the colours produced are so similar as to be indistinguishable. This applies to the Marquis, Mecke, Mandelin and Froehde reagents and to the Pellagri reaction and the colours produced with nitric acid. The colour reaction with ammoniacal copper nitrate described by Cooper[1] differentiates the two drugs on the macro scale but the production of the white precipitate is not sufficiently definite in quantities of less than 1 mg. of even pure material to be of practical value with micro quantities.

Absorptiometric methods of differentiating the two drugs have been described. Both show identical maxima and minima in the ultra-violet but they may be distinguished by their infra-red absorption and they may, in fact, be identified in the presence of each other by this method.[2]

An attempt has been made to devise methods of distinguishing between the drugs by microcrystalline reagents, by examination of the parent drugs and derivatives by means of X-ray diffraction and by separation by paper chromatography.

Experimental

Microcrystalline Reagents

A preliminary trial has been made of the reagents shown in Table I using the technique of the Official Methods of Analysis, Association of Official Agricultural Chemists[3], and the following were investigated further: Marme's reagent, Wagner's iodine reagent, picrolonic acid, hydriodic acid and potassium mercuric iodide.

Marme's cadmium iodide reagent. With morphine a silvery-white gelatinous precipitate is produced which rapidly crystallises into masses of fine white needles. Nalorphine produces only an amorphous mass.

Wagner's iodine reagent. With morphine a heavy red-brown precipitate is first formed which slowly forms shining red overlapping crystals extending in plates. Nalorphine produces only a red-brown precipitate and crystals are not formed even after long standing.

Hydriodic acid. As normally encountered this acid (s.g. 1·73) contains much free iodine and if used in this condition the reagent reacts similarly

TABLE I

Reagent	Product of reaction with	
	Morphine	Nalorphine
Marme's	Crystals	Amorphous
Wagner's	Crystals	Amorphous
Picrolonic acid	Crystals	Crystals
Picric acid	Amorphous	Amorphous
Styphnic acid	Amorphous	Amorphous
Kraut's	No precipitate	No precipitate
Mercuric chloride	No precipitate	No precipitate
Hydriodic acid	Crystals	Crystals
Potassium mercuric iodide	Crystals	Amorphous

to Wagner's iodine reagent. If, however, the acid is freed from iodine (by distilling over red phosphorus and using immediately) the crystals obtained are quite different. With morphine long needle-shaped crystals are formed either singly or in bundles or in fan-shaped sheaves according to the concentration (Fig. 1A). With nalorphine short tabular crystals are formed either singly or as agglomerates, again according to the concentration (Fig. 1B). The appearance of the crystals formed with

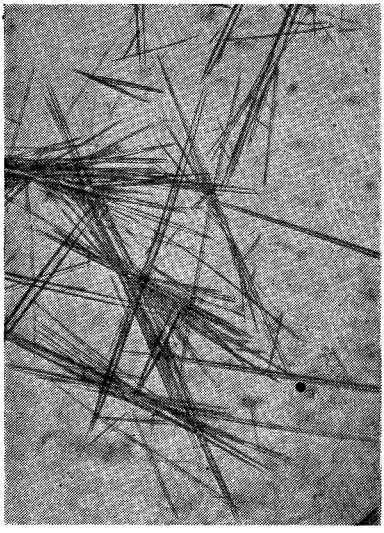


FIG. 1A. Morphine + hydriodic acid.

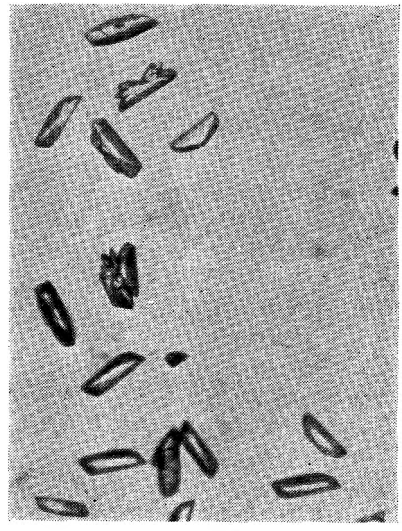


FIG. 1B. Nalorphine + hydriodic acid.

the two drugs is sufficiently striking to permit positive identification and it would seem that this reagent is useful in their differentiation.

Sensitivity of the reaction was determined by placing varying amounts of the drugs on microscope slides (by evaporation of known amounts of their ethanolic solution), dissolving the residues in 0·02 ml. of water or dilute hydrochloric acid and adding 0·03 ml. of iodine-free hydriodic acid. Results are shown in Table II.

The test proved more sensitive for nalorphine than for morphine and is more reliable in that morphine failed occasionally to give crystals

even in concentrations higher than those indicated in the table, whereas nalorphine always gave characteristic crystals with concentrations in excess of 1 in 80.

These experiments also illustrate a weakness of microcrystalline tests, that the form of crystal obtained may vary with the concentration of the drug. In testing tablets and other pharmaceutical preparations it is easy to obtain approximately equivalent amounts of drugs in each test. This cannot apply, however, in dealing with toxicological residues of unknown purity.

TABLE II

Concentration	Morphine	Nalorphine
1:250	No crystals	No crystals
1:125	No crystals	No crystals
1:83·5	No crystals	Occasional crystals
1:62·5	Occasional crystals	Individual crystals
1:50	Sheaves of crystals	Agglomerates
1:25	Large fan-shaped crystals	—

Picrolonic acid. A saturated solution of this reagent in 20 per cent. ethanol was used. With morphine, globular masses were formed which on standing formed bundles of needles (Fig. 2A). More often the globules were more in evidence than the needles. Nalorphine, on the other hand, gives a homogeneous mass which rapidly formed more or less discrete long silky needles (Fig. 2B).

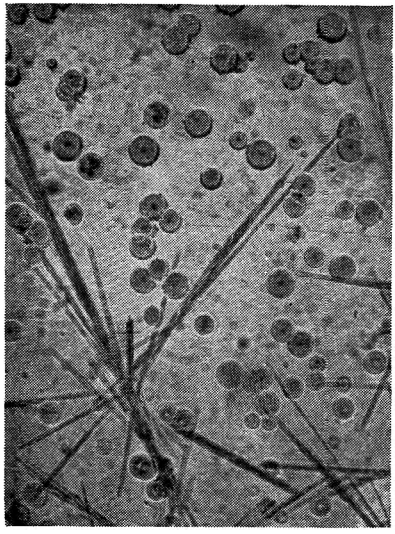


FIG. 2A. Morphine + picrolonic acid.

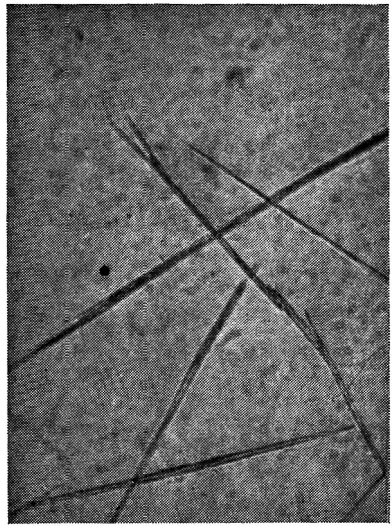


FIG. 2B. Nalorphine + picrolonic acid.

The crystals formed in each case were filtered off, recrystallised and melting point determinations made. The results obtained, however, were erratic and variable and unsuitable for identity purposes. The picrolonates, however, formed excellent crystals for determination by X-ray diffraction (see under).

Potassium mercuric iodide. This reagent has been recommended as a sensitive microcrystalline reagent for morphine[4] using a solution of potassium iodide saturated with mercuric iodide. Experiments confirmed the sensitivity and reliability of the reaction for morphine and the form of the recrystallised morphine–mercuric iodide complex. With nalorphine the reaction is different. The precipitate formed is heavy and amorphous (contrasting with the gelatinous precipitate with morphine) and no crystals were formed even after long standing in a saturated atmosphere for 24 hours. Attempts to crystallise the complex from aqueous acetone yielded a thin yellow oil.

Paper Chromatography

This proved to be a simple and excellent method for separation of the two drugs since they were found to have differing R_F values. Methods of Munier and Macheboeuf[5,6] were applied, using paper treated with 0·5 M potassium chloride and a modified method of Curry and Powell[7] using downward displacement. R_F values obtained are given in Table III. Amounts as low as 25 μg. were detectable and a mixture containing equal amounts (50 μg. of each) separated clearly and gave distinct spots.

TABLE III

	Solvent A R_F	Solvent B R_F	Solvent C R_F
Morphine ..	0·59	0·46	0·22
Nalorphine ..	0·68	0·70	0·39

A: *n*-Butanol–hydrochloric acid 100:2, saturated with water.
B: *n*-Butanol–glacial acetic acid 10:1, saturated with water.
C: *n*-Butanol–citric acid 100:1, saturated with water.

TABLE IV

INDEX LINES

Compound	Strongest lines (*d*) 1st	2nd	3rd	Innermost lines (*d*)
Morphine	6·00	6·60	4·18	10·04
Nalorphine	5·06	7·05	4·40	8·34
Morphine picrolonate ..	7·13	6·17	3·54	8·89
Nalorphine picrolonate	3·47	5·17	6·13	11·78

TABLE V

X-RAY DIFFRACTION DATA

Morphine		Nalorphine		Morphine picrolonate		Nalorphine picrolonate	
(*d*)	Intensity	(*d*)	Intensity	(*d*)	Intensity	(*d*)	Intensity
2·10		2·20		3·54		3·00	
2·29		2·58		4·20		3·24	
2·39		3·14		6·17	s.	3·47	vs.
2·71		3·64		7·13	s.	3·71	
3·46	s.	3·84	s.	8·89		3·78	
3·73		4·40	vs.			4·35	
4·18		5·06	vs.			4·58	s.
4·78	s.	5·60	s.			5·17	vs.
5·03	s.	5·92				5·71	
6·00	vs.	7·05	vs.			6·13	
6·60	vs.	8·34	s.			6·68	vs.
7·46	s.					8·15	vs.
10·04						11·78	

s.—Strong. vs.—Very strong.
Data: Phillips Metallix Equipment, Copper radiation (Kα), Nickel filter. Camera diameter, 115 mm.

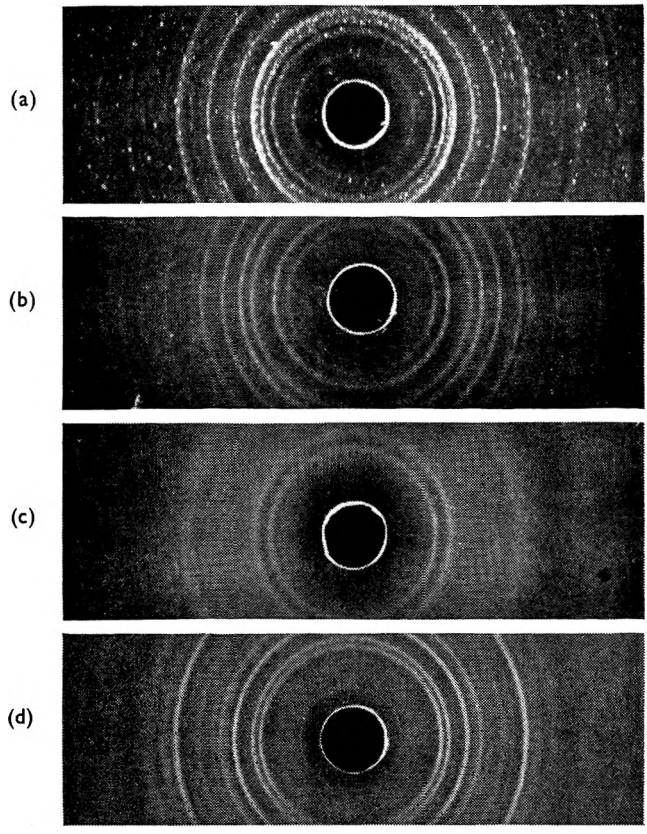


FIG. 3. X-ray diffraction pattern of, (*a*) Morphine, (*b*) Nalorphine, (*c*) Morphine picrolonate, (*d*) Nalorphine picrolonate.

X-Ray Diffraction Patterns

Morphine and nalorphine bases give well-defined X-ray diffraction patterns. These are reproduced in Figure 3. Index lines and diffraction data are given in Tables IV and V.

SUMMARY

1. Micro-methods of differentiating morphine and nalorphine have been investigated.

2. Many microcrystalline reagents give excellent crystals with morphine but failed with nalorphine. Iodine-free concentrated hydriodic acid is a notable exception and is a possible reagent for the identification of morphine. Picrolonic acid is less satisfactory.

3. Excellent separations of the two drugs may be obtained by paper chromatography.

4. X-ray diffraction data of morphine and nalorphine bases and their picrolonates are given.

References

1. Cooper, *Pharm. J.*, 1954, **173**, 145.
2. Seagers, Neuss and Mader, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 640.
3. *Official Methods of Analysis*, Ass. Off. agric. Chem., Wash., 7th Ed., p. 601.
4. Levi and Farmilo, *Analyt. Chem.*, 1954, **26**, 1040.
5. Munier and Macheboeuf, *Bull. Soc. Chim. biol. Paris.*, 1949, **31**, 1144.
6. Munier and Macheboeuf, *ibid.*, 1951, **33**, 846.
7. Curry and Powell, *Nature, Lond.*, 1954, **173**, 1143.

THIAMBUTENE AND BARBITURATE ANÆSTHESIA IN THE DOG

By L. N. Owen

From the Department of Pharmacology and Therapeutics, University of Liverpool

Received March 31, 1955

The analgesic and other properties of the dithienyl alkenylamines have been described by Green[1,2] and Green and his colleagues[3], who have shown that many of the members in this series of compounds have actions resembling those of morphine and also of pethidine. One of these compounds, 3-diethylamino-1 : 1-dithienylbut-1-ene hydrochloride (thiambutene) has also been found by the author to have marked sedative and hypnotic properties which are of considerable therapeutic value in veterinary practice. It has been found that premedication with this drug enhances and prolongs the action of barbiturates, and that the action of thiambutene is rapidly terminated by nalorphine. The purpose of this paper is to describe the advantages of the combined use of these drugs for surgical anæsthesia in the dog.

Methods

All the observations were carried out on dogs which had been admitted to the Liverpool University Veterinary Hospital for surgical treatment of localised lesions. The animals were injected with thiambutene and then anæsthetised either with pentobarbitone sodium or thiopentone sodium, and after completion of the operation a number of dogs in each series were injected with nalorphine to terminate the anæsthesia.

The pulse and respiration rates of each animal were recorded by conventional clinical examination for 1-minute periods at frequent intervals. Temperatures were recorded at intervals by means of a sub-clinical mercury thermometer inserted into the rectum. The environmental temperature of the dogs was not rigidly controlled, but was usually 15° C. Immediately after operation the animals were covered with blankets until they recovered from the anæsthetic.

The action of thiambutene varied from slight inco-ordination of gait to sluggish response to all stimuli and complete abdominal relaxation. These effects were conveniently scored as light, medium and deep narcosis which will be described more fully elsewhere. Deep anæsthesia was characterised by the abolition of pedal, anal, conjunctival and pupillary reflexes, and light anæsthesia by re-appearance of the pedal reflex. The time for complete recovery from the anæsthetic varied considerably, but for the purposes of assessing the action of nalorphine, recovery was denoted by ability of the dog to stand and walk.

Materials

Thiambutene solutions were prepared by dissolving tablets (50 mg.) of Themalon in sterile water to produce a concentration of 25 mg. to 100 mg./ml. according to the dose required; the volume of solution

injected was 0·5 to 5·0 ml. For pentobarbitone anæsthesia a solution containing 64 mg./ml. and for thiopentone anæsthesia fresh solutions containing 64 mg./ml. were used. Nalorphine was used at a concentration of 20 mg./ml.

Results

1. *Thiambutene–Pentobarbitone Anæsthesia*

18 dogs were injected subcutaneously with thiambutene (4·5 mg./kg.). 1 hour later, pentobarbitone sodium was injected intravenously in a dose sufficient to produce deep anæsthesia. 8 of these dogs subsequent to operation were injected intravenously with nalorphine (0·45 mg./kg.).

TABLE I

DURATION OF ACTION AND RECOVERY FROM ANÆSTHESIA WITH THIAMBUTENE AND PENTOBARBITONE IN 10 DOGS

Case	Operation	Age	Thiambutene narcosis	Weight in kg.	Dose of pentobarbitone (mg.)	Duration of anæsthesia (hours)	Ability to walk (hours)
1	Castration	3 mths.	deep	7·5	48	2¾	10¼
2	Fracture ulna	3 mths.	deep	6·8	114	3¾	11¼
3	Dental	5 mths.	light	6·4	160	2¼	7¾
4	Fracture, radius	5 mths.	deep	8·9	146	1¾	4¼
5	Mammary tumour	1½ yrs.	light	7·7	108	4	6½
6	Castration	2 yrs.	light	20·0	290	4¾	>12 <24
7	Mammary tumour	3 yrs.	light	11·0	290	3½	> 9 <19
8	Bilateral aural resection	6 yrs.	medium	8·9	160	4¼	8
9	Neoplasia eyelids. Abscess in foot	8 yrs.	light	9·3	160	2	10
10	Anal adenoma	8 yrs.	light	9·3	160	2	10

A summary of the results on 10 dogs is shown in Table I. Data for the remaining 8 dogs are later described in Table II. From these it will be seen that the age and weight of the dogs varied over a wide range and that the effect of thiambutene varied from light to deep narcosis.

TABLE II

DURATION OF ACTION AND RECOVERY FROM ANÆSTHESIA WITH THIAMBUTENE AND PENTOBARBITONE AFTER NALORPHINE IN 8 DOGS

Case	Operation	Age	Thiambutene narcosis	Weight in kg.	Dose of pentobarbitone (mg.)	Period of anæsthesia prior to nalorphine (hours)	Abolition of anæsthesia	Ability to walk after nalorphine (hours)
1	Spey	4 mths.	deep	6·4	145	1	Almost immediate	2½
2	Bilateral entropion	9 mths.	deep	14·5	245	2	Almost immediate	Unknown
3	Spey	5 yrs.	medium	8·0	108	1½	Almost immediate	4
4	Sub-maxillary cyst	5 yrs.	medium	8·4	227	3¼	1 hour	>24 <30
5	Abscess	5 yrs.	light	13·7	227	1	Almost immediate	3½
6	Sebaceous cyst	5 yrs.	medium	19·0	320	1	Almost immediate	4¾
7	Skin tumour	8½ yrs.	medium	8·0	108	1	Almost immediate	4
8	Tumour of lip	10 yrs.	medium	21·0	275	35 mins.	25 mins.	2½

In all cases, however, no excitement was observed and the subsequent injection of pentobarbitone was readily carried out. In all cases the dose of pentobarbitone required was less than the estimated dose which would have been necessary if the animals had had no premedication, and ranged from 6·4 to 26·4 mg./kg., the mean dose used in 18 dogs was 17·8 mg./kg. (S.D. $\pm$5·44), whereas the calculated mean expected dose is 30 mg./kg.

In the 10 dogs which were allowed to recover spontaneously the duration of anæsthesia varied from 1¾ to 4¾ hours with a mean of 3¼ hours recovery from the anæsthetic was quiet and uneventful and 7 dogs were able to walk within 12 hours. The shortest period of recovery was 4½ hours; the longest, 36 hours. There was a marked fall in body temperature ranging from 3·1 to 12·3° F.; this began after the injection of thiambutene and continued during anæsthesia. The maximum fall usually occurred within 2 hours; towards the end of the anæsthesia the temperature began to rise and returned to within normal limits within 5 hours of the recovery. A typical response is plotted in Figure 1, which also shows concurrent changes in respiratory and pulse rates. The change in pulse rate gave rise to no particular anxiety, but on one occasion the respirations fell to 4 per minute which required resuscitation. Complete muscular relaxation was observed in all cases and there was no obvious increase in hæmorrhage during surgical procedures.

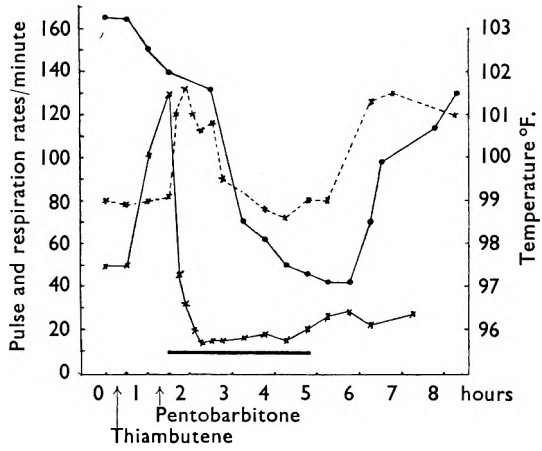


FIG. 1. Typical changes in pulse, respiration and temperature of a dog during anæsthesia with thiambutene and pentobarbitone.
3 year old bitch. Weight 11 kg. The bold line shows the duration of anæsthesia.
×– – –× Pulse. ●——● Temperature.
×——× Respiration.

It is clear that prior administration of thiambutene reduces the amount of pentobarbitone required, and that the total period of anæsthesia is considerably greater than that obtained when pentobarbitone alone is used.

2. *Termination of Thiambutene–Pentobarbitone Anæsthesia with Nalorphine*

The object of the following observations was to study the reversal of thiambutene–pentobarbitone anæsthesia by nalorphine.

8 dogs during deep anæsthesia with thiambutene–pentobarbitone were

each injected intravenously with nalorphine, as previously described. Anæsthesia terminated abruptly in 6 dogs, was reduced in depth in 1 dog, but 1 dog did not regain consciousness until 60 minutes after the injection. The recovery period was reduced, the ability to walk being restored within 2½ to 5 hours after nalorphine (Table II). The dog which had a delayed response to nalorphine did not fully recover until after 24 hours.

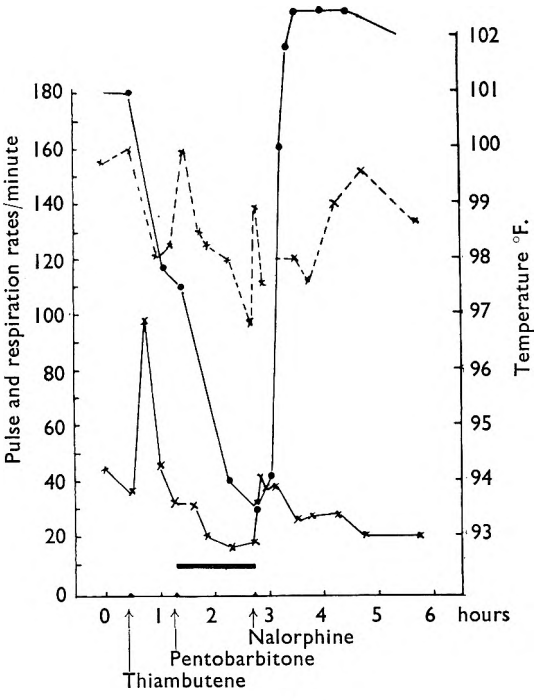


FIG. 2. Typical changes in pulse, respiration and temperature of a dog during anæsthesia with thiambutene and pentobarbitone and after the administration of nalorphine.
5 year old bitch. Weight 8 kg. The bold line shows the duration of anæsthesia.
×- - -× Pulse. ●——● Temperature.
×——× Respiration.

Violent shivering and a rapid return to normal or near-normal of body temperature were usually observed during recovery (Fig. 2). In some cases there was limb galloping and whining, as is often encountered during recovery from pentobarbitone alone.

4 dogs given pentobarbitone alone (30 mg./kg.) were similarly injected with nalorphine, but no changes were observed on the depth of anæsthesia, the recovery period, or the pulse and respiration rates.

3. *Thiambutene–Thiopentone Anæsthesia*

16 dogs were injected intravenously with thiambutene (0·9 mg./kg.); 10 to 15 minutes later thiopentone sodium was injected slowly during approximately 3 minutes. 6 of these dogs, subsequent to operation, were injected intravenously with nalorphine (0·45 mg./kg.).

A summary of the results on 10 dogs is shown in Table III. Data for the remaining 6 dogs are later described in Table IV. The age of dogs varied from 9 weeks to 9 years and the weight from 3·6 to 18·2 kg. Medium or deep narcosis occurred in all except 2 dogs.

The dose of thiopentone required ranged from 5·2 to 20·0 mg./kg., the mean dose was 13 mg./kg. (S.D. ±4·77), which is considerably less than the mean dose calculated to be necessary if the animals were anæsthetised with thiopentone alone (30 mg./kg.).

TABLE III

DURATION OF ACTION AND RECOVERY FROM ANÆSTHESIA WITH THIAMBUTENE AND THIOPENTONE IN 10 DOGS

Case	Operation	Age	Thiambutene narcosis	Weight (kg.)	Dose of thiopentone (mg.)	Duration of anæsthesia (mins.)	Ability to walk (hours)
1	Remove dew claws	9 wks.	deep	5·0	48	19	1¼
2	Spey	3 mths.	deep	3·6	32	20	1
3	Reduce and set fracture, tibia	1 yr.	deep	16·0	320	32	2½
4	Castration	1½ yrs.	medium	10·7	160	23	1
5	Reduce dislocation, hock	2 yrs.	medium	12·4	208	31	2
6	Reduce dislocation, hip	2 yrs.	medium	6·8	130	24	Unknown, left to rest
7	Open abscess. Scale teeth	3½ yrs.	deep	13·7	240	42	1¾
8	Remove plaster cast	5 yrs.	light	12·2	227	42	2¼
9	Dental	7 yrs.	deep	17·0 (fat)	195	23	1
10	Dental	7 yrs.	medium	15·0	146	27	1¼

In the 10 dogs which were allowed to recover spontaneously the duration of anæsthesia varied from 19 to 42 minutes, with a mean of 28 minutes. Recovery was uneventful, no case of post-anæsthetic excitement occurred and ability to walk returned in 1 to 2½ hours after the induction with thiopentone.

The fall in body temperature was not so marked as that described in the previous method; the maximum fall was 4° F., beginning after the injection of thiambutene and continuing during anæsthesia. Pulse rates rose immediately after the thiopentone injection but fell again within a few minutes, a further slight fall occurring during anæsthesia. Respiratory rates were satisfactory throughout (Fig. 3). Muscular relaxation was complete in all cases and no obvious increase in hæmorrhage occurred during surgical procedures.

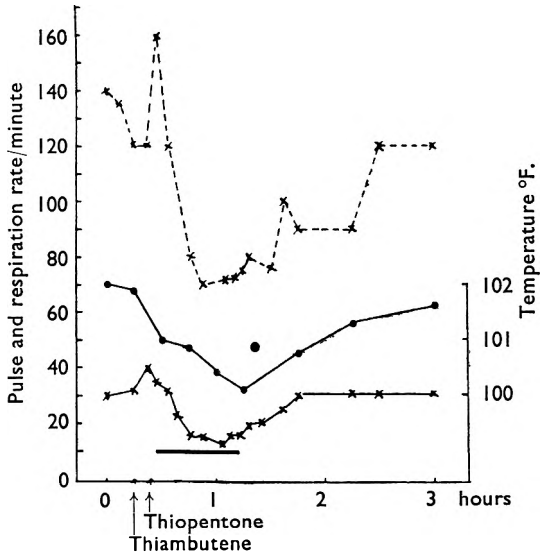


FIG. 3. Typical changes in pulse, respiration and temperature of a dog during anæsthesia with thiambutene and thiopentone. The bold line shows the duration of anæthesia.
5 year old dog. Weight 12·2 kg.
×– – –× Pulse. ●——● Temperature.
×——× Respiration.

Induction was smooth and uneventful except in 1 fat dog, deeply narcotised, which became apnœic and required artificial respiration for 15 minutes. A second dog also became apnœic for 3 minutes, but was readily resuscitated and recovered without further incident.

Thiambutene reduced the amount of thiopentone required and prolonged the total period of anæsthesia to approximately twice that obtained when thiopentone alone is used.

4. *Termination of Thiambutene–Thiopentone Anæsthesia with Nalorphine*

The object of the following observations was to study the reversal of thiambutene–thiopentone anæsthesia by nalorphine.

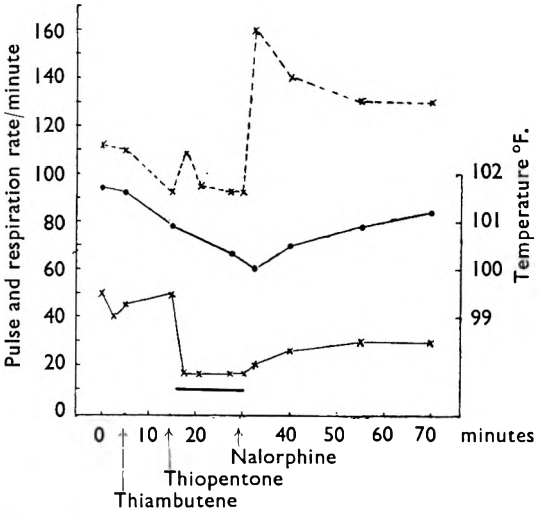


FIG. 4. Typical changes in pulse, respiration and temperature of a dog during anæsthesia with thiambutene and thiopentone and after the administration of nalorphine. The bold line shows the duration of anæsthesia.
1½ year old dog. Weight 18·1 kg.
×– – –× Pulse. ●——● Temperature.
×——× Respiration.

During the subsequent deep anæsthesia of the 6 dogs previously mentioned, the effects of an intravenous injection of nalorphine on the depth of anæsthesia and the period of recovery was noted (Fig. 4, Table IV). After nalorphine, anæsthesia terminated abruptly within 1 minute in all 6 dogs. Ability to walk returned in from 5 to 12 minutes and the recovery was uneventful and without excitement.

Four dogs given thiopentone alone (30 mg./kg.) were similarly injected with nalorphine but no changes were observed on the depth of anæsthesia, the recovery period or the pulse rates.

The duration produced by the two methods and its termination bynalorphine are conveniently summarised in Table V.

TABLE IV

DURATION OF ACTION AND RECOVERY FROM ANÆSTHESIA WITH THIAMBUTENE AND THIOPENTONE IN 6 DOGS

Case	Operation	Age	Thiambutene narcosis	Weight (kg.)	Dose of thiopentone (mg.)	Period of anæsthesia prior to nalorphine (mins.)	Ability to walk after nalorphine (mins.)
1	Umbilical hernia	3 mths.	deep	5·0	48	14	10
2	Amputation, abnormal tail	4 mths.	light	8·6	146	17	5
3	Examination of œsophagus	1½ yrs.	medium	18·1	97	14	10
4	Skin tumour	6 yrs.	deep	13·6	162	35	12
5	Removal of mammary tumour	8 yrs.	medium	15·5	80	18	7
6	Removal of mammary tumour	8 yrs.	medium	13·6	195	15	7

TABLE V

EFFECT OF NALORPHINE IN REDUCING THE TIME OF RECOVERY FROM ANÆSTHESIA WITH THIAMBUTENE–PENTOBARBITONE AND WITH THIAMBUTENE–THIOPENTONE

	Hours		Minutes	
	Thiambutene–pentobarbitone		Thiambutene–thiopentone	
Dog No.	alone	+ nalorphine	alone	+ nalorphine
1	18	4¼	108	12
2	14	4	108	10
3	8¼	4	89	10
4	7¼	3¼	60	7
5	5¼	2¼	40	7
6	2¼	2¼	37	5

DISCUSSION

Although various hypnotics and analgesics have been combined with barbiturates to produce anæsthesia in dogs there is no evidence from the literature that these drugs have produced the potentiating effect similar to that now reported with thiambutene.

Wright[4] stated that the duration of anæsthesia after pentobarbitone sodium varied from 15 minutes to an hour or more depending on the depth initially produced; with thiopentone injected slowly the expected duration varied from 10 to 20 minutes[5]. When morphine was administered prior to pentobarbitone, Wright[4] found that the amount of the latter drug required to produce anæsthesia was reduced but that the duration was shortened though the total period of narcosis was prolonged due to the morphine. Burns[6] in his review of veterinary anæsthesia stated that pethidine prolonged the duration of thiopentone anæsthesia.

The mechanism of the potentiating effect of thiambutene on the action of thiopentone and of pentobarbitone is not known. In view of the observations of Brodie, Bernstein and Mark[7] that the short duration of action of thiopentone is due to its rapid localisation in fat, it is relevant to emphasise the prolonged effects observed in fat dogs with combined thiambutene anæsthesia. It is not clear in what way thiambutene influences the clearance of barbiturate, though there is some evidence that thiambutene is localised in the body fat (Green—personal communication).

The indications and advantages of thiambutene–pentobarbitone combination in veterinary canine practice are similar to those of morphine–pentobarbitone anæsthesia described by Wright[4]. The premedication drug is particularly valuable in restraining vicious dogs and the long period of anæsthesia is useful in prolonged surgical techniques and prolonged recovery period with absence of galloping movements is also a valuable feature. No particular hazards have been encountered during these observations in respect to the fall in temperature and the change in respiration and pulse rate. The rapid reversal of anæsthesia and of depression of respiration by nalorphine is an additional advantage and safeguard.

The period of anæsthesia after thiambutene and thiopentone is usually sufficient for the majority of canine operations. The recovery period is reasonably short, but if more rapid recovery is required this is quickly obtained by nalorphine. Termination of anæsthesia by this drug has the additional advantage that after operation, dogs can be returned to the owner in a very short time. The termination also reduces the risks of post-operative anæsthetic mishaps associated with the peculiar pharynx and larynx of the brachycephalic breeds.

To eliminate the danger of apnœa when injecting thiopentone after premedication with thiambutene it is recommended that the injection be made very slowly, over a 3 to 4-minute period.

Summary

A method of producing anæsthesia in dogs by thiambutene and barbiturates is described. Thiambutene was injected subcutaneously into 18 dogs and 1 hour later the dogs were injected intravenously with pentobarbitone sodium. The amount of pentobarbitone sodium was considerably less than that which would have been required if this drug had been injected alone, and the duration of anæsthesia was considerably greater than that obtained with pentobarbitone alone. When 16 dogs were injected intravenously with thiambutene and 13 minutes later with thiopentone similar effects were observed. In each series of observations nalorphine by intravenous injection abruptly terminated the anæsthesia and reduced the recovery period. A marked fall in body temperature occurred during anæsthesia with pentobarbitone sodium. A reduction in respiration and pulse rates was also observed, but these effects did not adversely influence the recovery of the dogs.

Acknowledgments

I wish to thank Professors A. Wilson and J. G. Wright for their interest and encouragement, and Mr. A. C. Shuttleworth for his valuable co-operation in the management of certain cases.

References

1. Green, *Vet. Rec.*, 1952, **64**, 803.
2. Green, *Brit. J. Pharmacol.*, 1953, **8**, 2.
3. Adamson and Green, *Nature, Lond.*, 1950, **165**, 122.
4. Wright, *Veterinary Anæsthesia*, 3rd Ed. Baillière, Tyndall and Cox, 1952.
5. Jaudin, *Les Cahiers Med. Vet.*, 1949, **18**, 187.
6. Burns, *Aust. Vet. J.*, 1952, **28**, 313.
7. Brodie, Bernstein and Mark, *J. Pharmacol.*, 1952, **105**, 421.

THE STABILITY AND SENSITIVITY OF INSTRUMENTS USED IN SPECTROPHOTOMETRIC ANALYSIS

By C. Morton

From the School of Pharmacy, Chelsea Polytechnic, London, S.W.3

Received March 22, 1955

An important advance in the development of the absorptiometric method of analysis has been made by the recent introduction of differential spectrophotometry by Hiskey and his colleagues[1,2,3]. Various applications of the differential technique have been described[4,5,6], and it is claimed that precision equal to or exceeding that of gravimetric processes may be achieved. The technique differs from that of conventional methods of absorptiometric analysis in that, instead of measuring the intensity of the light transmitted through the solution of unknown concentration against the incident light, the comparison is made with the intensity of light emerging from a solution of the absorbing substance in known concentration. It is also customary in differential spectrophotometry to use solutions of exceptionally high optical density and concentration. In other respects the technique resembles that of conventional methods of absorptiometric analysis and, as Hiskey[2] has pointed out, is subject to a similar limitation, viz., that the precision is determined ultimately by the magnitude of various "uncertainty" factors: chief amongst these are the discrimination, sensitivity or response, and over-all stability of the instrument.

A substantial improvement in discrimination may be effected by replacing the logarithmic optical density scale of the Unicam SP500 spectrophotometer by a linear absorption potentiometer of the type recently described by the writer[7]. By this means the scale-length of the instrument is expanded to such an extent that the discrimination is multiplied by factors of 5, 10, 20 and 25 throughout the density regions 0·0 to 0·2, 0·2 to 0·5, 0·5 to 0·8 and 0·8 to 1·3 respectively. The present communication is concerned with the remaining "uncertainty" factors referred to above, viz., with methods of increasing the over-all stability and sensitivity of the instrument.

The power supplies required for the operation of the amplifier and tungsten lamp of the Unicam spectrophotometer are normally derived from two 6 volt accumulators, which must be changed at intervals of 8 hours and recharged every 24 hours; the stability of the instrument is thus dependent to a high degree on the care which is taken to maintain the batteries. During a demonstration at the Exhibition of the Physical Society in April, 1954, stabilised power for the operation of a Unicam spectrophotometer was obtained from the public electricity supply mains with the aid of a voltage regulator, and it has been suggested to the writer that a description of this device would be of interest. In addition to affording a substantial improvement in zero stability, a

stabilised mains unit of this type offers the advantages that the inconvenience and delay occasioned by the frequent changing and re-charging of accumulators are avoided. The degree of stabilisation afforded by the regulator is such that the readings of the spectrophotometer are unaffected by variations of $\pm$ 50 per cent. in the voltage of the power supply.

Of the various methods available for increasing the sensitivity or response of a spectrophotometer, that commonly used has the drawback that the setting of the sensitivity control determines the bandwidth which must be used in the examination of a solution of given optical density. The auxiliary sensitivity control described in the present communication provides for a twelvefold increase in the over-all response without affecting the optical performance of the instrument.

The Stabilisation of the Source of Power

In preliminary experiments, rectified current derived from public supply mains was stabilised by means of an electronic voltage stabiliser having a self-regulated cathode heater supply[8]. Owing to the high gain of the amplifier incorporated in the stabiliser and the high voltage required for efficient regulation by electronic means, difficulty was experienced in eliminating disturbances of the pointer of the spectrophotometer arising from neighbouring electrical fields or leakage from

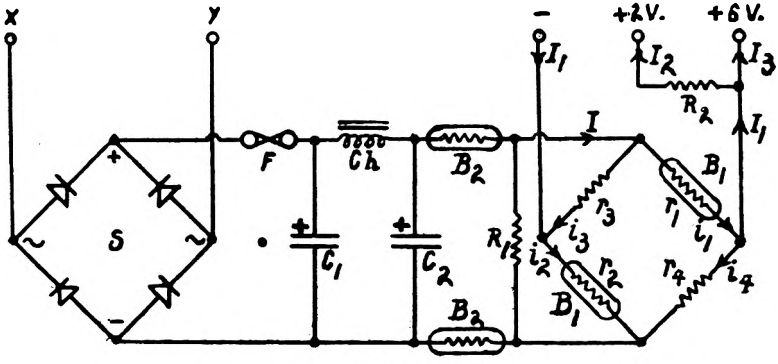


Fig. 1.

the high voltage source. It was eventually decided to rely upon a bridge voltage regulator as a means of stabilising the power supply. Regulators of this type are insensitive to external electrical disturbances and, except when high output voltages are required, are considerably more economical in power consumption than electronic stabilisers. A bridge voltage regulator also has the advantage over an electronic stabiliser that its performance remains unimpaired after 2000 hours of service.

The arms r_1 and r_2 of the regulating bridge (Fig. 1) consist of the twin filaments of a Siemens 100 mA. barretter B_1, the bridge being completed by the ohmic resistances r_3 and r_4. As the author has shown[9], a barretter to which a voltage e is applied is electrically equivalent to

an ohmic resistance r in series with a constant internal potential v, the value of which may be calculated from the relationship $v = ir - e$, where i is the current flowing through the filament at the applied voltage e, and $r = de/di$ is the differential resistance of the filament. Thus, if $r_1 r_2$ and $v_1 v_2$ are the differential resistances and equivalent internal E.M.F.s of the twin filaments of the barretter B_1, i_1, i_2, i_3, i_4 are the currents through r_1, r_2, r_3, r_4, I is the current supplied to the bridge, I_1 is the output current from the bridge, and r_0 is the resistance of the load, the following relationships apply to the circuit of Fig. 1:—

$$I_1 r_0 = v_1 + i_3 r_3 - i_1 r_1 = v_2 + i_4 r_4 - i_2 r_2 \quad .. \quad .. \quad .. \quad (1)$$

$$I_1 = i_1 - i_4 = i_2 - i_3 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$I = i_1 + i_3 = i_2 + i_4 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

From these equations it is readily deduced that

$$r_0 i_2 - (r_0 + r_1 + r_3) i_3 + r_1 I = v_1 \quad .. \quad .. \quad .. \quad .. \quad (4)$$

and $$(r_0 + r_2 + r_4) i_2 - r_0 i_3 - r_4 I = v_2 \quad .. \quad .. \quad .. \quad .. \quad (5)$$

By eliminating I from (4) and (5) we obtain

$$(r_1 r_2 + r_0 r_1 + r_1 r_4 + r_0 r_4) i_2 - (r_3 r_4 + r_0 r_1 + r_1 r_4 + r_0 r_4) i_3 = r_4 v_1 + r_1 v_2 \quad .. \quad (6)$$

If the resistances of the ohmic arms r_3 and r_4 of the bridge are adjusted in such a manner as to satisfy the relationship $r_1/r_4 = r_3/r_2$, equation (6) reduces to

$$I_1 = i_2 - i_3 = (r_4 v_1 + r_1 v_2)/[r_1(r_0 + r_2) + r_4(r_0 + r_1)] \quad .. \quad (7)$$

As equation (7) does not contain any term which involves the power supplied to the bridge, it follows that the output current I_1 is independent of fluctuations in the supply voltage.

In order to extend the regulating range, the twin filaments of a Siemens 140 mA. barretter B_2 are connected in series with the input leads to the bridge. As the current passed by the filaments of this barretter at the mid-points of their respective regulating ranges, viz., approximately 128 mA., is somewhat greater than the input current I required by the regulating bridge, it is necessary to connect a resistance R_1 across the input terminals of the bridge in order to bypass the excess current. Alternating current derived from public supply mains is applied, via a step-down transformer, to the input terminals X and Y of a Westinghouse bridge rectifier S, and the rectified output from the bridge S is passed through a filter consisting of the choke Ch and electrolytic condensers C_1 and C_2 in order to remove the ripple voltage. The fuse F is included in the circuit with the object of protecting the rectifier from damage in the event of a breakdown of the electrolytic condensers.

The 3-way cable which emerges from the case of the Unicam spectrophotometer is normally connected to a 6-volt accumulator, the black lead being joined to the negative terminal, the green lead to a 2-volt tapping on the accumulator, and the red lead to the 6-volt positive terminal; the currents flowing along these leads are approximately 76,

14 and 62 mA. respectively. In the circuit of Figure 1 the resistances associated with the regulating bridge are so chosen that an output of 76 mA. at 6 volts is obtained and, in order to ensure that the currents I_1, I_2 and I_3 are 76, 14 and 62 mA. respectively, a resistance $R_2 = 4/0{\cdot}014 =$ 286 ohms is connected in series with the +2 V. terminal. The power required for the tungsten lamp of the spectrophotometer is supplied by an additional secondary winding, delivering 6 amperes at 6 volts, on the mains transformer, and the input to this transformer is stabilised by means of a constant voltage transformer. Siemens 100 and 140 mA. barretters have twin tungsten filaments, and their regulating properties are due to changes in the density of the gas surrounding the filaments; in consequence, these barretters are almost completely free from the thermal time lag which is associated with ballast lamps of the conventional iron–hydrogen type.

The stabilisation ratio $\delta V_0/\delta V_i$ of the regulating bridge and series barretter, i.e., the change in output voltage produced by unit change in the voltage V_i across the condenser C_2, depends upon the linearity of the current–voltage characteristics of the barretters and upon the accuracy with which the ohmic resistances in the bridge network are adjusted. The mains unit used at the Exhibition of the Physical Society was constructed by the Doran Instrument Co., Stroud, all resistances being adjusted with an accuracy of $\pm 0{\cdot}1$ per cent. For this unit the value of $\delta V_0/\delta V_i$ was found to be 2×10^{-4}; in other words, a variation of ± 1 V. in the voltage across the condenser C_2 gives rise to a change of $\pm 0{\cdot}2$ mV. in the voltage across the 6 V. output terminals. Additional regulation is provided by the constant voltage transformer, and the over-all stability is such that no movement of the pointer of the meter can be detected as a result of changes of ± 50 per cent. in the voltage of the supply mains.

Due to the high internal resistance of the mains unit as compared with an accumulator, the dark current and sensitivity controls are in some degree interdependent; if the setting of the sensitivity control is altered to an appreciable extent after the dark current adjustment has been carried out, it will in general be found that the latter adjustment has been disturbed. For this reason, when the mains unit is in use, the sensitivity control should normally be set about $3\frac{1}{2}$ turns from the fully clockwise position before carrying out the dark current adjustment, and should not be disturbed during subsequent measurements.

The Sensitivity of the Instrument

A simplified circuit diagram of the electrical connections of the spectrophotometer is given in Figure 2. The photoelectric current i_p generated by the photoelectric cell P flows through the 2000 MΩ resistor R_2, producing across this resistor a voltage drop E which is applied, in series with an opposing potential derived from the density potentiometer R_{13}, to the grid of the input valve V_1 of a two-stage D.C. amplifier. The measurement consists in adjusting the potentiometer until the output current i_a flowing through the microammeter M attains a predetermined

value (300 μA.); the opposing voltage impressed upon the grid of V_1 by the potentiometer R_{13} is then equal to the P.D. across R_2 due to the photoelectric current, and is directly proportional to the percentage transmission of the solution under investigation. The voltage sensitivity $S_v = \delta i_a/\delta E$ of the amplifier is approximately 15 μA./mV., and the current sensitivity $S_i = \delta i_a/\delta i_p = S_v R_2$ is accordingly about 30 μA./$\mu\mu$A.

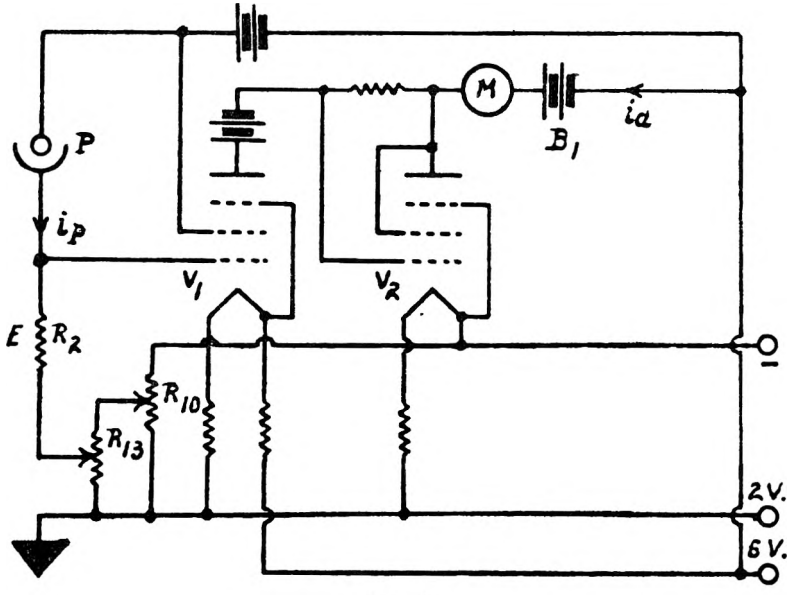


FIG. 2.

No means of controlling the gain or voltage sensitivity is provided, but the P.D. between adjacent subdivisions of the transmission scale of R_{13} may be continuously adjusted between the approximate limits of zero and 14 to 16 mV. by means of the sensitivity control R_{10}, which regulates the current flowing through R_{13}; each of these subdivisions corresponds to 1 per cent. change in transmission. The ratio $S_t = \delta i_a/\delta t$, that is, the increment in the output current i_a produced by unit change in the percentage transmission t, may conveniently be termed the transmission sensitivity; it is evident that if, at a given setting of the control R_{10}, the voltage drop between adjacent subdivisions of the transmission scale is V, the transmission sensitivity is $S_t = \delta i_a/\delta t = V\delta i_a/\delta E = VS_v$. For example, assuming that V may be varied from zero to 16 mV. by adjustment of R_{10}, and that the voltage sensitivity of the amplifier is $S_v = 15$ μA./mV., the transmission sensitivity of the instrument, i.e., the output current obtained for 1 per cent. change in transmission, is adjustable between the limits of zero and $15 \times 16 = 240$ μA. by means of this control.

The sensitivity control R_{10} and the slit width control are interdependent; only one of these controls may be varied at will, the other being a dependent

variable. For all normal purposes the manufacturers of the instrument recommend that the sensitivity control should be set to a predetermined value, and that balancing adjustments should be effected by means of the slit width control, which thus becomes the independent variable. Under these conditions the transmission sensitivity is maintained at a constant high level throughout the measurements, regardless of the optical density of the solutions. On the other hand, Neal[5] recommends the use of a constant narrow slit width in differential spectrophotometry on the grounds that variation in bandwidth may lead to interference from other absorbing species present in the solution. When, however, the sensitivity control is used as the independent variable in the manner recommended by Neal, the transmission sensitivity decreases rapidly as the optical density of the solution increases, and the response of the instrument may be reduced to such a low level as to render adequate discrimination impossible.

From the foregoing considerations it is evident that some auxiliary means of increasing the sensitivity without affecting the optical performance of the instrument would be an advantage. An auxiliary control of this nature, known as the "meter sensitivity control", is included amongst the components of the Uvispek spectrophotometer, the provision of this control being facilitated by the use of twin triodes in the output stage. The corresponding stage of the Unicam amplifier contains only 1 valve (V_2 in Fig. 2); nevertheless, the circuit of the amplifier may be modified in a simple and inexpensive manner in order to provide for the addition of a similar auxiliary control.

The microammeter incorporated in the spectrophotometer is a moving coil instrument requiring 600 μA. for full-scale deflection; if this is replaced by a 50 μA. galvanometer, a twelvefold increase in over-all sensitivity is obtained. Inspection of Figure 2 reveals the fact that the meter carries the full anode currents (300 μA.) of the valves; in order that the microammeter may be replaced by a sensitive galvanometer, it is necessary to make provision for a counter current of the same magnitude. The slight alterations in the circuit which are required for this purpose are shown in Figure 3, the counter current i_c being obtained by connecting the stabilised 6 V. power supply, in series with a current-limiting resistor R, across the galvanometer G. As $i_c = i_a = 300\,\mu$A., the appropriate ohmic value of R is $6/300 \times 10^{-6} = 20{,}000\ \Omega$. A high stability resistor of the inexpensive radio type, having a tolerance of ± 1 per cent., is suitable for the purpose. In the circuit of Figure 3, the anode leads of the valves are returned to the negative pole of the 6 V. source instead of to the positive terminal: the voltage tapping on the battery B_1 should accordingly be increased by 6 V. in order to restore the anode voltages to their normal values. A variable shunt (not shown in the diagram) is connected across the galvanometer terminals for use as the auxiliary sensitivity control.

In the modified circuit of Figure 3 the voltage drop across the galvanometer is $i_g R_g = i_c R - E_0 = (i_a - i_g)R - E_0$, where R_g is the resistance of the galvanometer, i_g is the galvanometer current, and E_0 is the voltage

(nominally 6 V.) of the source of counter-current; the galvanometer current is thus

$$i_g = (i_a R - E_0)/(R_g + R) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

and the increment in galvanometer current produced by unit increase in the photoelectric current i_p is

$$\frac{\delta i_g}{\delta i_p} = \frac{\delta i_g}{\delta i_a} \cdot \frac{\delta i_a}{\delta i_p} = \frac{R}{R_g + R} \cdot Si \quad .. \quad .. \quad (9)$$

As $R \gg R_g$, equation (9) reduces in practice to $\delta i_g/\delta i_p = S_i$.

The circuit arrangement of Figure 3, in addition to providing for a substantial increase in the over-all sensitivity of the instrument, has the advantage that drift of the pointer due to fluctuation in the voltage E_0 of the power supply is further reduced. The change in the galvanometer

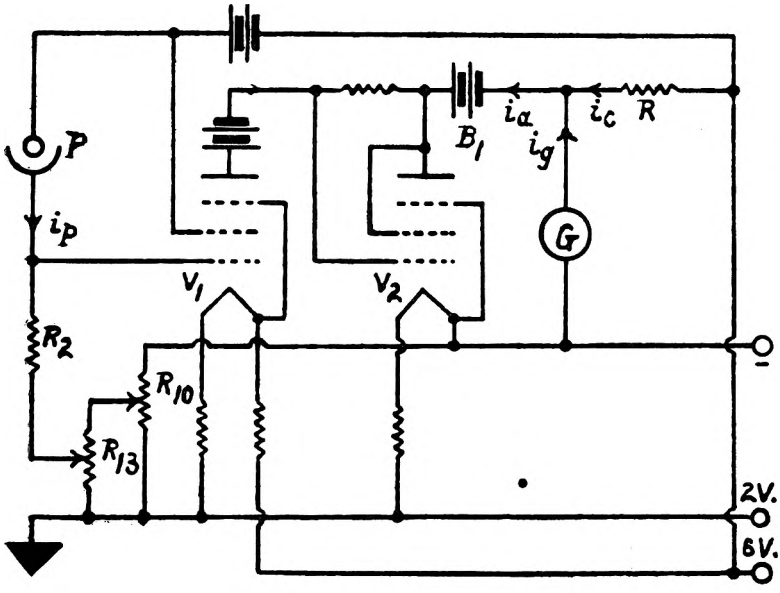


FIG. 3.

current produced by fluctuations in the supply voltage is zero when the slopes of the i_a/E_0 and i_c/E_0 curves are identical. From equation (8) it follows that the conditions which must be satisfied in order that the galvanometer current may be zero and may be unaffected by variations in the supply voltage are $R = E_0/i_a$ and $R = \delta E_0/\delta i_a$ respectively. These two conditions cannot be satisfied simultaneously unless, at the normal operating voltage, the tangent to the i_a/E_0 curve passes through the origin of the graph. In practice, this requirement is not accurately fulfilled and, in consequence, perfect compensation for fluctuations in the supply voltage cannot be achieved; nevertheless, a substantial further improvement in the zero stability of the instrument is obtained.

Summary

1. With the aid of a bridge voltage regulator the power required for the operation of the amplifier and tungsten lamp of the Unicam SP500 photoelectric spectrophotometer may be derived from public electricity supply mains. The readings of the spectrophotometer are unaffected by changes of ± 50 per cent. in the voltage of the power supply, and are also independent of variations in the frequency of alternation of the supply.

2. A description is given of a simple means of effecting a twelvefold increase in the over-all sensitivity of the spectrophotometer, together with a substantial further improvement in zero stability.

The author is indebted to the Royal Society and the University of London for research grants.

References

1. Hiskey, *Analyt. Chem.*, 1949, **21**, 1440.
2. Hiskey, Rabinowitz and Young, *ibid.*, 1950, **22**, 1464.
3. Hiskey and Firestone, *ibid.*, 1952, **24**, 342.
4. Bastian, *ibid.*, 1949, **21**, 972; 1951, **23**, 580.
5. Neal, *Analyst*, 1954, **79**, 403.
6. Milner and Phennah, *ibid.*, 1954, **79**, 414.
7. Morton, *J. Pharm. Pharmacol.*, 1954, **6**, 148
8. Morton, *Electron. Engng.*, 1952, **24**, 65.
9. Morton, *J. sci. Instrum.*, 1944, **21**, 15.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

p-Aminosalicylic Acid Solutions, Assay and Stability of. A. Ågren. (*Farm. Revy*, 1955, **54**, 225.) The violet colour given by *p*-aminosalicylic acid with ferric salts in acid solutions may be used for colorimetric determination, as the reaction is not affected by the presence of *m*-aminophenol. The solution, containing about 0·5 mg. of the compound, is treated with 1·00 ml. of ferric chloride solution (M/60 in 0·09N hydrochloric acid) and 0·50 ml. of 0·1N hydrochloric acid. The solution is made up to 100 ml., and the extinction is determined at 500 mμ. The colour does not fade rapidly, losing only 2 per cent. in intensity after 4 hours. Aqueous solutions of *p*-aminosalicylic acid are stable at ordinary temperature if the *p*H is not less than 6·0. At *p*H = 5·0 about 50 per cent. is decomposed after 10 weeks; and at *p*H 4·0 in about 10 days.

G. M.

Calcium and Magnesium, Spectrophotometric Determination of. A. Young, T. R. Sweet, and B. B. Baker. (*Analyt. Chem.*, 1955, **27**, 356.) A method for the determination of small quantities of calcium and magnesium in water has been developed, depending on the relative light absorption of the Eriochrome Black T complexes at *p*H 9·5 and 11·7. Measurement of a dye blank against a solution containing calcium and magnesium at *p*H 11·7 gives a reading which is proportional to the total calcium and magnesium present, while at *p*H 9·5 the reading is essentially a measure of the magnesium. The light absorption was measured at 630 mμ; as the blank absorbs more light at 630 mμ than the sample, the instrument was balanced with the sample in the light path the absorption of the blank then being determined. For 43 known mixtures containing 0·3 to 6·0 p.p.m. calcium (as $CaCO_3$) the average absolute error of magnesium was 0·09 p.p.m. and of calcium 0·12 p.p.m. R. E. S.

Cantharidine in Cantharides, Determination of. C. G. van Arkel and M. Meyst. (*Pharm. Weekbl.*, 1955, **90**, 38.) A comparative study was made of 5 methods for the assay of cantharides. The following procedure is recommended (based on that of the Veterinary Addendum of the Danish Pharmacopœia). 15 g. of the material (in no. 20 powder) is allowed to stand overnight with 148·5 g. of chloroform and 2 ml. of hydrochloric acid, and then shaken for a couple of hours. After filtering, 10 g. of the filtrate is concentrated to a few ml., then dried by a current of dry air at 50 to 55° C. The residue is refluxed for 5 minutes with 100 ml. of water, and the solution is filtered through a wet filter. The filter paper (9 cm. diameter) is extracted with a further 50 ml. of boiling water, which is again filtered through a wet paper, which is finally washed with 50 ml. of boiling water. The combined aqueous solutions are acidified with 2 ml. of hydrochloric acid and shaken out 3 times with 25 ml. of chloroform. The chloroform is removed as described above, and the residue is treated with 5 ml. of a mixture of 9·5 ml. of light petroleum and 0·5 ml. of absolute ethanol, with shaking for 30 minutes. The solution is decanted through a plug of cotton wool, with two washings with the above mixture, then with 5 ml. of chloroform. The chloroform is removed, the residue is dried for 30 minutes at 60° C. and weighed. A correction of 10 mg. is added to the weight obtained. G. M.

Morphine in Opium, Determination of. A. B. Svendsen and E. D. Aarnes. (*Sci. Pharm.*, 1955, **23**, 18.) The determination of morphine as dinitrophenyl-ether gives high results, as the product contains other alkaloids in addition to colouring matter. These errors may be avoided by using an absorbent in the extraction: 1 g. of the opium is rubbed down with 3 ml. of methanol and 1 ml. of 25 per cent. ammonia, and the mass is then mixed with 15 g. of alumina (Brockmann). The resulting dry mass is packed into a chromatograph tube and eluted with a mixture of 180 ml. of chloroform and 60 ml. of *iso*propanol. Morphine is extracted from the resulting solution by shaking 3 times with 0·1N sodium hydroxide (20 + 15 + 15 ml.). The solution is immediately neutralised with hydrochloric acid, and evaporated to about 25 ml. To this is added 0·25 g. of 4-fluor-1 : 3-dinitrobenzene in 30·0 ml. of acetone and 5 ml. of 25 per cent. ammonia. After standing for 4 hours the precipitated morphine ether is filtered off, washed with 2 ml. of acetone, then twice with 2 ml. of water, and dried for 1 hour at 80° C. The product is only slightly yellow and contains only traces of methoxyl. Comparative trials showed that the results obtained are appreciably lower than those by the Mannich method, and compare well with those of the lime method of the Swiss Pharmacopœia. G. M.

Piperazine, Assay of, by Titration of the Monoperiodate. A. Wickström and A. Valseth (*Ann. pharm. franç.*, 1954, **12**, 777.) The rate at which piperazine reduces periodic acid depends on the hydrogen ion concentration of the solution, being a maximum between *p*H 7·5 and 8·0. In solutions containing bicarbonate, 1 molecule of piperazine reduces about 2 molecules of periodic acid in 2 hours, after which the reaction continues more slowly until a further 1 to 1½ molecules are reduced. Two reaction mechanisms seem to be involved, one producing ammonia and formaldehyde and the other ammonia and glyoxal which is further oxidised to formic acid. It is possible to assay piperazine by precipitation as the sparingly soluble monoperiodate, as follows. To 5 ml. of solution containing 15 to 40 mg. of anhydrous piperazine in ethanol (95 per cent.) add 5 ml. of ether and 1 ml. of 0·4 M hexamine in ethanol. Precipitate the salt by the addition of 0·5 M periodic acid, allow to stand for 10 minutes at 10° C., filter through sintered glass and wash the precipitate with 4 quantities of 3 ml. of a mixture of equal volumes of ethanol (95 per cent.) and ether. Dissolve the precipitate in 10 ml. of sulphuric acid (10 per cent.), dilute to 100 ml., add 10 ml. of potassium iodide solution (10 per cent.), allow to stand for 5 minutes and titrate the liberated iodine with sodium thiosulphate. Hexamine prevents the formation of diperiodate under the conditions described and counteracts the solubilising effect of any excess of periodic acid on the monoperiodate. G. B.

Protoveratrine, Methods for the Determination of. E. W. Grant and E. E. Kennedy (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 129.) The total quantity of protoveratrine in a mixture of protoveratrines may be determined by dissolving the alkaloids in 0·02 N sulphuric acid and titrating with sodium hydroxide using bromocresol green as indicator. Alternatively, titration with perchloric acid in a non-aqueous medium may be used. The intensity of the infra-red absorption band at 5·8μ due to the carbonyl linkage may also be used for the quantitative determination of protoveratrines. The proportion of protoveratrines A and B in mixtures of the alkaloids may be calculated from the ratio of the absorption coefficients at 8·52 and 8·68μ or 9·18 and 9·60μ. The accuracy of the determination is affected by the presence of other alkaloids or impurities. A method of analysis which is not affected by the presence of other veratrum alkaloids depends on the separation of protoveratrines A and B by paper

chromatography, using benzene saturated with formamide as the developing solvent. Protoveratrine A travels with the solvent, while protoveratrine B remains almost stationary. The position of the alkaloids is marked on a guide strip by the application of Dragendorf's reagent, and the appropriate portions of the other strips are extracted with ethanol, the residue after evaporation being treated with sulphuric acid. The colour is measured at 540 mμ after 18 hours, and the content of protoveratrines A and B is calculated with reference to standard solutions, similarly treated. G. B.

Quinine, Alkalimetric Determination of. W. Poethke and D. Horn. (*Pharm. Zentralh.*, 1954, **95**, 414.) Quinine affects the colour of certain indicators such as bromocresol purple and chlorophenol red, and it is therefore necessary with such indicators to titrate against an artificial comparison solution. In the case of a mixed bromocresol green-chlorphenol red indicator a suitable standard (when the end-point occurs in 20 per cent. ethanol) is an ammoniacal solution containing 2 mg. of copper and 0·1 mg. of chromium (as chromate) in 50 ml. Methyl red is unsuitable for quinine in aqueous solutions, but quite useable in ethanolic; in the latter case the end-point is sharpened by the addition of methylene blue. This mixture is especially suitable for the titration of slightly coloured solutions such as may be obtained in alkaloidal assays. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

***p*-Aminosalicylic Acid, Metabolism of, in Man.** E. L. Way, C.-T. Peng, N. Allawala and T. C. Daniels. (*J. Amer. pharm. Ass., Sci., Ed.*, 1955, **44**, 65). Studies were carried out in 5 subjects, 3 of whom received *p*-aminosalicylic acid for 1 day only and 2 over prolonged periods. Urine samples were examined for content of free and conjugated amine, and the metabolites produced from *p*-aminosalicylic acid were isolated by paper chromatography, using dioxan: water (5 : 1) or butanol : ethanol : 3N ammonium hydroxide (4 : 1 : 5) as solvent. Ehrlich reagent was used for the detection of free amines and ferric nitrate solution (1 per cent.) for phenols. Metabolites were also isolated by the countercurrent distribution technique using 2M acetate buffer and *iso*amyl alcohol. The following were isolated: *p*-aminosalicylic acid (14–33 per cent. of the dose administered), acetyl *p*-aminosalicylic acid (28–63 per cent.), *p*-aminosalicyluric acid (0–26 per cent.) and a small proportion of *m*-aminophenol. Traces of 4 other substances were detected. G. B.

Urethane, Tumour-initiating Action of, and its Inhibition by Purine Precursors. F. J. C. Roe. (*Nature, Lond.*, 1955, **175**, 636.) Experiments are described which were undertaken with the object of confirming the hypothesis that urethane competes with one or more of the precursors in purine synthesis with the formation of unphysiological purine-like substances. Glycine and formate as known purine precursors were supplied in high concentration both separately and together, with urethane to groups each of 20 to 30 male mice of stock albino strain. Glycine and sodium formate were given in drinking water during the first 11 days and urethane was applied directly to the skin on the 4th and 7th days. This was followed in each case by 18 weekly applications of croton oil starting on the 22nd day. Large numbers of tumours appeared in that group in which urethane was administered without sodium formate or glycine. Tumour incidence was not significantly different in the groups in which either glycine or sodium formate were administered with urethane. Only a few tumours appeared

in the group of mice treated with both sodium formate and glycine, as well as urethane. J. B. S.

BIOCHEMICAL ANALYSIS

Azovan Blue in Plasma, Estimation of. G. A. Bedwell, J. Patterson and J. Swale. (*J. clin. Path.*, 1955, **8**, 61.) A chromatographic method is described for the estimation of azovan (Evans) blue in plasma, which is not invalidated by the presence of opalescence or hæmolysis. A 10 ml. venous sample of blood is obtained exactly 10 minutes after the intravenous injection of 20 ml. of a 0·1 per cent. solution of the dye. The packed cell volume is determined by Wintrobe's method and the remainder of the dyed sample is centrifuged for 15 minutes. To 4 ml. of the dye-plasma 2 ml. of a dilute teepol solution is added, the mixture is warmed to 50° C. for 10 minutes and transferred to a column of degraded amorphous cellulose. The dye separates as a narrow band on the surface of the column and is eluted with aqueous acetone. The eluate is collected and the intensity of colour determined in a Spekker absorptiometer against a blank of aqueous acetone. The concentration of the dye is estimated from a standard calibration curve. Consistent recoveries of 97 per cent. were obtained and the analyses were not affected by the presence opalescence or hæmolysis. G. F. S.

Cobalt in Biological Materials, Microdetermination of. B. E. Saltzman. (*Analyt. Chem.*, 1955, **27**, 284.) A method is given in which the cobalt compound of 1-nitroso-2-naphthol is formed at *p*H 3 to 4 in aqueous solution and is extracted by shaking with chloroform. Poor recovery was obtained at lower *p*H values; the chloroform extract was purified by shaking with dilute hydrochloric acid to decompose any copper complex and to remove entrained salts. After evaporation of the chloroform extract the residue is ashed by heating with nitric acid and sodium sulphate. The final determination is made by a nitroso R salt method which has been improved to give reliable results and very close adherence to Beer's law. Analyses using the method given showed a 95 per cent. recovery on microgram quantities of cobalt added to 25 g. samples of bone. The cobalt content of normal tissues ranged from 0·03 μg. per g. (rabbit bone) to 0·11 μg. per g. (rabbit kidney). R. E. S.

Hypertensin, Preparation and Assay of. W. S. Peart. (*Biochem*, J., 1955, **59**, 300.) The rapid concentration of hypertensin was achieved by adsorption on to charcoal from serum and subsequent elution by glacial acetic acid; the hypertensinase activity of the serum did not affect the yield. The method involved a preliminary treatment of the serum with charcoal, followed by incubation with renin in the presence of more charcoal, the hypertensin then being eluted from the charcoal with acetic acid. Large quantities of the hypertensin could be prepared by this method, the yield in terms of (—)-noradrenaline being 0·5 to 1·0 mg./l. of serum and the dry weight of material in the acetic acid eluate 100 to 200 mg./l. The yield was assayed by the pressor response produced in the anæsthetised rat (urethane 100 mg./kg. intraperitoneally); the blood pressure was lowed by pentapyrollidinium tartrate in polyvidone solution (2·5 mg./100 g. subcutaneously). Comparisons were made with stock solutions of hypertensin and with (—)-noradrenaline; details of the assay method are given. R. E. S.

Pepsin in Gastric Juice, Estimation of. A. W. Williams. (*J. clin. Path.*, 1955, **8**, 85.) A simple method is described enabling the determination of low concentrations of pepsin in gastric juice to be made, and through dilution the

removal of pepsin inhibitors. The method is based on the conversion by pepsin of edestin to edeston which does not become opalescent when mixed with a saturated solution of sodium chloride. To 1·0 ml. of stock edestin solution add 1·8 ml. of 0·1 N hydrochloric acid and 0·2 ml. of gastric juice. Incubate at 37° C. and at intervals of 2 minutes remove a few drops of the mixture into a saturated solution of sodium chloride. Digestion is complete when there is no further opalescence (usually 10 to 15 minutes). The time taken is compared with that for a standard solution of pepsin treated in the same way. G. F. S.

Pepsinogen (Uropepsin) in Urine, Determination of. M. B. Jørgensen. (*Scand. J. clin. Lab. Invest.*, 1954, **6**, 303.) A method for the quantitative determination of urinary pepsinogen is described, in which hæmoglobin is used as a substrate. 15 ml. of urine is dialysed against distilled water in cellophane tubes for 4 hours. The sample is then quantitatively transferred to a 20 ml. flask and the volume made up to 20 ml. with water. 1 ml. of this dilution is incubated for 30 minutes at 37° C. with 2 ml. of a hæmoglobin substrate and the reaction is then stopped by the addition of 10 ml. of 0·3 N trichloroacetic acid. The amount of aromatic amino-acids is determined in the filtrate, after filtration through Whatman No. 50 filter paper, by reading the extinction coefficient against 0·3 N trichloroacetic acid at 2750 Å. The results are read from a standard calibration curve for known dilutions of L-tyrosine in 0·1 N hydrochloric acid. Blank determinations are run alongside. The pepsinogen activity is expressed as mg. tyrosine liberated (1 mg. tyrosine is equal to approximately 10 μg. of Armour crystalline pepsin). In 70 routine determinations (in duplicate) the mean pepsinogen was equivalent to 0·354 mg. of tyrosine, with a standard deviation of 0·023. G. F. S.

CHEMOTHERAPY

***N*-Bis(β-chloroethyl)amino-acids and Related Compounds as Tumour Growth-retarding Agents.** M. Ishidate, Y. Sakurai and M. Izumi (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 132.) The nitrogen mustard derivatives *N*-bis(β-chloroethyl)glycine and -alanine were prepared by acid hydrolysis of the corresponding *N*-bis(β-chloroethyl)alkyl cyanides, obtained by the simultaneous condensation of bis(β-chloroethyl)amine with the hydroxyalkyl sulphonate and sodium cyanide. *N*-Bis(β-chloroethyl)taurine was made by the alkylation of taurine with ethylene oxide and chlorination with thionyl chloride. The compounds were more hydrophilic and less toxic than the aliphatic nitrogen mustards, and had no vesicant action on the skin. When the free carboxyl group was converted into an ester or an amide, toxic compounds were produced. The minimum effective dose against ascite sarcoma cells was about the same as for nitrogen mustard. The activity of the *N*-oxide derivatives of these compounds was lower than that of the parent substance. In neutral aqueous solution the *N*-oxides were found to undergo gradual transformation into hydroxylamine derivatives. G. B.

Cycloserine, Antibacterial Activity and Blood and Urine Concentrations of. H. Welch, L. E. Putnam and W. A. Randall. (*Antibiotic Med.*, 1955, **1**, 72.) Cycloserine is the generic name for a new antibiotic produced by *Streptomyces orchidaceus*. It is a water-soluble product of relatively low molecular weight and appears to differ in its mode of action from other known antibiotics. It has a wide but relatively low antibacterial activity. *In vitro* studies on 117 strains of

organisms representing 15 genera (gram-positive and gram-negative) showed this activity to be not significantly different among the genera tested. This lack of selective activity may indicate that the antibiotic exerts its inhibitory effect by interfering with a component of an enzyme system or essential metabolite common to all the bacteria tested. Its acute or chronic toxicity in mice, rats, dogs and monkeys is low and not unlike that observed with penicillin. With doses of from 1 to 4 g. daily by mouth, blood and urine concentrations are higher than those obtained with other antibiotics. The percentage of drug excreted in urine with a given dose is apparently higher than that seen with the broad spectrum antibiotics. The drug has been found effective in certain difficult urinary infections caused by organisms whose *in vitro* resistances are considerably higher than the concentration of the antibiotic obtained in the blood. Its effectiveness in these infections and in preliminary trials in pulmonary tuberculosis, in spite of its ineffectiveness in mouse tuberculosis, warrants further laboratory and clinical studies. S. L. W.

PHARMACY

NOTES AND FORMULÆ

Methacholine Iodide and Succinylcholine Iodide in Solution, Stability of. L.-E. Tammelin and L. Larsson. (*Svensk farm. Tidskr.*, 1955, **9**, 229.) A theoretical treatment is made of the previous work of Larsson (*Acta chem. scand.*, 1954, **8**, 1017) and Tammelin (*Acta chem. scand.*, 1953, **7**, 185) on the hydrolysis of choline esters. The *p*H values at which the lowest rate of hydrolysis occurs were 4·2 for acetyl-β-methylcholine iodide and 3·6 for succinylcholine iodide; at these minimum hydrolysis *p*H values the times for 10 per cent. hydrolysis at 25° C. for both esters was 2 years. A stabilised solution could be prepared by dissolving the appropriate ester in 5 per cent. glucose solution and adjusting the *p*H to the minimum hydrolysis value. R. E. S.

Folic Acid in Liquid Pharmaceutical Preparations, Stabilization of. R. P. Tansey and G. H. Schneller. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 34.) Solutions of folic acid with riboflavine, *p*H 4·5 to 5·5 were investigated. The solutions contained propylene glycol as a solubiliser for folic acid, and methyl and propyl *p*-hydroxybenzoate to prevent the growth of moulds. Stabilisers were added and the content of *p*-aminobenzoylglutamic acid (the decomposition product of folic acid) determined at intervals by diazotisation and coupling with Bratton and Marshall reagent. The total folic acid (free and decomposed) was determined similarly after treatment with zinc amalgam. When solutions were stored in diffused daylight in bottles of amber glass, 0·02 per cent. of nordihydroguaiaretic acid or 0·05 per cent. of butylated hydroxyanisole or ethyl hydrocaffeate effectively retarded the decomposition of the folic acid. Propyl gallate and dihydrobenzopyrone were less effective, while sodium formaldehyde sulphoxylate, sodium bisulphite thioglycerol, thiourea, glycine and ascorbyl palmitate had no significant effect. Decomposition was more rapid in solutions kept in direct daylight in flint glass bottles, but the same substances were effective in retarding the decomposition. At higher *p*H levels the protective effect of these substances was less marked.

G. B.

Procaine Solutions, Aniline as a Decomposition Product of. E. Zöllner and G. Vastagh. (*Pharm. Zentralh.*, 1955, **94**, 3.) The normal course of decomposition of procaine solutions leads to *p*-aminobenzoic acid. In view

of the possibility of decarboxylation of the latter compound, and of the reported presence of butylaniline in amethocaine solutions, this question was investigated. After 1 hour's heating at 100° C. at *p*H 4·5, no appreciable amount of aniline could be detected, but at *p*H 2 appreciable quantities were formed. Of 60 preparations for injection, 14 were found to contain aniline in quantities of 10 to 120 μg./ml. These were mostly the ones which were the subject of complaints of undesirable side reactions. G. M.

Sorbic Acid as a Fungistatic Agent. D. D. Puls, L. F. Lindgren and F. P. Cosgrove. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 85.) Solutions containing 0·05 to 0·4 per cent. of sorbic and benzoic acids were tested for fungistatic activity against *Aspergillus niger* and an unidentified species of penicillium. Paper discs soaked in the solutions under test were placed on plates of inoculated medium and the zones of inhibition measured after incubation at 30° C. Sorbic was superior to benzoic acid in these tests and appeared to be a satisfactory preservative for mucilages of acacia and tragacanth and for solutions of guar gum and sucrose when stored at 5, 25 and 37° C. for 30 days. G. B.

PHARMACOGNOSY

***Datura stramonium*, Effect of Removing the Flowering Tops on the Alkaloidal Content.** F. H. L. van Os, E. Drijfhout and F. K. Klompsma. (*Pharm. Weekblad*, 1955, **90**, 209.) A significant increase in the alkaloidal content of *D. stramonium* var. *inermis* plants was found as a result of removing the flowering tops during growth. Removal when the plants were about two thirds developed produced better results than when they were mature. The chlorophyll content was also increased by this earlier treatment. The yield of fresh weight was, however, diminished, since the development of many side shoots was prevented. Removal of the flowers also produced an increase in alkaloidal content. This increase was maintained by repeated removal but the ultimate increase was less than that obtained by removing the tops. None of the treatments described had any effect on the ratio of hyoscyamine to hyoscine. J. W. F.

***Mentha* Species, Composition of Essential Oils of.** A. G. Rooth and R. Hegnauer. (*Pharm. Weekbl.*, 1955, **90**, 33.) The composition of a number of oils of *Mentha* species is given in the table below.

Species	Acid value	Ester per cent. as menthyl acetate	Acetylation value	Alcohols per cent. as menthol	Carbonyl compounds percent. as menthone	
					by hydroxyl-amine	by dinitro-phenylhydrazine
M. aquatica ..	7·9	10·2	26·5	7·4	4·9	—
M. verticillata ..	2·6	21·6	24·9	7·0	4·5	5·2
M. longifolia ..	10·2	31·9	73·4	20·5	18·7	21·5
M. niliaca	5·3	4·3	35·1	9·8	72·4	67·0
M. velutina ..	3·2	—	28·6	8·0	—	66·4
M. rotundifolia ..	11·0	22·3	84·1	23·5	20·5	32·6
M. spicata	6·0	25·5	41·7	11·7	62·5	63·7
M. dalmatica ..	18·1	—	49·2	13·7	64·9	66·3
M. gentilis	4·7	22·9	40·4	11·3	36·8	39·0

It may be noted that none of the wild and non-hybrid forms give a pharmaceutical oil. It appears that an intensive synthesis of menthol or of carvone results only from the combination of the genes of 2 or 3 species. The interpretation of the course of the biosynthesis of the different terpenes is made very difficult by the great number of different chromosome races which appear to exist. It is known that in practically all *Mentha* species there are both morphological and biochemical varieties. G. M.

PHARMACOLOGY AND THERAPEUTICS

Aldosterone, Anticortisol Action of. H. Selye. (*Science*, 1955, **121**, 368.) The question whether aldosterone is an antagonist of glucocorticoids has been investigated. 96 female rats averaging 160 g. were bilaterally adrenalectomised and then subdivided into 4 groups. Hormone treatment was commenced on the day of adrenalectomy. Cortisol as hydrocortone acetate microcrystals (400 μg. daily in 0·2 ml. of aqueous suspension) was given subcutaneously in the chest region, and aldosterone (20 μg. daily in 0·2 ml. of sesame oil) was injected into the inguinal region. The inflammation produced was quantitatively assessed by preparing granuloma-pouches 48 hours later by injecting 25 ml. of air under the dorsal skin, following this immediately by introducing 0·5 ml. of 1 per cent. croton oil into the air space. 14 days after adrenalectomy the animals were killed. The results showed that aldosterone slightly but significantly diminished the bodyweight loss and also the inhibition of inflammatory-exudate formation, but did not suppress the thymus and spleen. In a second experiment, 36 female rats averaging 160 g. were treated by preparing the pouch on the first day, adrenalectomising and giving the steroids 48 hours later and increasing the dose of aldosterone to 25 μg. twice daily. Cholesterol and deoxycorticosterone were given to controls. All the animals were killed on the 12th day. The dose level of aldosterone was found to inhibit a variety of characteristic cortisol actions, being approximately equally as active as deoxycorticosterone. Thus the concept according to which the balance between two opposing naturally secreted corticoids can regulate the course of various biologic phenomena has been demonstrated. J. R. F.

α-Amino-acids, Mercurated Amides of. W. O. Foye and R. A. Mode. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 76.) A series of *N*-(3-acetoxymercuri-2-methoxypropyl) hippuramides of the general formula shown below were prepared from benzoylated glycine, methionine and phenylalanine.

$$C_6H_5{\cdot}CO{\cdot}NH{\cdot}CHR{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH(OCH_3){\cdot}CH_2{\cdot}HgOOC{\cdot}CH_3$$

N-Allylhippuramides were prepared by refluxing with allyl *iso*thiocyanate. The mercury derivatives were obtained by reaction between the *N*-allylhippuramides and mercuric acetate in methanol. The compounds were found to exhibit good diuretic activity when administered intravenously to dogs although the leucine and phenylalanine derivatives had only a weak effect. The glycine derivative was effective when administered orally. G. B.

Butylamine, Substituted, Hypotensive Properties of. R. Charlier, M. J. Dallemagne and E. Philippot. (*Arch. int. Pharmacodyn.*, 1954, **100**, 127). The meriquinone of 4-(4′-oxyphenyl)-4-(3″-methyl-4″-oxyphenyl)-butylamine-2 and 4′: 4-dehydro-4 (4′-oxyphenyl)-4 (3″-methyl-4″oxyphenyl) butylamine-2 (designated L 1935), when injected intravenously into dogs caused a sharp, prolonged fall in blood pressure, which was in the main due to histamine release. That other factors were also concerned in the vasodepressor action was demonstrated by the drug's being effective after oral administration; also the fall was never entirely blocked by antihistamines. In addition, L 1935 had a direct vasodilator action, blocked transmission through the superior cervical and vagal ganglia, and blocked the vasopressor effects of bilateral carotid occlusion and of injections of lobeline and of acetylcholine after atropine. Sometimes the ganglia were stimulated before being blocked. Like other hypotensive

agents, L 1935 decreased the cardiac output. The drug also decreased the neuromuscular blocking activity of decamethonium, while being itself almost without blocking activity. On the isolated frog rectus abdominis muscle L 1935 caused a slow contracture and antagonised the action of acetylcholine, but not that of potassium chloride. G. P.

Carbimazole in Thyrotoxicosis. K. Kirkeby and O. Rømcke. (*Lancet*, 1955, **268**, 374.) Carbimazole differs from methimazole in the replacement of the hydrogen of the sulphydryl group by a carbethoxy group and it was hoped that, in addition to being tasteless, the drug would give a more constant supply of active methimazole to the thyroid gland. The drug has been given to 56 patients with thyrotoxicosis, in 29 of whom the diagnosis was recent; the remainder had relapsed after operation or treatment with other substances. Initial dosage was 30 to 50 mg. daily; 40 mg. is probably the optimal dosage in most cases, producing an average fall of 0·9 per cent. per day in the basal metabolic rate. In most cases a normal B.M.R. was produced in 3 to 12 weeks; 4 cases needed 3 to 5 months, the delay probably being due to low initial dosage. In nearly all patients a rapid subjective improvement occurred, with disappearance of nervousness and palpitations, gain in weight and a rise in serum cholesterol. The only side effect was the appearance of a rash in 1 patient necessitating discontinuance of treatment. Goitrogenic reactions occurred in 4 patients but disappeared during continued treatment. Although the dosage by weight is about the same as that of methimazole, the difference in molecular weight due to the carbethoxy group results in the amount of active substance in a given dose being much less than in the same weight of methimazole and this probably accounts for the lowered incidence of side effects. H. T. B.

Carbonic Anhydrase Inhibitors and Antacids, Effect of, on the Development of Gastrointestinal Ulcers during Treatment with Depot-Histamine. J. W. E. Harrisson, E. W. Packman, P. S. Guth, N. Back and W. S. Chernick. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 123.) Guinea-pigs were treated with diphenhydramine and injection of histamine diphosphate in a mixture of oil and beeswax. This treatment generally produced gastro-intestinal ulceration within 24 hours. A series of antacids was administered orally, twice daily, to the animals in the form of aqueous suspensions. Dihydroxyaluminium sodium carbonate was the most effective in the prevention of ulceration, followed by dihydroxyaluminium aminoacetate, calcium carbonate and sodium carbonate. The carbonic anhydrase inhibitors Diamox (2-acetylamino-1 : 3 : 4-thiadiazole-5-sulphonamide and Dirnate (*p*-carboxybenzenesulphonamide), administered by subcutaneous injection did not inhibit ulceration. G. B.

Chlorpromazine as a Therapeutic Agent. J. H. Moyer, V. Kinross-Wright and R. M. Finney. (*Arch. intern. Med.*, 1955, **95**, 202.) Chlorpromazine was employed in the treatment of 412 unselected patients suffering from neuro-psychiatric disorders; 217 were out-patients with neuroses and other minor emotional disorders, and 195 were psychotic patients. The ambulatory patients were given 5 to 50 mg. of chlorpromazine 3 or 4 times daily by mouth (after meals). Treatment in the psychotic patients was initiated with 50 mg. intramuscularly every 4 to 6 hours, the dose being doubled after 24 hours; by the 3rd or 4th day the route of administration was gradually changed from intramuscular to oral, and the dose increased daily by 200 mg. until improvement in the basic illness was observed. The usual maximum dose in these patients ranged between 1000 and 1600 mg. per day; the maximum dose was continued for a week or ten days and gradually reduced to a maintenance level. In the group of ambulatory patients excellent results (total remission or relief of

symptoms) were obtained in 135; good results in 61, and poor in 21. In the psychotic patients remission was obtained in 96, improvement occurred in 92, and 17 remained unchanged. A further series of 338 patients suffering from nausea and vomiting due to various causes (including a wide range of drugs) were treated with chlorpromazine in doses of 10, 25 or 50 mg., orally or intramuscularly, at varying intervals as needed to control symptoms. Excellent results (complete cessation of vomiting and relief of nausea) were obtained in 243 of the patients, good or fair results in 81, and poor results in 28. The nausea and vomiting of pregnancy responded very well. 55 out of 78 patients obtained complete relief and only 4 were complete failures. During the course of these studies 10 patients with persistent and intractable hiccoughs were given chlorpromazine. In 6 of the patients hiccoughs were arrested within 20 minutes of an intramuscular injection of 25 mg. of chlorpromazine; two required a second dose, and two did not respond. Among the side-effects noted was dermatitis (27 cases), confusion, with disorientation (4 cases), and Parkinsonian syndrome (14 cases). S. L. W.

Codeine and Morphine, the Action of, on Cardiac Arrhythmias. A. Leimdorfer. (*Arch. int. Pharmacodyn.*, 1955, **100**, 333.) Experiments in dogs, under light pentobarbitone or thiopentone anæsthesia, have shown that codeine and morphine can prevent the appearance of cardiac arrhythmias provoked by adrenaline or (—)-noradrenaline. Ventricular extrasystoles were induced by intravenous injections of 0·005 to 0·014 mg./kg. of adrenaline or 0·002 to 0·007 mg./kg. of noradrenaline. The dose of codeine phosphate was 1–2 mg./kg. and of morphine sulphate 2·5 to 4 mg./kg., both being given in four equally divided doses. The results obtained were in agreement with the clinical reports of the disappearance of ventricular extrasystoles after oral administration of codeine and the successful treatment of paroxysmal ventricular tachycardia with small intravenous injections of morphine. G. F. S.

Cycloserine in the Treatment of Pulmonary Tuberculosis. I. G. Epstein, K. G. S. Nair and L. J. Boyd. (*Antibiotic Med.*, 1955, **1**, 80.) This is a preliminary report on the treatment with cycloserine of two groups of patients suffering with pulmonary tuberculosis. In one group of 8 patients, only recent infection was present and the patients had not received any prior antimicrobial therapy. A second group of 29 patients presented active, far-advanced pulmonary tuberculosis of long duration; all had been in hospital for at least a year and had been under intensive treatment with streptomycin, isoniazid and *p*-aminosalicylic acid, to all of which they were clinically and bacteriologically resistant. The cycloserine was administered orally, in capsules containing 250 mg., in doses of from 1 to 1·5 g. daily, for from 6 weeks to 4 months. In all of the acute cases there was a rapid and marked clinical response, with a temperature return to normal and disappearance of cough and expectoration; within 8 weeks sputum and gastric washings became negative and there was marked roentgenographic improvement. In the 29 chronic patients, clinical improvement occurred in all but one, and roentgenographic improvement in 20. Up to the present, sputum concentrate determinations show conversion in 76 per cent. of the patients, and, in cases with a negative sputum, gastric washings showed 85 per cent. free from tubercle bacilli. Cycloserine was well tolerated and there was a low incidence of side-reactions. On the basis of neurologic symptoms it was necessary to discontinue treatment in 4 of the patients; no other toxic manifestations were observed. The authors conclude that although the study has not progressed sufficiently to allow judgment as to the relative efficacy of cycloserine as compared with streptomycin and isoniazid, the data

available are sufficient to indicate that cycloserine is a potent antituberculosis antibiotic. S. L. W.

Pentolinium Tartrate Combined with Rauwolfia in Hypertension. C. W. C. Bain, F. Ashton and B. P. Jones. (*Brit. med. J.*, 1955, **1**, 817.) This is a report on a series of 17 cases in which stabilisation had been achieved on pentolinium tartrate (Ansolysen) alone and which were later restabilised on that drug with the addition of rauwolfia alkaloids (Rauwiloid), 4 mg. daily, taken in a single dose each evening. All the patients had essential hypertension. Their blood pressure before treatment varied from 300/160 to 240/130. None had evidence of renal involvement. The average dose of pentolinium tartrate when given alone was 840 mg. a day. The average daily dose of that drug when combined with rauwolfia alkaloids was 240 mg.; one patient who required 440 mg. a day was restabilised on the combination at 60 mg. a day. The corresponding decrease in the undesirable effects following the use of pentolinium tartrate, e.g., constipation, dry mouth and visual disturbances, was very great, and some patients who had found it impossible to continue with pentolinium tartrate alone are now well stabilised and able to lead active lives. Where formerly postural hypotension was a problem, this has been less evident on the combination. The authors conclude that the combined use of these two drugs seems to offer a promising treatment of hypertension. S. L. W.

Piperazine Derivatives, Effect of, on Intestinal Helminths. T. L. Dunn. (*Lancet*, 1955, **268**, 592.) The drugs diethylcarbazine, piperazine hydrate and piperazine adipate were tested against roundworm and hookworm infestation of children of 1 to 14 years in New South Wales. Tests for ova were made by the salt flotation method. The highest egg counts before treatment were 153,000/g. of fæces for ascaris, 54,000 for necator and 13,000 for tricocephalus. Piperazine adipate (75 mg./kg.) proved the most effective against *A. lumbricoides* and *T. dispar* in 45/47 and 28/31 cases the children being ova-free 2½ weeks after treatment. (Ova in the cases not cured were very scanty). The hydrate (85 mg./kg.) was effective in 20/26 cases and 4/22 cases respectively under the same conditions. Diethylcarbazine (13 mg./kg.) was successful in 12/54 against *A. lumbricoides*. 41 children with *Necator americanus* treated with diethylcarbazine, 28 with piperazine hydrate and 7 with the adipate showed no change in egg counts, and 4 children with *Hymenolepsis nana* treated with the adipate were also unaffected. The hydrate caused most worms to be passed on the second day of treatment, whereas with the adipate evacuation extended to the 3rd and 4th day. No toxic or unpleasant side reactions occurred with either the hydrate or the adipate. Contrary to manufacturers' directions a single daily dose was given and no aperient. It is suggested that factors such as food intake may have affected the results. J. R. F.

Reserpine in Hypertension. I. Singh. (*Brit. med. J.*, 1955, **1**, 813.) Twenty-three cases of hypertension were treated with reserpine; 24 had benign hypertension, 2 had malignant hypertension, 1 was associated with chronic nephritis, 2 with arteriosterosis, and 3 with anxiety state. All had been under care for periods varying from 2 months to 7 years. Fifteen patients whose basal systolic blood pressure at the time of treatment was above 200 mm. Hg. were treated with reserpine 1·5 mg. daily; 18 patients whose pressure was below 200 were treated with 0·75 mg. daily. In the former group 2 patients were reduced to below 145/90, 5 below 160/100, 7 below 170/110, and 10 below 185/115. In the latter group 12 patients were reduced to below 145/90 and 18 below 160/100. Lack of adequate response was associated with chronicity of hypertension and myocardial and/or renal failure. Periodic fluctuations up to plus 45/20 mm.

(ABSTRACTS *continued on p. 560.*)

BOOK REVIEW

PAPER CHROMATOGRAPHY, by Friedrich Cramer. Second edition, translated from the German by Leighton Richards. Pp. xi + 124 (including 68 illustrations, and Index). Macmillan and Co., Ltd., London, 1954. 25s.

This is a translation of the second German edition of a book based on a series of articles in *Angewandte Chemie*. The first edition was a praiseworthy attempt to fill the need for an introductory book on paper chromatography and was the first of its kind. The present text claims to be "a practical manual and attempts to give precise working directions". This is a curious claim, since the details given are too few. Particularly is this so in the inorganic section. To the reviewer, unversed though he is in this great and expanding branch of the subject, seven pages seem totally inadequate for a practical manual, particularly when compared with the gratuitous introduction to paper electrophoresis occupying six pages. One half-page, devoted to quantitative inorganic analysis, contains generalities of a most arbitrary selection. A most irritating feature of the work is the almost complete disregard of the conventions and nomenclature of chemistry as published in the English language. A number of errors, including the mis-spelling of authors' names, have been carried over from the German edition, and obscurities and errors have been introduced.

More serious is the adoption of the term development to mean the application of chemical agents to produce colour effects to help locate the material on the paper. This term has a long-established meaning in chromatography and refers to the operation of irrigating the particulate chromatographic medium with the fluid phase. Thus paper chromatography depends largely on elution development, with minor components of displacement development and frontal development.

The presentation of the introductory matter is good, and there are many excellent illustrations and tables of R_F values. It is evident, however, that what is still needed at the present moment, particularly on the inorganic side, is a detailed practical manual, co-ordinating and summarising the already great volume of published work. TUDOR S. G. JONES.

(ABSTRACTS *continued from p. 559.*)

Hg. continued to occur, and required suitable adjustment of dosage. The blood pressure started falling between the 4th and 19th days, reaching its lowest in 9 to 40 days. In some cases it was possible to discontinue the drug for 3 to 13 days before a rise started again. Appreciable additive effects were obtained, with less fluctuation of blood pressure, when reserpine was administered together with hexamethonium bromide. The severity of side-effects varied with individual tolerance and the dosage of reserpine. Nasal congestion, anorexia, dryness of the mouth, depression, muscular weakness, giddiness, diminished vision and drowsiness occurred with varying severity and frequency, and were more frequent and pronounced when the dose of reserpine exceeded 0·75 mg. daily. Under the influence of the drug slowing of the pulse rate from 20 to 36 beats was a consistent finding. When the side-effects passed off, usually within a week or so, all patients showed subjective improvement, the symptoms most relieved being headache, giddiness, insomnia, palpitation and worry. Constipation had a tendency to be relieved. In some patients the bradycrotic effect was very marked and precordial discomfort was complained of. The bradycrotic effect was abolished by atropine. Some degree of postural hypertension was present in those receiving reserpine 1·5 mg. daily; this was also abolished by atropine. S. L. W.

PRINTED BY W. HEFFER & SONS LTD., CAMBRIDGE, ENGLAND